

HEALTH EFFECTS

OF THE CHORNOBYL ACCIDENT

a Quarter of Century Aftermath



**National Academy of Medical Sciences of Ukraine
Research Center for Radiation Medicine
Nagasaki University
Global Strategic Center for Radiation Health Risk Control**

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PREFACE

It is a great honor and pleasure for Nagasaki University, Japan, to jointly support the English edition of the "Health Effects of the Chernobyl Accident — a Quarter of Century Aftermath" together with the State Institution "Research Center for Radiation Medicine of the National Academy of Medical Sciences of Ukraine" (RCRM), according to the existing academic cooperative agreement between our institutions.

For nearly 20 years we have been jointly working in the radio-contaminated field of Chernobyl in Ukraine. These investigations were of particular importance for two successive frameworks of Center of Excellence (COE) programs. These are 21st Century COE Program entitled "International Consortium for Medical Care of Hibakusha and Radiation Life Science" from FY2002 to FY2006 for five years and a newly established Global COE Program entitled "Global Strategic Center for Radiation Health Risk Control" from FY 2007 until now for four years. We are intended to establish a pivotal center for education and research with a close focus on local population in Chernobyl.

Now, on the special occasion of the 25th anniversary of the Chernobyl accident, we will summarize the studies that continued for 25 years, especially here in Ukraine. Note that as a whole the studies are not yet complete or comprehensive enough to clarify the accurate details of cause-and-effect relationship of diseases discovered around Chernobyl. Since this worst ever technogenic catastrophe was accompanied by an unprecedented massive release of varying spectra of radioisotopes which led to radioactive contamination of vast territories through fallouts and exposure of millions of residents of all ages, consequences for health and radioecology could not be reliably estimated within a relatively short time.

To overcome the uncertainty and vagueness of chronic radiation syndrome, Ukrainian researchers have done an enormous effort and reached mutual understandings of acute and chronic radiation syndromes as well as of the late effects of radiation on such illnesses as leukemia, thyroid cancer, non-cancer diseases and so on. A wide-ranged and tremendous exertion on compiling the results obtained by RCRM is highly appreciated to realize the past and current situation of Chernobyl victims. Moreover, the comparative studies with Belarus and Russia will be desired to further strengthen their impact on various aspects of health effects of the Chernobyl accidents.

Among several important collaborative research projects in the framework of our Global COE program such as radiation-associated leukemia and blood disorders, and metabolic and psychiatric disorders, here I would like to emphasize, as an important example, the necessity of joint studies on radiation-induced thyroid cancers around Chernobyl. The incidence of thyroid cancer in children grew dramatically in the territories affected by the Chernobyl accident and there is a broad range of latency periods in children and adolescent and at present young adults who developed thyroid cancer. The experience from Chernobyl childhood thyroid cancer has substantially improved our understanding of the disease in several fields, including clinical practice, pathology and molecular carcinogenesis.

Childhood thyroid cancers have a fairly good prognosis based on early diagnosis and appropriate treatment combining surgery and radioactive iodine therapy. The mutational spectrum of childhood thyroid cancers demonstrates that gene rearrangements leading to activation of mitogen-activated protein kinase signalling seem to have a pivotal role; point mutations are rare. Recently another important finding has been reported that a common genetic predisposition factor exists in both sporadic and radiation-induced thyroid cancers in Chernobyl. Therefore the characteristics of thyroid cancer, including its clinico-pathological manifestations, may associate with patients' age at exposure and also depend on an individual's genetic susceptibility, indicating the urgency of continuous large-scale molecular epidemiology studies of thyroid cancer in Chernobyl-affected areas in the framework of our academic cooperative research projects and the necessity of further strengthening Chernobyl Tissue Bank.

Based on the tight cooperative relationship with RCRM, Global COE program of Nagasaki University clearly aims at the achievement of new multidisciplinary academic outcome, an establishment of "Radiation Health and Life Sciences" in cooperation with the leading education and research institutes and universities in Eurasia and developed countries. Nagasaki University is now forwarding its unique education and research activities in three major research fields: International Radiation Health Science, Atomic Bomb Disease Medicine, and Radiation Basic Life Science. It would be ideal to share our knowledge on Atomic bomb survivors with and expand it on the Chernobyl survivors.

For this purpose, at the occasion of Chernobyl 25th anniversary, RCRM and Nagasaki University recently reached another mutual agreement for intensive cooperation at the standpoint of environmental sensing and human body monitoring for nuclear workers who are currently managing and handling the aftermaths of destroyed Chernobyl nuclear reactor in the high risk and constrained working areas. We separately plan to provide necessary support and start a pilot study of a remote individual real-time dose sensing and health monitoring system. We have further agreed to search for a possibility of preparing a new publication with a tentative title of "Hiroshima-Nagasaki-Chernobyl" in future.

After I wrote up this preface, the biggest earthquake and Tsunami in the north-eastern Japan region seriously damaged the Fukushima Daiichi Nuclear Reactor Power Plant on March 11, 2011. I myself started to be deeply involved in crisis task force from the beginning as an advisory board member of radiation health risk control of Fukushima prefecture. There are still a lot of confusion and mess, and so many rooms for improvement at the site of accident as well as at the radio-contaminated areas. It takes time to solve the critical problems of damaged reactors. It, too, takes time to monitor not only health status of local residents affected by radiation fallout but also the radio-contaminated lands for agriculture and living. The lessons learnt from Chernobyl, therefore, will be definitely useful and contribute to early recovery from the worst nuclear reactor disaster in Japan.

Finally, radiation effects on human beings are complicated but may be categorized simply into two groups: acute and chronic effects, and high and low dose effects. International cooperative projects are, therefore, of vital importance for solving problems in radiation emergency medicine as well as for identifying potential impact of low dose and low dose-rate radiation effects and models. Risk communication based on well-established regulatory science is really critical for maintaining a healthy life and safeguard at the Nuclear Age of the 21st century.

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PREFACE FROM UKRAINIAN EDITORS

On April 26, 1986 the largest accident in the history of the nuclear industry occurred at the Chernobyl NPP Unit IV. More than 300 MCi ($14 \cdot 10^{18}$ Bq) of radioactive materials were released from the reactor core, causing the contamination of 50,500 km² of the territory of Ukraine, where in 2,218 settlements had home to over 2.4 million people. Central part of Ukraine, south-eastern regions of Belarus and some regions of European Russia underwent the highest contamination. These territories were inhabited by 17.5 million people, including 2.5 million children under the age of 7 years. Over 600,000 people took part in 1986–1989 in clean-up works of accident. Collective effective dose only for Ukraine's total population (thyroid doses not included) was about 50,000 person-Sievert for 10 years. Collective thyroid dose (to 21 regions of Ukraine and Kyiv) comprised 1.306 million person-Gray, of which 607,000 person-Gray were doses in children and adolescents.

Lessons from Chernobyl present an unique and valuable experience of the minimisation of its consequences, its effects on health and environment. It has elucidated gaps in the field of nuclear technologies, emergency response, radiation protection of population influenced by the accident. To our mind, such radiation accidents as the 2011 Great East-Japan earthquake and tsunami, and the last fact of workers overexposure at an irradiation facility in Bulgaria show the existing necessity of continuous assessment of the experience of Chernobyl at national levels as well as standardized approaches of the international scientific evaluation.

Early response after the accident included estimation of the scale of the accident, reporting, prehospital care, patients sorting and evacuation. It has shown timely evacuation of highly exposed patients to the specialized clinics. Limited and delayed use of sheltering in Pripyat, adjacent settlements and later in Kyiv was explained by the need of changes in emergency plans re-directing them from civil defense actions after nuclear attack to situation with radioactive contamination after a substantial damage to nuclear reactor, that was regarded secondary or even impossible. One of the first challenges was the evacuation of residents of Pripyat and 30-km zone. Photos of the process, captured by the U.S. satellites have fixed a high organizational level of this action and quick implementation of civil defense plans. Not later than 24 hours after the accident more than 1,000 buses were provided for evacuation with needed staff, police and emergency health care support. Substantial part of the population of adjacent area was evacuated using 3,000 private cars. At the same time, radiation protection actions for adjacent area were regarded secondary and used later. An increase of radioactive contamination of transportation corridors, absence of facilities for timely decontamination of big groups of staff and population, clothes and transport led to an increase in radiation doses. City coaches contaminated with radionuclides after the evacuation were on the second day returned for providing public transportation to Kyiv, increasing the radiation tail.

Before the accident for Ukraine's population that lived near the Chernobyl nuclear power plant, the radiation protection limits were used that were appropriate in the USSR. After the dosimetry data analysis by the USSR State Committee on Hydrometeorology after the accident the temporary accidental regulations were introduced on radioactive contamination of foodstuffs and annual population doses. There are different views on the efficiency of health care system response to the Chernobyl accident. In the summary of the international conference "Fifteen Years after the Chernobyl Disaster. Lessons Learned" (2001) the gaps were mentioned in psychological support and stated that lack of expertise in radiology did not allow people to personally assess the accuracy of information distributed by press, radio and television. As a result, the subjective perception of the possible consequences of the accident was much superior to the actual situation. Worsening economic conditions, the collapse

of the Soviet Union — all this taken together made the accident really a disaster for millions of people, translating them into the category of "affected by the Chernobyl accident". To comment, it should be noted that the stability of the existing political and economic situation and lack of information had not reduced the health consequences of the Southern Urals accident, and only provided their concealment for decades. Differences between the official information presented at Fig. 1 and the real situation (Fig. 2) show its inadequacy. To the contrary, the individual human reactions directed to escape from the factor known to be extremely harmful for human life to them and their progeny could be estimated as adequate and could not be averted by psychological support in the absence of radiation protection actions known to population from the civil defense trainings of the "cold war" times.

In analyzing the effectiveness of countermeasures the Ukrainian-Russian group (2007) has indicated that in general, by the end of 1986 a total of 116,000 inhabitants were evacuated from 188 settlements, including 24,700 in Belarus. The population was evacuated in the hope of returning, however, till now the specific criteria on returning the population to areas with residual contamination are discussed. It was planned to evacuate from the radiation control zone 80 thousand children and 5 thousand pregnant women. In fact, only in Ukraine evacuated were 200 thousand children, which allowed to reduce by 15–20% the expected annual dose of radiation for 1986, although the unit cost of averted dose was high. Of substantial effect was the self-evacuation. The evacuation of children contributed also to the improvement of psychological status of the population.

The not-timely administration of stable iodine was among the most serious drawbacks of the emergency response system. The science-based action plans were elaborated, published and known but to radiation safety professionals ("Radioactive iodine in the system of radiation safety" edited by L.A. Ilyin, 1972). The mandatory recommendations approved by the USSR Ministry of Health (1981) on population protection after nuclear power plant accident were classified as "Confidential", not used to justify the local emergency plans and staff training, and as a result, never implemented at the critical moment of Chernobyl. Of negative effect was also the excessive authority centralization at that period. Due to the absence of clear and understandable for local administration and population criteria that could enable an immediate its start, the decision on iodine prophylaxis had to be approved at the regional levels, agreed with higher authorities and invited experts. Administration of the stable iodine to population was started at the end of the acute "iodine" period.

Information on the stable iodine administration is still contradictory. According to the Ministry of Health of the USSR about 5 million iodine tablets were distributed, but there is a reason to believe that only about 20% distributed pills were used. According to the IAEA International "Chernobyl Project" the decisions on stable iodide tablets distribution were in line with sustainable practices and intervention was justified. In the city of Prip'yat stable iodine administration covered 70% of the population, including 60% on April 26th. Information from the former Soviet Republics has shown differences in time and volume of stable iodine administration. The Ministry of Health of Ukraine has reported that blocking of ra-

Четверг, 1 мая 1986 года

От Совета Министров СССР

Несмотря на то, что в реакторе
произошла авария, радиоактив-
ного топлива не выгорело, реактор
остановлен и находится в
безопасном состоянии.

... chain fission reaction of
nuclear fuel does not oc-
cur, the reactor is in the OFF
state

Fig. 1. Example of official information from the USSR Council of Ministers. May 1, 1986

ected people were conducted, and the dosimetric certification of all settlements contaminated by radionuclides from the Chernobyl accident carried out. The Radiation Safety Standards of Ukraine (NRBU-97) were introduced with amendments in 2000. Several methods were proposed for dose reconstruction within the years after Chernobyl accident, the most reliable results were obtained using the RADRUE approach. Technologies of biological dosimetry were introduced and implemented including the restoring of biological doses using electron spin resonance of tooth enamel. The accuracy of the method meets global standards, confirmed by the results of intercalibration with the IAEA Reference Laboratory and the National Institute of Standards and Technology, USA. Cytogenetic laboratories performed chromosome aberrations tests in clean-up workers and population. Fluorescent *in situ* hybridization, identification of mutations in the Glycophorin A, T-cell receptor locus were introduced and implemented.

In the first 20 years after the accident reliable data were collected and data presented to international scientific community on the health effects of Chernobyl, the mechanisms of low-dose effects and treatment of radiation-exposed. Of special importance were studies of the early and late effects of the acute radiation syndrome, local radiation skin and eye injuries, epidemiology and molecular mechanisms of radiation-induced thyroid cancer in children, leukemia in clean-up workers and epidemiology of specific types of cancers. Among the priorities are the studies on the excess of chronic lymphocytic leukemia that are the subject of the ongoing international research now. The same is the situation with myelodysplastic syndrome and multiple myeloma.

For some other effects a substantial amount of data is collected but still there is no consensus on their radiation origin, due to longer period of induction after radiation exposure (solid cancers), wide prevalence in the unexposed population (cardiovascular diseases), difficulties in reconstructive dosimetry of combining external irradiation and incorporation of radionuclides (thyroid cancer in clean-up workers of the accident, evacuees exposed in adulthood, benign thyroid disease, breast cancer), or contradictory data.

Important results also include the impact of ionizing radiation on the health of children, particularly exposed *in utero*, the influence of ionizing radiation on the regulatory mechanisms, the human brain. The book gives a scientific analysis of data accumulated in recent years.

A 25-years period is the period of real assessment of accident consequences, conclusions and prognoses for the future. Fundamental data proving that the Chernobyl disaster and its aftermath have become a factor both the direct and indirect effects of ionizing radiation on the human body, its organs and systems, cell populations. In the range of low doses new mechanisms were described — positive and negative bystander effect, adaptive response and a low-dose radiosensitivity. Among the results of the last studies are molecular studies of leukemia, bone marrow microenvironment, CD34⁺ stem cells, telomere length, gene polymorphism and expression. These studies have to be continued. The important contribution of the State Institution "Research Centre for Radiation Medicine of the National Academy of Medical Sciences of Ukraine" are also the late effects of acute radiation syndrome, changes of brain function and epidemiological studies on non-tumor diseases, which are detailed in the next sections.

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ABBREVIATIONS

12-HETE ... 12-hydroxyelicosatetraenoic acid	BD birth defects
8-OHdG 8-hydroxydeoxyguanosine	BER-EP4 differentiation epithelial antigen
AA auditory analyzer	BFU-E early erythroid progenitors
ACE angiotensin converting enzyme	BLHX bleomycin-hydrolase
ADHD attention deficit and hyperactivity disorder	BM bone marrow
ADR analytical dose reconstruction	BME body mass excess
AF atrial fibrillation	BMI body mass index
AH arterial hypertension	BMS bone marrow syndrome
AH arterial hypertension	BP blood pressure
AICC acute impairment of cerebral circulation	BPDM daily monitoring of blood pressure
AIHA autoimmune hemolytic anemia	BPH benign prostate hyperplasia
AIP auditory induced potentials	BWW Biggers-Whitten-Whittingham medium
AIS automated information systems	CA-605 Construction Administration № 605
AIT autoimmune thrombocytopenia	CAT catalase
AL acute leukemia	CCT conditionally clean territories
ALT alanine aminotransferase	CD cluster of differentiation
AML acute myeloid leukemia	CD10 cluster of differentiation (CALLA); common acute lymphoblastic leukemia antigen
ANOVA analysis of variance	CD13 Gp 150, aminopeptidase N
AOS antioxidant system	CD14 Lipopolysaccharide receptor, LPS R
APE1 apurinic/pyrimidinic endonuclease 1	CD16 Low affinity Fc receptor for IgG, Fcγ R III; NK-cells, granulocytes, macrophages
APh acid phosphatase	CD19 B4 B-lymphocyte antigen
APL acute promyelocytic leukaemia	CD22 BL-CAM; Precursors B-cells and B-cells
Ara-C cytarabine	CD3 T3; T-cells
AREM acoustic reflex of erotic muscles	CD33 pan myeloid antigen; majority of monocyte cells
ARS acute radiation syndrome	CD4 helper/inducer T-cells, monocyte subset, cortical thymocyte subset
ARS NC acute radiation syndrome non-confirmed	CD45 leucocyte common antigen
AST aspartate aminotransferase	CD56 neuronal cell adhesion molecule, NK-cells, some T-cells
ATA antitryptic activity	CD8 cytotoxic/suppressor T-cells, NK-cells subset
ATM ataxia-telangiectasia-mutated protein	CD95-L Fas-ligand
ATR attributable risk	CDD congenital defects of development
AURCRM All-Union Centre for Radiation Medicine, Academy of Medical Sciences of the USSR	CDK cyclin-dependent kinase
BAL bronchoalveolar lavage	CDS Civil Defense Service
Bax bcl-2-associated X protein	CEA condensate of expired air
bcl-2 (B-cell lymphoma 2) apoptosis regulator protein	
B-CLL B-cell chronic lymphocytic leukemia	
BCR B-cell receptor	

CPS.....	chronic fatigue syndrome	EA.....	extrasystolic arrhythmia
CFU	colony-forming units	EAR.....	excess absolute risk
CPubm.....	bone marrow CFU activity	E-CAT.....	erythrocyte catalase
CFUpb.....	circulating progenitor cells in peripheral blood	ECC.....	external Corti's cells
CGI.....	cephalography index	ECG.....	electrocardiogram
ChA	Chemobyl accident	ECG DM	electrocardiography daily monitoring
ChB	chronic bronchitis	ECOG-WHO..	54f
CHD	coronary heart disease	EDD.....	end-diastolic dimension
ChNPP.....	Chemobyl Nuclear Power Plant	EEG.....	electroencephalogram
CIDB	lipid peroxidation compounds with isolated double bonds	EF	ejection fraction
CIDI	Composite International Diagnostic Interview	EGFR.....	epidermal growth factor receptor
CIS	Commonwealth of Independent States	ENG.....	electromyostagmography
CIS	carcinoma in situ	EOG.....	electrooculography
CKRS.....	chemokine receptor 5	EP	evoked potentials
CLL.....	chronic lymphocytic leukemia	EPR	electron paramagnetic resonance
CML.....	chronic myelogenous leukemia	ERD.....	external radiation doses
CMMI.....	chronic myelomonocytic leukemia	E-RPC	E-rosette forming cells
CMPD.....	chronic myeloproliferative diseases	ERR.....	excess relative risk
CMV.....	cytomegalovirus	ERV	expiratory reserve volume
CNS.....	central nervous system	E-SOD.....	erythrocyte superoxide dismutase
COE.....	Center of Excellence	F _{ADH}	factor of antioxidant system
Con A	concanavalin A	FAS-receptor..	death receptor on the surface of cells that leads to apoptosis
COPD.....	chronic obstructive pulmonary disease	FC.....	functional class
COX	cyclooxygenase	FCM	flow cytometry
CR	cancer registry	FEV1	forced expiratory volume in one second
CRR.....	contaminated by radionuclides regions	FGFR.....	fibroblast growth factor receptor
CRW.....	Chemobyl accident recovery operations workers	FGI.....	French-German Initiative
Cik	cytokeratin	FHIT.....	fragile histidine triad gene
CVD	cardiovascular diseases	FISH.....	fluorescent in situ hybridization
CZ.....	contaminated zone	FISH-WCP	fluorescence in situ hybridization of human metaphase chromosomes with DNA-probes to whole chromosomes
CER.....	Clinical and Epidemiological Registry	FITC.....	fluorescein isothiocyanate
DAG	1,2-diacylglycerol	FL-1.....	fluorescence parameter
DB	database	FRO	free-radical oxidation
DBP.....	diastolic blood pressure	FT ₄	free thyroxin
DC.....	diene conjugates	FVC.....	forced vital capacity
DCC.....	data coordinating center	GAF.....	Global Assessment of Functioning
DEP	discirculatory encephalopathy	GAI.....	growth age index
DHE.....	dihydroethidine	G-banding.....	differential G painting of chromosomes
DLCO.....	diffusing capacity for the lungs measured using carbon monoxide	GDP.....	gross domestic product
DNA.....	deoxyribonucleic acid	GHQ-28.....	General Health Questionnaire-28
DSB.....	double strand breaks	GIT	gastrointestinal tract
DSM.....	Diagnostic and Statistical Manual of Mental Disorders	GM	geometric mean
E/A	ratio of maximal velocity early and lately diastolic filling	GPA.....	glycophorin A
		GPs.....	glutathione peroxidase
		GR.....	glutathione reductase
		GSD	geometric standard deviation
		GSH.....	glutathione reduced

GSSH.....	glutathione oxidized	IISW.....	Integral Index of Social Well-being
HbF.....	fetal hemoglobin	IL.....	interleukine
HC.....	haemopoietic cells	IMC.....	intima-media complex
HCDR3.....	heavy chain complementarity-determining region-3	IMGT.....	International Immunogenetics Database
HCV.....	hepatitis C virus	imp.....	impulse
HDG.....	high dose group, Heidelberg UICC classification	INOS.....	inducible NO synthase
HEp-2.....	epithelial cell line	inter-SSR-PCR.....	inter-simple sequence repeats - polymerase chain reaction
HER-2.....	human epidermal growth factor receptor 2	IPHECA.....	International Program on the Health Effects of the Chernobyl Accident
HF.....	heart failure	IPM.....	interpeak interval
HFp.....	high frequency	IPMDS.....	54f
HHd.....	hypertensive heart disease	IPSS.....	International Prognostic Scoring System
HIV.....	human immunodeficiency virus	IQ.....	Intellectual quotient
HLA-DR.....	MHC class II cell surface receptor encoded by the human leukocyte antigen complex on chromosome 6 region 6p21.31	IR.....	ionizing radiation
HR.....	high risk (IPSS)	IR.....	intermediate risk (IPSS)
HRD.....	heart rhythm disorders	ISCN-2005.....	International System for Human Cytogenetic Nomenclature-2005
HRV.....	heart rate variability	ITD.....	internal tandem duplications
HSC.....	hemopoietic stem cells	ITGV.....	intra thoracic gas volume
HSV1+2.....	herpes simplex viruses, types 1 and 2	ITIM.....	tyrosine phosphorylation-based activation
IAEA.....	International Atomic Energy Agency	IVRT.....	isovolumetric relaxation time
IARC.....	International Agency for Research of Cancer	IVSTh.....	interventricular septum thickness
IC.....	index centralization	LA.....	left atrium
IC.....	inspiratory capacity	LD.....	low doses
ICA.....	internal carotid artery	LDG.....	low dose group,
ICD.....	International Classification of Diseases	LDH.....	lactate dehydrogenase
ICD-10.....	International Classification of Diseases, 10th revision	LE.....	Life expectancy at birth
ICD-O.....	International Classification of Diseases for Oncology	LET.....	linear energy transfer
ICRP.....	International Commission on Radiological Protection	LE/HF.....	low to high frequency range
ID.....	index of differentiation	LFp.....	low frequency
IDH.....	inosine-5-monophosphate dehydrogenase	LGL.....	large granular lymphocytes
Ig.....	immunoglobulin	LL.....	long-latent
IGHD.....	immunoglobulin stereotyped combinations of heavy-chain diversity	LOH.....	loss of heterozygosity
IGHJ.....	immunoglobulin stereotyped combinations of heavy-chain joining	LP.....	latent period
IGHV.....	immunoglobulin heavy chain variable region	LPO.....	lipid peroxidation
IGK.....	immunoglobulin kappa light chain gene	LR.....	low risk (IPSS)
IGL.....	immunoglobulin lambda light chain gene	LRP.....	lung resistance protein
IHD.....	ischemic heart disease	LV.....	left ventricular
		LVDD.....	left ventricular diastolic dysfunction
		LVEF.....	left ventricular evacuation function
		LVH.....	left ventricular hypertrophy
		LVMM.....	left ventricle myocardial mass
		LVPWTh.....	left ventricular posterior wall thickness
		M.....	mutated
		MANOVA.....	multivariate analysis of variance

MAPK	mitogen-activated protein kinase	PCR-SSCP	polymerase chain reaction - single strand conformation polymorphism
mtDNA	mitochondrial DNA	PFS	progression-free survival
MDR	multi-drug resistance	P-gp	P-glycoprotein
MDS	myelodysplastic syndrome	PI	propidium iodide
MEK	MAP/ERK kinase	PL	phospholipid
MH	mental health	PNA	peptide nucleic acid
MH of UkrSSR	Ministry of Health of Ukrainian SSR	Ptx	peroxiredoxin
MHC	major histocompatibility complex	PTSD	posttraumatic stress disorder
MI	myocardial infarction	qEEG	quantitative EEG
MIA	Ministry of Internal Affairs	Q-MI	Q-wave myocardial infarction
MMI	myocardial mass index	R123	rhodamine 123
MMMB	Ministry of Medium Machine Building	RA	refractory anemia
MMP	midmolecule (medium molecular weight) peptides	RAAS	renin-angiotensin-aldosterone system
moAb	monoclonal antibody	RADRU	Realistic Analytical Dose Reconstruction and Uncertainty Analysis
MOD	Ministry of Defence	RAEB	refractory anemia with the excess of blasts
MPH	Ministry of Public Health	RAEB-T	refractory anemia with the excess of blasts in transformation
MPO	myeloperoxidase	RAPD-PCR	random amplified polymorphic DNA polymerase chain reaction
MRI	magnetic resonance imaging	RARS	refractory anemia with ringed sideroblasts
MRP	multidrug resistance associated protein	RBC	red blood cells
MSX	metabolic syndrome X	RBL	reaction of lymphocyte blast transformation
mU	milliunit	RCA	radiation contaminated areas
MVD	microvessels density	RCCs	renal-cell carcinomas
NADH ₂	nicotinamide adenine dinucleotide reduced form	RCD	cardiol
NADPH ₂	nicotinamide adenine dinucleotide phosphate reduced form	RCR	Regional Cancer Registry
NCRU	National Cancer Registry of Ukraine	RCRM	State Institution "Research Centre for Radiation Medicine, National Academy of Medical Sciences of Ukraine"
NP-κB	nuclear factor-κB	RCT	radiation contaminated territories
NK	natural killer cell	RERF	Radiation Effects Research Foundation
NOS	nitric oxide synthase	RF	risk factors
NPP	Nuclear Power Plant	RIA "Prypiat"	Research Industrial Association "Prypiat"
NRBU 97	Radiation Safety Standards of Ukraine, 1997	RMSSD	the square root of the mean squared differences of successive NN interval
NVD	neurovascular dystonia	ROS	reactive oxygen species
NYHA	New-York Heart Association	RR	relative risk
ODC	oxadine conjugates	RRP	Radiation Registry Polyclinics
ODR	official dose records	RTL	relative telomere length
OEDP	ototoxic emission of distorted products	RT-PCR	reverse transcription polymerase chain reaction
OI	ossification index	RV	residual volume
OMP	osteomyelofibrosis		
OPM	oxidative protein modification		
p53	protein 53		
PB	peripheral blood		
PC	personal computer		
P-CAT	plasma catalase		
PCNA	proliferating cell nuclear antigen		
PCR	polymerase chain reaction		

S	light sum index	TLC	total lung capacity
SA	small abnormalities	TLD	teem luminescent dosimetry
SBP	systolic blood pressure	TNF	tumor necrosis factor
SCD	sudden cardiac death	TNM	Tumour - Node - Metastasis Classification of Malignant Tumors
SCID	severe combined immunodeficiency	TP	total power spectrum
SDANN	standard deviation of the NN intervals with a long cycle duration	TP53	tumor protein 53 gene
SDNN-I	standard deviation of the NN intervals	TR	thioredoxin reductase
SE	standard error	TRH	thyrotropin-releasing-hormone
SH-groups	sulphydryl groups	Trx-SS-Trx	thioredoxin oxidized
SI	stress index	Ts	time slump
SI "UCIT and NR"	State Institution «Ukrainian Centre for Information Technologies and the National Registry»	TSH	thyroid-stimulating hormone
Slth	superficial intension of a drop hanging	TUE	transuranium elements
SIR	standardized incidence ratio	TUNEL	terminal deoxynucleotidyl transferase (TdT)-mediated UTP-nick and end-label
SL	short-latent	UACOS	Ukraine-American Ocular Chornobyl Study
SLL	lymphoma with small lymphocytes	Ub	ubiquitin
SNDH	sensor neural diminished hearing	UL	urolithiasis
SNP	single-nucleotide polymorphism	UM	unmutated
SOD	superoxide dismutase	U-MDS	unclassified myelodysplastic syndrome
SPM	specific pulmonary moisture	UNSCEAR	United Nations Scientific Committee on the Effects of Atomic Radiation
SPOCCC	State Program of Overcoming Consequences of the Chornobyl Catastrophe	USE	ultrasound examination
SRU	State Registry of Ukraine of the Persons Affected by the Chornobyl Catastrophe	UZ	uncontaminated zone
SSEP	somatosensory evoked potentials	VA	visual analyzer
SUMO	small ubiquitin-related modifier	VC	vital capacity
SVE	supraventricular extrasystolic	VD	vegetative dysfunction
Ta	time of active action	VeD	vestibular dysfunction
TASR	truncated age-adjusted incidence rate	VE	ventricular extrasystolic
TBA-AP	thio-barbituric acid active products	VeA	vestibular analyzer
TC	thyroid cancer	vEGF	vascular endothelium growth factor
TCh	total cholesterol	VEPCP	visual evoked potential to chess pattern reversion
TCR	T cell receptor	Vim	vimentin
TF-52	transfer factor "soil-milk"	VVD	vegetative-vascular dystonia
TC	thyroid gland	WBC	white blood cells
TGF- β 1	tumor growth factor β 1	WbBC	whole body counter
Ti	time index	WCP	whole chromosomes painting
Ti	time increase	WHO	World Health Organization
TK6	human lymphoblastoid cell line	WISC	Wechsler Intelligence Scale for Children
Tl	time lengthening	WTK1	human lymphoblastoid cell line
TL	total lipids	XPA	xeroderma pigmentosum A
		ZAP-70	zeta-chain-associated protein kinase 70

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CHAPTER 1 DOSES OF EXPOSURE

Chornobyl radioactive release started April 26th, 1986 and according to some estimates, lasted 10–15 days. Short-lived radionuclides (radioisotopes such as niobium, zirconium, iodine, etc.) played a role of dose factors in the early phase of the accident. With the distance from the start of the accident, the increasing role in shaping the dose was played by radioisotopes of caesium (^{137}Cs , ^{134}Cs) and much less — strontium (^{90}Sr). Radioisotopes of transuranic elements ($^{238,239,240,241}\text{Pu}$, ^{241}Am), though were present in radioactive emissions, but the physical and chemical properties of the inactive carriers in which they were fixed, stipulated that the role of the transuranic dose was small.

During the emergency radioactive release the weather conditions constantly changed, causing significant heterogeneity of radioactive fallout in Ukraine: ^{137}Cs fallout density varied from units to several hundred $\text{kBq}\cdot\text{m}^{-2}$. ^{90}Sr contamination levels were approximately 10 times lower. In the area of population residence levels of fallout ^{239}Pu varied from 0.004 to 0.9 $\text{kBq}\cdot\text{m}^{-2}$ (at the average level of global fallout $^{239,240}\text{Pu}$ for Ukraine — 0.037 $\text{kBq}\cdot\text{m}^{-2}$).

While in the area of radiation influence of the Chornobyl accident virtually all the residents of Ukraine were somehow involved, but four critical forces that are most experienced accidental exposure are highlighted:

- 1) clean-up workers of Chornobyl NPP accident known as "liquidators" those who were directly involved in work on the industrial site or in the Chornobyl 30-km zone;
- 2) evacuated residents of Prypiat and Chornobyl, as well as settlements of 30-km zone;
- 3) children and adolescents (age — at the time of the accident), whose thyroid gland has undergone irradiation by radioactive isotopes of iodine, which came into the body with food or inhaled in May–June 1986;
- 4) rural residents living on contaminated territories.

The population of the contaminated areas is the largest in number among the four contingents outlined above and makes millions of people in Ukraine.

Dose load were formed by two basic ways of irradiation for population of contaminated areas:

- 1) external whole body gamma irradiation from the complex of gamma-emitting radionuclides in the fall-out on the soil;

- 2) *Internal irradiation* of thyroid gland from *radiocaesium* (in 1986) and whole body irradiation from *radiocesium* (and much less from other radionuclides) due to consumption of contaminated foodstuffs produced (grown) at the radioactively contaminated territories.

(I. Likhtarev)

1.1 RADIATION DOSES OF CLEAN-UP WORKERS OF THE ACCIDENT. RETROSPECTIVE DOSE RECONSTRUCTION OF CLEAN-UP WORKERS OF THE ACCIDENT

Clean-up workers of the Chernobyl NPP accident (also known as "liquidators") compose one of the largest and probably the most exposed cohort affected by the Chernobyl accident. Despite the importance of this population — both in terms of medical and social aspects and position of studying the consequences of accidental exposure — exposure picture of the situation with the liquidators for a long time remained uncertain. Thus, among the clean-up workers years of 1986–1990 included in the State Register of Ukraine (SRU) of persons affected by the Chernobyl accident only about a half have records of individual doses. Remained unclear both the quality of available dosimetric data, and the overall success or failure of radiation protection during clean-up works.

Traditional were ideas of almost total falsification of data on radiation doses and the mass excess of fixed dose limits. It was also uncertain situation with radiation dose of crystalline lens, one of the most radiosensitive organs, especially beta radiation from the Chernobyl radionuclide mixture. Therefore a large range of activities was planned and largely implemented for the past ten years aimed at bringing clarity to the real exposure of clean-up workers and retrospective evaluation of dosimetric monitoring results during clean-up works.

A special place in the complex problems of exposure assessment dosimetry of clean-up workers took the need of dosimetric support of post-Chernobyl epidemiological studies. These studies require information on subjects' individual doses, and solving the problem of dosimetric support needs the implementation of integrated approaches. The main practices of individual retrospective dosimetry, which was developed in recent years and successfully used to reconstruct individual doses of clean-up workers is RADRUE (Realistic Analytical Dose Reconstruction and Uncertainty Analysis) — analytical calculation method jointly developed by Russia (Institute of Biophysics), Ukraine (Research Centre for Radiation Medicine, Chernobyl NPP), USA (National Cancer Institute) and France (International Agency for Research on Cancer).

An important feature of this method based on an inquiry of clean-up workers, the reliability analysis of responses by expert monitoring team and the use of expansive databases of radiation situation in the places of clean-up works, is that it can be universally applied to any clean-up worker, including the deceased (through the inquiry of colleagues and relatives).

RADRUE method was widely used to reconstruct individual doses for whole body and bone marrow of subjects (cases and controls) of Ukraine-US research of leukemia among liquidators. Altogether 1,010 doses were reconstructed for clean-up workers using RADRUE method, in particular 79 subjects who died (through questioning colleagues and relatives of the deceased clean-up workers).

Taking into account that the cohort studied included clean-up workers of 1986–1990, which have official status and registered liquidators in the SRU, the dose range was from zero to about 3.2 Gy, arithmetic mean dose (expectation) — 90 mGy, geometric mean — 12 mGy. Such a huge range of doses suggests extreme heterogeneity of cohort of clean-up workers composed at the same time with the people who received large doses in the first days after the accident, and household sector employees or persons who visited the 30-km zone during a short business trip.

Doses of certain occupational categories among the clean-up workers differ (Table 1.1). Thus, the employees of the Ministry of Internal Affairs (MIA) for which there were fewer opportunities to effectively influence on the levels of exposure, and nuclear power professionals (NPP employees, workers of Construction Administration-605) received relatively higher doses. It should be noted that the last group (nuclear power professionals) also includes so-called early liquidators, i.e. people from Chernobyl NPP personnel exposed to radiation at the initial crash, when there were no established effective system of radiation protection and radiation control.

Obviously, the highest doses received participants of clean-up works for which no effective system of radiation protection and exposure management was used, first of all, early liquidators (those who participated in the clean-up works during April-May, 1986) and certain categories of workers who carried out works on individual tasks, mostly outside the scope of attention of radiation monitoring services. Dynamics of military liquidators exposure by years (Table 1.1) adequately reflects the evolution of the radiation situation in the 30 km zone and a gradual reduction of the dose limits within 1987–1988. It should be noted that the average dose of military liquidators were significantly lower than officially registered and established in the public consciousness value. This finding agrees well with the independent analysis of official dose records and qualitative considerations concerning radiation monitoring features of the troops during the clean-up works.

Table 1.1. Results of individual doses reconstruction by RADRUE for some categories of occupational accident

Category	Number	Average dose (mGy)	Median dose (mGy)	Geometric standard deviation
Military (total)	218	76	54	2.1
Separately by year of participation in the clean-up works				
1986	99	105	82	1.89
1987	52	78	46	2.32
1988	44	29	17	2.41
1989	20	31	17	2.22
1990	3	60	24	2.89
Professional atomist	35	381	277	1.78
Staff of MIA	27	203	173	1.86
Sent in official journey	340	70	48	1.95
Drivers	213	64	41	1.98

It should be noted that the cohort of subjects of Ukraine-US study of leukemia among liquidators is quite representative, because data on levels of doses, type of work and distribution of professional groups (categories) of clean-up works (Table 1.2) are very informative and can characterize the total set of Ukrainian clean-up workers.

A separate scientific and practical problem is to determine the lens exposure in clean-up workers, including assessment of individual doses of beta-radiation. The essence of the problem is that during the clean-up works beta-irradiation doses were not controlled (because of shortage of existing logistical and methodological basis) while in the Chernobyl mixture of radionuclides were amply represented hard beta emitters ($^{144}\text{Ce}/\text{Pr}$, $^{106}\text{Ru} / \text{Rh}$, $^{90}\text{Sr} / \text{Y}$), which could form a substantial remote dose beta-irradiation. Under certain conditions, beta doses on lens of clean-up workers may exceed an order of the appropriate dose of gamma-radiation.

A large study on reconstruction of individual doses of lens beta-irradiation of 8,607 participants of Ukraine-US ocular Chernobyl research (UACOS) RCRM specialists conducted jointly with the Institute of Occupational Health, AMS of Ukraine (the main contractor of the project UACOS). Developed dosimetric model took into account the time elapsed after the accident (and, accordingly, changes in the composition of the Chernobyl nuclide mixture), peculiarities of exposure (which were determined through questioning of liquidators) — character of work, workplace characteristics, using of protective eyewear. To assess an adequate exposure model was used (phantom, taking into account the anatomical location of the lens, exposure from horizontal and vertical surfaces with different textures) and the method of calculating the distance doses of beta radiation by the Monte Carlo method.

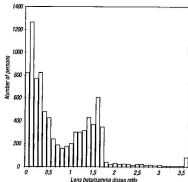


Fig. 1.1. The distribution ratio of beta-irradiation to doses of gamma radiation for the 8,607 subjects of Ukraine-American Chernobyl ocular research (UACOS)

Although the ultimate goal was to assess the total doses to the lens, a correlation between doses of gamma and beta radiation is also of interest (Fig. 1.1). It was found that approximately 32% of subjects for study beta-irradiation doses were higher than the corresponding gamma doses (i.e., the total radiation dose in the lens was more than two times higher than the assessment only gamma dose), while for about 53% of subjects of beta irradiation did not exceed a half of the corresponding dose of gamma radiation.

Parameters of total doses (beta + gamma) of lens irradiation in certain groups of clean-up workers — the study

Table 1.2. The structure of clean-up workers, who were the subjects of Ukraine-US study of leukemia

Category	All cohort study		Kyiv and Kyiv region		Other regions		Assessment for all Ukrainian clean-up workers, %
	number (%)	duration of work-me-dian (minimum, maximum)	number (%)	duration of work-me-dian (minimum, maximum)	number (%)	duration of work-me-dian (minimum, maximum)	
Witnesses of the accident	3 (0.5)	7 (1, 11)	2 (0.7)	6 (1, 11)	1 (0.4)	7 (7, 7)	<1
Directly affected	2 (0.3)	2 (1, 2)	2 (0.7)	2 (1, 2)	0 (0.0)	— (—, —)	<1
Early liquidators	66 (11.5)	7 (1, 185)	50 (17.1)	7 (1, 185)	16 (5.7)	7 (3, 16)	~10
Staff of Chernobyl NPP	9 (1.6)	317 (36, 1420)	8 (2.7)	379 (36, 1420)	1 (0.4)	225 (225, 225)	~1
Temporary submitted NPP staff	1 (0.2)	31 (31, 31)	1 (0.3)	31 (31, 31)	0 (0.0)	— (—, —)	<1
Staff of CA-605	5 (0.9)	31 (19, 63)	1 (0.3)	24 (24, 24)	4 (1.4)	46 (19, 63)	~1
Staff of the Institute of Atomic Energy, named by I. Kurchatov	2 (0.3)	157 (138, 175)	0 (0.0)	— (—, —)	2 (0.7)	157 (138, 175)	<1
Military clean-up workers	220 (38.5)	67 (6, 835)	33 (11.3)	65 (7, 356)	187 (66.8)	69 (5, 833)	48
Civil liquidators sent to the 30-km zone	181 (31.6)	19 (1, 1710)	121 (41.4)	18 (1, 1710)	60 (21.5)	21 (2, 103)	26
Staff of Industrial Association «Combina-	4 (0.7)	458 (164, 1450)	4 (1.4)	458 (164, 1450)	0 (0.0)	— (—, —)	<1
Mixed type	79 (13.8)	250 (4, 1710)	70 (24.0)	258 (4, 1710)	9 (3.2)	111 (9, 1710)	10
Total	572		292		280		

subjects are listed in the table 1.3. The table shows that the largest radiation dose received so-called early liquidators, i.e. staff persons from the Chernobyl NPP and other units that were involved in the clean-up works in at the first days and weeks after the accident. It should be noted that in view of the nature of exposure (dose gamma irradiation + beta; see Fig. 1.1) and characteristics of the cohort (clean-up workers of 1986–1987) doses of the largest group of studied subjects — military liquidators — are in a good agreement with the dose reconstruction by RADRUE (Table 1.1).

Another original feature of ocular dosimetry project was the sharing of various methods of dosimetry that were relatively uniform across a single calibration standard ("gold standard"). Thus, to assess individual doses of gamma irradiation the high-quality data of personal dosimetry monitoring (staff of Construction Administration № 605 — CA-605), the results of calculation and analytical dose reconstruction (ADR), the official dose records (ODR) of military liquidators were used.

Dose estimates from independent sources were calibrated against EPR dosimetry on tooth enamel as the most accurate and valid method for retrospective dosimetry. This caused the introduction of calibration correction in the primary dose assessment. Thus, it appears that ODR of military liquidators approximately twice overstate the actual levels of exposure in this category of clean-up workers.

To correct the primary ODR and take into account of doses uncertainty it was to multiply the initial ODR by an adjustment of logarithmically normal distribution type with the following parameters: geometric mean — 0.5, geometric standard deviation — 2.2. These options of the corrective distribution reflect large systematic shift of registered doses, as well as a considerable uncertainty of radiation monitoring data of military liquidators. Unique data on individual doses of liquidators' lens irradiation enabled to succeed ocular research and to analyze the risks of cataract under the influence of ionizing radiation.

Table 1.3. Characteristics of lens exposure of certain groups of clean-up workers-participants of Ukraine-American ocular Chernobyl research (UACOS)

Group of clean-up workers / dosimetry method	Number in the tested cohort	Dose distribution parameters of lens (beta + gamma) only: Median (5% and 95% percentile)
Employees of CA-605 / individual monitoring by TLD dosimeters	410	16 (2, 235)
Representatives of various groups / EPR dosimetry in tooth enamel	104	94 (19, 426)
Early liquidators / analytical dose reconstruction (ADR)	712	502 (142, 1143)
Sent to the 30-km zone / ADR	126	16 (1, 242)
Military liquidators / Official dose records (ODR), received by the group and group-calculating method	7,255	121 (30, 287)
Total	8,607	123 (15, 480)

Serious attention was paid to assessing the quality and reliability of existing dose records of radiation levels of clean-up workers. These attempts were made on several areas:

Collection and unification of all existing databases on individual doses and the corresponding paper archives.

Studying the practice of radiation protection and radiation control of various contingents of clean-up workers, including institutional and regulatory aspects.

Study of dosimetric registration accuracy by statistical methods.

Retrospective evaluation of dosimetric uncertainty of SRU data by comparing the official results with the results of dose reference EPR dosimetry.

In the direction of the collection of available dosimetric information with funding from the National Cancer Institute (USA) all the electronic databases of radiation control during the clean-up work were acquired. Unfortunately, this information gathered by the efforts of Russian colleagues from the Institute of Biophysics (Moscow) was not useful for practical use due to lack of reliable identifiers for linking records in dosimetric databases with the data in SRU.

Noteworthy achievement was the transfer of dosimetric data archives of the Ministry of Defence of Ukraine in an electronic database. Results of this work, carried out jointly by specialists of Military Archive, Military Medical Academy and the RCRM was the database containing more than 45 thousand entries of Ukrainian troops (regular and reserve), those that include the terms of participation in the clean-up works, the number of units and individual dose during the clean-up works. Comparison of data from the archive of Ministry of Defence (MOD) with ODR of the State Register of Ukraine proved to be very instructive. It appeared that the dose of military liquidators who are registered on the SRU practically coincide with the data contained in the military archive. Moreover, the degree of overlapping data sets and SRU and Military Archive was quite high — almost all of the records contained in the Military Archive is reflected in the SRU. This conclusion confirms the high importance of SRU data as a source of dosimetric information for military liquidators, who are about 90% of people with registered dose in the SRU.

Study of regulations and practices of radiation protection and radiation monitoring during the clean-up works that has been done in the framework of Franco-German Initiative "Chornobyl", allowed to better assess the quality, reliability and completeness of dosimetric data on clean-up workers. It was found that in terms of quality and level of contingent coverage by dose control, five periods can be singled out (Table 1.4).

If at the preaccidental period the dosimetry and radiation safety service satisfactorily fulfilled its tasks, sudden accident revealed a complete failure of the existing dosimetry to effectively determine individual dose of workers and provide the necessary level of personnel radiation safety in emergency conditions. It should be noted that the period of confusion was quickly overcome and since the second decade of May, 1986 dosimetry system in support of emergency workers began to be made gradually and in early June (about a month after the accident) the foundations of effective radiation protection and radiation control of the main forces involved to overcome the disaster were laid.

It was found that the quality of dosimetry data (a radiation monitoring) essentially depends on which service or agency conducted radiation protection of liquidators and dosimetry control. During the clean-up works (1986–1990) in the 30-km zone four large independent dosimetry services (Table 1.5) and several smaller independent service pro-

viders (USSR Academy of Sciences, USSR Academy of Medical Sciences, Integrated Expedition of Institute of Atomic Energy, etc.) were in function. These dosimetric services began at different times after the accident, covering various contingents and, most importantly, practiced radically different approaches to the assessment of individual doses of clean-up workers. Therefore the completeness, quality and reliability of their dosimetric data differ significantly (Table 1.5).

Table 1.4. Periods of dosimetric maintenance of clean-up works

Period	Time interval	Description
Preaccidental	1978–26.04.1986	Normal functioning of Chernobyl NPP dosimetry service in accordance with Radiation Standards-78
Initial	26.04.1986 – about 10.05.1986	Inability of Chernobyl NPP dosimetry service, the use of the war-time approaches to dose control of forces
Interim	about 10.05.1986 – 01.06.1986	Parallel operation of dosimetry services Chernobyl NPP and the army, introducing a single exposure limit (250 mGy), CA-605 organization with its own dosimetric service
Main	June–October, 1986	Operation of dosimetry services of Chernobyl NPP, CA-605 and MOD units based on different approaches
Routine	after November, 1986	Parallel operation of dosimetry services of Chernobyl NPP, CA-605, IA «Combinat» and MOD departments. Gradual return to normal operation, reduction of dose limits (1987–1988)

Table 1.5. Basic dosimetric services that performed dosimetric control of clean-up workers

Service	Owner of Department	Period of function	Coverage of clean-up workers	Data quality
1. Chernobyl NPP Radiation Monitoring Service	Ministry of Energy and Electrification of the USSR, from July 1986 — USSR Ministry of Atomic Energy	May, 1986 – to present	NPP personnel and temporarily attached to the Chernobyl NPP personnel	satisfactory-high (depending on the period after the accident)
2. Departments of Ministry of Defence	USSR Ministry of Defence	May, 1986 – end of 1990	military liquidators	low
3. Division of Radiation Control CA-605	Medium Machine Building Ministry of the USSR	June 1986–1987	civil and military constructors of CA-605	high
4. Office of Radiation Control of IA «Combinat» and its successors	USSR Ministry of Atomic Energy	November, 1986 – to present	civilian personnel who worked in the 30-km zone outside the building site of Chernobyl NPP	satisfactory

Dosimetry control of CA-605 staff of the Ministry of Medium Machine Building (MMMB) of the USSR, a specialized construction organization created to build a sarcophagus ("Shelter" Confinement) was the best organized. The result of this exemplary work

was 100% coverage of quality TLD individual control more than 20 thousand employees of CA-605, mostly business travelers from MMMB enterprises, based in Russia.

Dose control, performed by Chernobyl NPP Radiation Safety Service is characterized by failure in the first weeks after the accident (when the regular dosimetric means have proved inadequate for measuring high levels of doses) and the gradual restoration of high-quality dose monitoring, which ended only in June–July, 1986. Negative consequence of such failure of the regular radiation control of Chernobyl NPP was that doses of early liquidators — probably the highest among clean-up workers — remained unknown. Consequently, the completeness of dosimetry data of Chernobyl NPP personnel was insufficient (including — in the sense of covering by dosimetric data for each liquidator during the entire period of clean-up works) that determined the need for reconstruction of individual doses. Throughout the years 1986–1996 in total 1,600 individual doses of Chernobyl NPP workers and those temporarily assigned to the station were evaluated ADR calculation method. Since July, 1986 dosimetry monitoring and recording of individual doses were carried out at the Chernobyl NPP properly and that dosimetric information is characterized by high quality and completeness.

Dose control of civilian personnel (permanent and temporarily assigned), working in 30-km zone, due to organizational problems practically was not carried out during 1986 and part of 1987, while this function gradually took over the Office of Dose Control of IA "Combinat" / RIA "Prypiat". Thus, doses of this contingent, especially in 1986–1987, are characterized by a lack of completeness and not always high quality.

The largest contingent of clean-up works were military liquidators — professional soldiers, soldiers (initially) and, the most, persons temporarily called to the army from reserve. The importance of this category is greater, as about 95% of ODR in SRU belong just to military liquidators (Fig. 1.2).

Such a situation with ODR of military liquidators is the result of a 100% coverage of this contingent by dose control and peculiarities of input dosimetric information to SRU — through the dose certificate (in case of military personnel — a supplementary sheet to military ticket) which were on hand in all military but in very few civilian

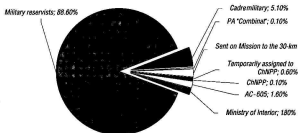


Fig. 1.2. Distribution of clean-up workers included in SRU according to departmental affiliation (per survey)

clean-up workers. However, concurrently with the exemplary coverage the dosimetry of military liquidators differed by the lowest precision of individual doses because of rude and inaccurate methods of dose assessment. For military liquidators generally used group (one dosimeter per group) and group-calculated (when the dose of all group members was calculated in advance on the basis of dosimetric situation and the planned work) dosimetry methods.

It was found during the retrospective assessment of accuracy and bias of the dose estimates of military liquidators that the average doses estimated by these methods exceeded twice the actual level of exposure, and geometric standard deviation (uncertainty) was very high and comprised about 2.2. It is significant also that widespread interpretation of abnormal distribution of individual doses of the military liquidators (Fig. 1.3) as the evidence of fraud dosimetry information to bring the reporting of exposure levels of military personnel in accordance with the applicable dose limits (250, 100 or 50 mSv) not was confirmed.

Statistical methods showed that the possible contribution of false (falsified) results of the dose does not exceed 10% of the total, and the atypical form of distribution (left depleted and a sharp precipice at doses above the limit) corresponds to fairly unusual practice of doses management when the person who received allowance dose exempted from the armed forces and their replaced by a new reservist.

In general, dosimetry control, which was carried out for different groups of liquidators and the system of radiation protection of contingents involved in clean-up works overcoming the Chernobyl accident consequences, allowed to comply with current norms and exposure limits. Mass overirradiation of clean-up workers was characteristic only for the initial phase of the accident and concerned rather limited group of so-called early liquidators.

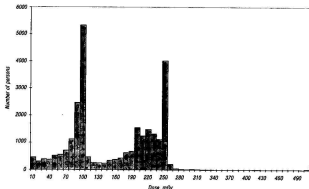


Fig. 1.3. The distribution of individual doses of military reservists (so called "partisans") of 1985-1987

In the future (from late May, 1986) contingents of many thousands was provided with adequate radiation protection forces, and cases of exceeding the established dose limits (250 mSv in 1986 and graduated limits of 100 and 50 mSv in subsequent years) were rare and usually occurred in cases prescribed by current at that time Radiation Standard-76.

Conclusions:

1. The largest individual doses received so-called early liquidators — people who participated in the clean-up works in April–May, 1986, under conditions of poorly explored radiation situation and lack of adequate facilities for radiation control.
2. Doses of clean-up workers of later periods (since June, 1986) are largely in accordance with established at the time dose limits. Radiation protection system, introduced during the clean-up works, mostly fulfilled its function and provided non-exceeding of permissible exposure.
3. The quality and completeness of dosimetry information significantly differ for different groups of clean-up workers, causing the need for a critical approach to the use of existing dose records, retrospective assessment of individual doses and revision and correction of available dosimetric data.
4. Doses of military liquidators are not characterized by strong falsification, but are displaced (in the direction of overstating actual exposure) and not accurate (with large uncertainty).
5. Studying health consequences of Chernobyl NPP accident among clean-up workers cohort requires broad application of retrospective dosimetry to determine individual doses of subjects research.
6. Developed in recent years retrospective dosimetry methods, in particular instrumental EPR dosimetry on tooth enamel and analytical calculation method RADRUE allow to effectively support the post-Chernobyl epidemiological studies.
7. Beta-radiation gave a significant contribution to the lens doses of clean-up workers. Consideration of this factor is needed in assessing risk of cataract in clean-up workers.

1.2 DOSIMETRY OF EVACUEES

Individual effective dose of irradiation for 12,632 inhabitants of Prypiat (group A, about 25% of the city evacuated population) and 14,084 residents of other villages of 30 km area (group B) were recovered and analyzed by the method of limitation and stochastic simulation based on direct measurements of dose rate and inquiry of evacuees. Overall evacuees represent 104 village of 30-km zone, including the cities of Prypiat and Chernobyl; 223 residents who lived in 40 settlements of the Belarusian part of the 30-km zone were also interviewed and included in the total number of investigated.

The average effective dose of group A contingent that was accumulated prior to evacuation, was 10.1 mSv. Doses of 534 persons from this group exceeded the level of 25 mSv and only 18 people received doses of 50 mSv. Maximum value of effective doses among this group of inhabitants of Prypiat was 75 mSv.

Frequency distribution of individual effective doses is shown in Fig. 1.4. Parameters of this dose distribution are shown in Table. 1.6.

Table 1.6. Parameters of external exposure individual doses distribution for individuals evacuated from Prypiat. Results of deterministic calculation

Number of persons in the group	12,632
The average portion of time outdoors	0.21
Distribution of effective doses of external gamma radiation exposure (mSv)	
The arithmetic mean	10.1
Median	8.93
Geometric mean	6.79
Geometric standard deviation	3.1
75 percentile	14
95 percentile	24
The coefficient of variation (%)	74

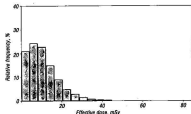


Fig. 1.4 shows that the distribution of individual doses is left-asymmetric and quite broad. This view of dose distribution agrees well with the generally accepted model of logarithmically normal distribution of population exposure doses. 95% of the evacuated residents of Prypiat (group A) received a dose not above 24 mSv.

Fig. 1.4. The distribution of individual doses of irradiation for 12,632 persons evacuated from Prypiat. Results of deterministic calculation**Table 1.7. The dependence of the distribution of individual external exposure from the age of persons who were evacuated from Prypiat. Results of deterministic calculation**

Birth years	1963-1966	1979-1982	1974-1978	1970-1973	1961-1969	1931-1960	before 1931
Age group (years)	0-3	3-7	7-12	12-16	16-25	25-55	>55
Number of persons in the group	1597	2104	2133	601	1159	4455	582
The average portion of time outdoors	0.14	0.17	0.18	0.21	0.25	0.25	0.21
Distribution of effective doses of external gamma radiation exposure (mSv)							
The arithmetic mean	8.32	9.09	9.0	10.5	10.8	11.5	10.2
Error in mean	0.15	0.15	0.14	0.31	0.24	0.12	0.32
Median	7.35	7.89	7.63	9.35	9.63	10.5	8.56
Geometric mean	5.50	6.15	6.32	7.16	6.77	7.85	7.07
Geometric standard deviation	3.18	3.04	2.79	3.02	3.6	3.13	2.74
75 percentile	11.2	12.2	12.2	15.1	15.3	15.6	14.2
95 percentile	20.0	21.2	21.3	24.8	25.9	25.8	23.9
The coefficient of variation (%)	74	75	73	72	75	70	75

Table 1.8. The dependence of the distributions of individual doses of irradiation from a professional affiliation of persons who were evacuated from Prypiat. Results of deterministic calculation

Professional group	Preschoolers	Schoolboys	Teachers	Medical workers	Consumer services	Workers	Engineers	Municipal economy workers	Chernobyl NPP staff members	Housewives	Pensioners
Number of persons in the group	3494	2945	376	202	799	2629	670	210	452	156	375
The average portion of time outdoors	0.16	0.19	0.22	0.23	0.23	0.26	0.24	0.27	0.28	0.20	0.20
Distribution of effective doses of external gamma radiation exposure (mSv)											
The arithmetic mean	8.81	9.4	10.2	11.5	10.9	11.5	10.8	12.6	13.0	8.94	9.51
Error in mean	0.11	0.13	0.36	0.59	0.28	0.16	0.30	0.73	0.40	0.54	0.35
Median	7.72	7.99	9.66	10.8	9.6	10.5	9.66	10.8	12.4	8.54	8.11
Geometric mean	5.92	6.51	7.35	7.57	7.66	7.7	7.11	8.27	8.39	5.74	6.92
Geometric standard deviation	3.11	2.68	2.72	3.16	2.86	3.21	3.46	3.09	3.84	3.25	2.53
75 percentile	11.89	12.8	13.7	16.5	14.5	15.9	14.9	16.7	18.0	12.8	13.4
98 percentile	20.64	22.6	22.4	27.4	25.1	26.2	24.7	29.7	28.2	20.1	20.4
The coefficient of variation (%)	74	74	68	73	72	71	72	84	66	75	70

1.3 THE CALCULATION OF EXTERNAL EXPOSURE OF PEOPLE EVACUATED FROM VILLAGES OF 30 KM ZONE

Dosimetric models for the rural population were used to calculate individual doses of 14,084 persons evacuated from the settlements of the 30-km zone. The calculation covered the period from the beginning of the accident and till the moment of evacuation beyond the 30-km zone. The average effective dose among this group (about 25% of the evacuated population) was 15.9 mSv. Among this group the dose of 1,260 persons exceeded 50 mSv for 120 effective doses were higher than 100 mSv and only one person with a dose of 214 mSv dose exceeded 200 mSv. Calculated in the course of revision the average dose of 15.9 mSv was slightly (14%) lower than the one that was identified in preceding study — 18.2 mSv. This difference has several reasons.

Firstly, from this review 3,119 persons were excluded, who lived in remote from Chernobyl NPP villages and whose doses were, respectively, quite small. On the other hand, a critical review of dose rate measurement forced to reject some, perhaps, false values. In addition, a more sophisticated method of spatial interpolation has prevented the development of artificial unreasonably high values of the interpolated dose rates. For these two reasons, number of persons with high doses slightly decreased. So, if according to the preliminary consideration 644 persons had dose more than 100 mSv, the results of the audit assessed the number of 120 with doses of such magnitude. Moreover, just as in the problem of dose assessment of the urban population, use of age-dependent factors of transition to an effective dose also contributed to a certain reduction of effective doses received as a result of the audit.

Table 1.9 shows the general parameters of the effective doses distribution of 14,084 inhabitants of 30 km zone.

Table 1.9. Parameters of individual doses distribution of external exposure of individuals who were evacuated from villages of 30 km zone. Results of deterministic calculation

Group strengths	14,084
The average share of time outdoors	0.41
Distribution of effective doses of external gamma radiation exposure (mSv)	
average	15.9
median	7.77
geometric mean	7.13
geometric standard deviation	4.08
75 percentile	16.1
95 percentile	67.7
coefficient of variation (%)	142

Noteworthy is a very large coefficient of variation of individual doses — 142%. Such a large variation can be explained by significant differences in dose in different villages of the 30-km zone, varying duration of the population in the zone of radioactive contamination (different time of evacuation), and individual differences in proportion of time spent outdoors. The distribution of individual doses (Fig. 1.5) presented evidence of heterogeneity of presenter in the calculated population, comprising the residents of faintly contaminated villages (such were majority) and dwellers of localities with high dose rate.

Quite a wide was distribution of shares of time outdoors. Fig. 1.6 shows that half of the population spent more than 42% of time on the street and level of 65% of time outdoors exceeded 5% evacuees among the rural population. If the urban population was characterized by average share of 21% time outdoors, (Table 1.7), the rural residents

more time spent in the court, that certainly contributed to higher levels of exposure. Another factor that has considerable influence on the resulting dose was the duration of stay of residents in the area of increased radiation.

The parameters of individual doses distributions for the settlements included in the reconstruction of doses are presented in Table. 1.10. For villages with a number of surveyed less than 20, the median and 95% quantile were not determined. The table shows that for all sectors except Sector B (Fig. 1.7),

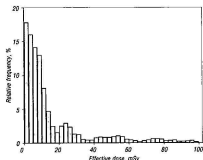


Fig. 1.5. The distribution of individual doses of irradiation 14,084 persons evacuated from settlements in the 30-km zone. Results of deterministic calculation

maximum (95% quantile) dose for the individual settlement exceeded the level of 50 mSv. On average, the highest doses were received in the villages Usiv — 154 mSv, Mashevo — 79 mSv (both — Sector C) and Chistogalivka — 70 mSv (Sector A). The parameters of the distributions are shown in the Table. 1.11. For comparison, the table contains parameters for the distribution of Chernobyl — much less affected settlement of the most populated settlements among the 30-km zone population.

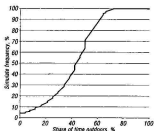


Fig. 1.6. Cumulative distribution of shares of time outdoors for people who were evacuated from villages of 30 km zone

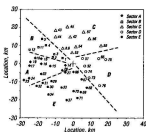


Fig. 1.7. Location of 30-km zone settlements and distribution per sectors. Line numbers of settlements and their names see in Tables 1.10, 1.11

Table 1.10. Results of deterministic calculation of individual doses for particular settlements of 30-km zone. Location of settlements see Fig. 1.7

Code	Name of settlements	Quantity of persons	Day of evacuation	Part-time in the open air as, %	Average	Median	95% percentile
Sector A							
14	Yaniv	63	3	34	22	9.5	84
20	Chystogalivka	331	7	44	61	70	98
15	Buriskivka	56	8	45	29	24	81
16	Nova Krasnytsia	89	8	48	23	23	29
18	Rechysia	382	7	50	24	26	33
17	Tovsty Lis	408	8	51	52	60	81
19	Krasne (of Tovstols rural district)	297	8	49	34	15	97
10	Rud'ky	43	7	32	10	12	19
22	Stara Krasnytsia	20	7	36	9.2	8.4	17
23	Vilshanka	24	10	34	10	11	13
24	Lub'ianka	304	10	46	12	12	16

Code	Name of settlements	Quantity of persons	Day of evacuation	Part-time in the open air as %	Average	Median	95% percentile
Sector B							
1	Novoshepelychi	815	6	37	15	13	31
2	Staroshepelychi	209	7	50	21	22	31
3	Beniowka	101	8	48	18	18	26
4	Bila Sovoka	7	6	41	16	—	—
5	Dovliady	10	5	38	12	10	23
12	Khatky	19	9	44	10	9	12
Sector C							
54	Mashevo	162	8	54	75	79	96
41	Usiv	89	8	48	150	154	165
43	Melochky	2	7	34	31	—	—
55	Zalissia	2	6	36	12	—	—
56	Kriuky	14	7	44	66	—	—
57	Kulazhyn	2	8	12	28	—	—
Sector D							
51	Zymovyshe	431	7	37	37	42	56
52	Kryva Gora	146	8	40	47	51	67
74	Starosilla	100	8	40	3	3	4
73	Koshovka	126	8	45	9	8	12
76	Paryshiv	286	8	46	4	4	5
65	Chapalyka	211	8	39	6	7	8
75	Ghykalovychy	3	8	50	8	—	—
Sector E							
66	Copachi	432	8	40	45	53	66
67	Lelliv	604	8	42	22	23	30
68	Chornobyl	4558	7	32	6	6	14
69	Zalissia	1611	8	52	7	8	10
70	Zapillia	69	9	48	6	7	8
71	Cherevach	263	9	47	5	5	8
37	Novosilky	202	9	48	6	6	8
33	Kerogod	601	8	52	4	5	6
29	Stochanka	333	8	43	4	4	5
30	Roz'yizhe	49	8	37	3	3	4
31	Ilinty	366	8	45	3	3	4
32	Rudnia Illinetska	15	11	36	4	4	5
34	Glinka	227	7	34	2	2	2

Table 1.11. Parameters of distributions of individual external exposure doses to certain localities of 30 km zone. Results of deterministic calculation

Settlement	Usik	Mashevo	Chystogalivka	Chornobyl
Quantity of group	89	165	331	4558
Distribution of effective doses of external gamma radiation exposure (mSv)				
average	150	74.8	60.8	6.38
median	154	78.7	69.7	5.84
geometric mean	146	69.2	44.7	3.4
geometric standard deviation	1.28	1.66	2.77	4.14
95 percentile	165	95.7	98.1	14.4
coefficient of variation (%)	15	27	52	85

It is seen that within the same locality variation of individual doses is rather small. Geometric standard deviation size for small settlements equal to 1.4–2.8 proves for a great homogeneity of the population in terms of exposure, in particular mode of behavior. The exception to this rule are such large settlements like Chornobyl, many population groups of which are characterized by a variety of behavior modes. In addition, the migration of Chornobyl residents out of the city, as well as self-evacuation of some of them resulted in significant expansion of doses absorbed by residents of this settlement. Therefore, geometric standard deviation of the doses distribution of Chornobyl evacuees is very large and comprises 4.14, which is very close to the value of this index calculated for general distribution of doses of 30-km zone evacuees (Table 1.9).

In general, as it is seen from above quite typical examples, the uncertainty of individual dose estimates of the rural population is slightly higher than for the reconstruction of the urban population doses, but even with uncertainty at the level of 60–80% (coefficient of variation) can be stated that such precision is adequate for the purposes of retrospective assessment of individual doses of population evacuated from the accident.

Doses of evacuation route

The doses received by Prypiat residents during evacuation were estimated as a result of the study. Analysis of routing messages (formal form), completed in 1988–1989 during a large-scale survey of evacuees allowed to identify four main routes by which the people of Prypiat moved in the direction of evacuation points (Table 1.12).

The most significant itinerary was the road to Polisske which passed in the west direction. Prevalent quantity of evacuees took this route. Another, somewhat less important, was the route to Kyiv direction, the path of which went through the “Red Forest”, Kopachi, Chornobyl, Cherevach and further to Kyiv. Two less important itineraries were revealed: the railway in the direction of Chernihiv, and self-evacuation on their own transport towards Belarus (the road to the Bila Soroka).

These data, in general, are not contradictory to information about the organization and realization of evacuation of Prypiat population.

Analysis of the radiation situation at the evacuation routes showed that the entire itinerary can be divided into two essentially different parts (phases): phase 1, which

passed through the 5-km zone of Chornobyl NPP, and the rest of the route (phase 2) from 5-km border zone to the place of destination.

Table 1.12. Features of evacuation itineraries from Prypiat (per survey)

Current No	Direction of evacuation	Code of route	Number of persons	Percentage of total
1	Polissko	1	6831	42
2	Kyiv	2	4478	27
3	Chernihiv	3	938	6
4	Belorussian	4	612	4
5	The route is not specifically listed	5	2271	14
6	In the villages of 30-km zone	6	1083	7
Total:			16193	100

Crucial difference of phase 1 is that the evacuation route crossed several extremely powerful traces of radioactive emissions, the scale (characteristic size) of which sometimes reached only tens or hundreds of meters. The rest of the evacuation (phase 2) went through the less contaminated areas with much lower dose rate overfalls.

As a result, assessment of doses received on these or other routes of evacuation was carried out separately for phases 1 and 2. In the case of phase 1 doses reconstruction was conducted manually using cartograms of radiation environment provided by Chornobyl NPP. In phase 2 dose was estimated by integrating the dose rate profiles along the route.

It appears from the estimation of doses received during each of the routes of movement, that staff evacuation route, anticipated by the plan of civil defence in the Poliske direction was not really less safe than Belarussian direction. On the other hand, a small change of route — leaving the Prypiat through the railway crossing near the plant "Jupiter" instead of travel through the contaminated area near the overpass over the railroad, crossing Prypiat-Chornobyl NPP and cottages south of the station Yaniv — could significantly reduce the dose load of evacuees.

Route to Kyiv was also associated with significant dose load due to travel through a very active spot in the cross NPP-Chornobyl-Prypiat. While the rest of the route to the south laid in less contaminated areas, doses formed at the first site were high enough.

Dose was most intense on the route in the direction of Chernihiv. This route flew about 6% questioned evacuees. Evacuation in this direction was carried by rail, and electric trains leaved from relatively polluted station Yaniv, then went on the north by the fence of the building site where the dose rates reached a fairly large values. Finally, electric trains made stops provided by schedules at platform Semibody (north-eastern outskirts of Chornobyl NPP), which only led to greater accumulation of doses. As a result, the doses received by evacuations of rail in the direction of Chernihiv were the largest of all possible routes of evacuation.

Thus, the doses of the majority of Prypiat dwellers, received during the evacuation, were within 11–19 mSv, which can be compared with pre-evacuation irradiation.

For comparison at the individual level components of the dose before and during the evacuation of each evacuees of those who had the ordinary mode of conduct during the general evacuation and indicated clearly the point of evacuation (in total 6,908

people met these criteria) the dose received at the route of evacuation by individualized estimation was attributed. Then the partial contribution of dose received at the route of evacuation in the total dose of radiation was determined. The distribution of dose partial contributions during evacuation presented in Fig. 1.8, shows that this value varies quite widely, but most likely value is 0.5. The average (52 ± 19)% of dose evacuees received just during evacuation.

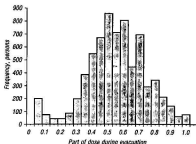


Fig. 1.8. Fractional contribution of dose received during the evacuation to the total dose of evacuees.

Thus, it is obvious that the correct estimation of doses during evacuation significantly change the pattern of irradiation of evacuated Prypiat population. For comparison, it should be emphasized that in case of evacuation of Prypiat in the direction of the Bila Soroka (Belarus route), the dose due to the evacuation would give the only contribution of 6% of the total dose.

As a result of these efforts, integral dose was estimated separately for each of the four tracks. It appeared that in terms of dose burden during evacuation, manning evacuation route in the Polisske direction, which was the plan of civil defense, was not optimal. Evacuation in the Belarussian direction resulted in the accumulation of an order of magnitude smaller doses than any of the other tracks. On the other hand, driving in the direction of Chornobyl-Kyiv and evacuation by train to Chernihiv gave even higher doses.

Comparison of individualized dose estimates on the evacuation route with individual doses received while staying within the Prypiat during the evacuation, showed that a large number of doses of the evacuation route were comparable or even larger than those that were received prior to evacuation. In general, the dose of the evacuation route had given the contribution to the total dose of evacuees at around 50%. Thus, consideration of dose on the evacuation route severely alters the overall picture of evacuees' irradiation.

1.4 POPULATION IRRADIATION DOSES

Radiological monitoring in 1986

In Ukraine in 1986 radiological emergency monitoring was organized so that literally the first hours and days the mass measurement of *gamma-field ratio* in the air from radioactive fallout on the ground, and later — *soil sampling* for gamma-spectrometric studies of radionuclide fallout on the basis of the most radioactively-contaminated areas were carried out.

As for the control of radiation exposure of the population-contaminated areas, the dosimetry monitoring was conducted in bulk and enough good quality, as in the earliest (acute) phase of the accident (May-June) when the main source of internal radiation were radioisotopes of iodine, which accumulate in thyroid gland, and at the beginning of the middle phase (August-December, 1986), when the basic role was played by radioisotopes of caesium, which are relatively evenly distributed in the soft tissues.

Total in Ukraine in May-June, 1986 more than 150 thousand direct measurements of radiiodine activity in the thyroid gland of residents of northern districts of Kyiv, Zhytomyr and Chernihiv regions (among them 130 thousand children and teenagers) were made. In addition, since July, 1986 in residents of Kyiv and Zhytomyr regions about 23 thousand measurements of the current content of caesium isotopes in the body ($^{137},^{134}\text{Cs}$) using whole body counters (WhBC) were made.

Doses of irradiation in 1986

Whole body irradiation

Table 1.13 shows values of average doses of internal radiation from caesium residents of districts of Kyiv and Zhytomyr regions, assessed by results of measurements on whole body counters (WhBC), conducted in summer and autumn of 1986.

Since WhBC monitoring covered the population far from all settlements of radioactively-contaminated areas a simulation of the transfer of dose forming radionuclides from Chernobyl release was conducted via the links of trophic chains in the climatic conditions of Ukraine using non-stationary models that are traditionally used for retrospection and prognosis kinetics and levels of radioactive contamination of environmental objects (food in particular) as well as exposure doses of the population consumed these products.

As it was expected, the main source of internal radiation doses in 1986 was the contamination of two components of the diet: leafy green, and milk (and milk products).

Table 1.13. Average by districts of Kyiv and Zhytomyr regions individual doses of internal exposure of children and adults from radiocaesium in 1986 (Results of WhBC measurements for areas with density of fallout ^{137}Cs in soil $>37\text{ kBq}\cdot\text{m}^{-2}$)

District	Children under 10 years			Children over 10 years			Adults		
	number of WhBC-measurements	dose, mSv		number of WhBC-measurements	dose, mSv		number of WhBC-measurements	dose, mSv	
	GM	GSD		GM	GSD		GM	GSD	
Zhytomyr region									
Korostens'kyi	—	—	—	—	—	—	34	0.13	2.9
Lugyns'kyi	—	—	—	—	—	—	12	0.32	4.8
Narodytskyi	1995	1.8	3.9	1120	1.8	3.9	2753	1.56	4.3
Ovruchskyi	134	1.1	2.8	28	0.43	2.5	18	0.19	3.5
Kyiv region									
Blizsarkivskyi	30	0.44	2.3	26	0.89	1.8	-	-	-
Boguslavskyi	28	0.28	2.4	17	0.30	3.0	-	-	-
Ivankivskyi	208	0.61	3.3	261	0.42	3.0	652	0.29	3.6
Kagarlitskyi	12	0.71	3.2	78	0.39	2.6	-	-	-
Polisskyi	1323	1.2	3.5	1061	0.86	3.3	1872	0.81	3.5
Stavyshechenskyi	-	-	-	31	0.11	3.6	527	0.09	2.8
Chornobylskyi	1195	1.3	5.6	518	0.58	3.3	304	0.61	5.5

GM — geometric mean, GSD — geometric standard deviation

Evaluations of effective doses of internal radiation from basic radionuclides of emergency release (per 1 kBq m⁻² ¹³⁷Cs in soil) obtained according to this model are presented in Table 1.14.

In terms of Western European countries the calculated ratio between doses of first emergency year and doses accumulated over subsequent years, may reach 10 or more in favor of the first year. At the same time a variety of soil characteristics, both in Ukraine and Russia, which makes high (uncharacteristically for European countries) coefficients of radionuclides transfer from soil to vegetation and further into the milk, as well as a retro enough technology of dairy production in the private sector of Ukraine, identified a wide range of internal radiation dose ratios in the first and subsequent years.

Table 1.14. Effective doses of internal exposure in 1986 from the main radionuclides of Chernobyl release (normalized to 1 kBq·m⁻² of ¹³⁷Cs at the soil) in adult rural dwellers of Ukraine, caused by consumption of milk and leafy green

Radionuclide	Relative contributions to total dose of internal radiation dose components					
	«milk»		«leafy green»		total	
	μSv per 1 kBq m ⁻² of ¹³⁷ Cs at the soil	relative contribution to dose (%)	μSv per 1 kBq m ⁻² of ¹³⁷ Cs at the soil	relative contribution to dose (%)	μSv per 1 kBq m ⁻² of ¹³⁷ Cs at the soil	relative contribution to dose (%)
⁹⁰ Sr	0.1	0.16	0.064	1.6	0.16	0.26
⁹⁰ Sr	0.39	0.7	0.094	2.4	0.48	0.8
⁹³ Nb	9.8E-07	1.7E-06	0.021	0.5	0.021	0.03
⁹⁵ Zr	4.8E-06	8.2E-06	0.042	1.1	0.042	0.07
¹⁰³ Ru	0.002	0.003	0.11	2.8	0.11	0.18
¹⁰⁶ Ru	0.015	0.028	0.37	9.5	0.39	0.6
¹²⁵ Sb	0.39	0.7	0.015	0.38	0.41	0.7
¹³⁴ Cs	6.8	12	0.26	6.6	7.1	11
¹³⁷ Cs	10	17	0.36	9.2	10	17
¹³⁶ Cs	0.08	0.1	0.014	0.35	0.10	0.16
¹³¹ I	41	70	2.3	57	43.3	69
¹⁴¹ Ce	1.0E-07	1.7E-07	0.038	1.0	0.038	0.06
¹⁴⁴ Ce	2.5E-06	4.3E-06	0.21	5.3	0.21	0.34
¹⁴⁰ Ba	0.001	0.002	0.099	2.5	0.10	0.16
All radionuclides	59	100	4.0	100	63	100

In Ukraine (Table 1.15) the ratio of effective dose of internal radiation of the first year (256 days) to the appropriate dose for the next nearly 20 years (1987–2005) essentially depends on the aggregate value of transfer factor «soil-milk» (TF-92) in a given settlement. In areas where the value TF-92 does not exceed 0.5 Bq·l⁻¹ per kBq·m⁻², the ratio of doses of the first and another 19 years is 8–50 in favor of the first year. For areas with TF-92 in the range 0.5–2 Bq·l⁻¹ per kBq·m⁻², this ratio is 1–2, but still in favor of the first year. At the same time for the settlements, where the value of TF-92 is 2–20 Bq·l⁻¹ per kBq·m⁻² doses in the middle and late phases of the accident exceed the first

Table 1.15. The ratio of internal radiation doses in 1986 to the appropriate doses for the 1987–2005 depending on interval values of the aggregated transfer factor “soil-milk” (TF-92). Doses were estimated by the Method-97 (total 4270 settlements of Ukraine)

TF-92* Bq · l ⁻¹ per kBq · m ⁻²	0-86/87-05 doses ratio	Number of settlements
<0,05	49	269
0,05–0,1	48	261
0,1–0,2	15	1106
0,2–0,5	8,0	1087
0,5–1	2,6	478
1–2	1,1	354
2–5	0,51	404
5–10	0,25	146
10–20	0,13	115
20–100	0,09	40

*TF-92 value was determined for a fixed time interval 1991–1993 based exclusively on results of monitoring radioactive contamination of soil and milk in every settlement

Irradiation of the thyroid

The most significant in terms of radiation exposure and expected radioinduced consequences after Chernobyl accident was thyroid irradiation of children by radioiodine that came mainly from contaminated milk, dairy products and leafy green in May-June, 1986. A three-level system of reconstruction of thyroid doses developed and verified on the results of direct measurements allowed to execute the average gender-age settlement-specific doses of the thyroid gland for all settlements of Ukraine (Table 1.16).

Table 1.16. Average regional thyroid doses (mSv) in different age groups of inhabitants of different regions of Ukraine

Region	The average thyroid dose (mSv) by age groups (years)				
	<7	7–14	15–18	>18	all
Vinnitsa	37	13	8,8	9,2	12
Volyn	87	33	25	21	31
Lugansk	12	4,0	3,1	3,1	4,1
Dnipropetrovsk	13	4,4	3,4	3,4	4,5
Donetsk	24	8,0	6,0	6,1	8,1
Zhytomyr	231	87	67	60	81
Transcarpathian	7,6	2,8	2,1	1,8	2,7
Zaporizhzhia	26	8,8	6,2	6,5	8,8
Ivano-Frankivsk	19	7,1	5,3	4,6	6,7
Kyiv	202	75	58	53	71
Kirovograd	89	31	23	23	30

year, so the first year dose is 13–50% of the dose for further years. In areas with very high values of TF-92 that exceed 20 Bq·l⁻¹ per kBq·m⁻² doses of the first year do not exceed 9% of the accumulated dose for subsequent years.

Thus, in some agro-ecological conditions (peat-swampy acidic soil of pastures) the population can receive significantly larger doses of internal irradiation in postaccidental period compared with doses of emergency first year (for example, a number of settlements Rokytno district, Rivne region, and Kamin-Kashirskyi district, Volyn region). On the other hand, where the black earth were prevailing, internal radiation doses accrued subsequent years may be a small fraction of the first year dose (for example, a number of settlements, Cherkassy region).

Region	The average thyroid dose (mSv) by age groups (years)				
	<7	7–14	15–18	>18	all
AR Crimea	34	12	8.8	8.4	12
Lviv	14	4.9	3.8	3.5	4.8
Mykolaiv	20	7.1	5.4	5.0	7.0
Odessa	15	5.2	3.8	3.7	5.1
Poltava	54	19	15	13	18
Rivne	177	64	49	42	62
Sumy	71	25	19	19	24
Terнопil	18	6.4	4.8	4.5	6.2
Kharkiv	26	8.7	6.5	6.6	8.6
Kherson	30	11	7.8	7.3	10
Khmelnitsky	39	15	11	10	14
Chernassy	142	52	39	37	49
Chernihvi	40	14	10	9.3	13
Chernihiv	151	55	43	37	50
Kyiv city	94	30	23	24	32
Sevastopol city	56	18	14	14	19
All Ukraine	55	20	15	14	19

Table 1.17 shows the distribution of children, adolescents and adults at intervals of absorbed radiation doses to the thyroid gland as a whole across Ukraine.

Table 1.17 The relative distribution of child, adolescent and adult population with a mean absorbed doses of thyroid

Dose interval, Gy	Relative (%) distribution of population				
	<7 years	7–14 years	15–18 years	>18 years	entire population
< 0.05	72	93	95	95	92
0.05–0.1	15	4.4	3.5	3.7	5.0
0.1–0.2	8.2	2.2	1.3	1.1	2.0
0.2–0.5	3.7	0.76	0.40	0.21	0.65
0.5–1.0	0.77	0.08	0.05	0.04	0.12
1.0–2.0	0.15	0.03	0.02	0.01	0.03
2.0–5.0	0.04	0.007	0.005	0.004	0.008
>5.0	0.01	0.0005	—	—	0.001

Dosimetric certification and radiological monitoring at the middle and late phases of disaster

"The Concept of the Annual Total Effective Dose", adopted in 1991, was important for planning and carrying out measures to reduce the impact of radioactive emissions on the population. Regulations of this concept were legalized in the Law of Ukraine № 791a-XII "On legal regime of territories which have undergone radioactive contamination

as a result of the Chernobyl disaster" (1991) and in the Law of Ukraine № 796-XII "On the status and social protection of citizens affected by the Chernobyl disaster" (1991).

The basic principle of Concept lay in that for the critical population group (children born in 1986), the value of calculated effective dose of additional radiation associated with the Chernobyl disaster, should not exceed 1.0 mSv (0.1 rem) per year and 70.0 mSv (7.0 rem) for life (over the dose received by the population in pre-accident period in certain natural conditions).

The density of soil contamination with radionuclides was taken as a provisional criterion. These laws set a *four territorial zone* of radioactive contamination (Table 1.18).

Table 1.18. Areas of contamination according to criteria of the annual dose and density of radionuclides fallout determined by The Law of Ukraine № 791a-XII (1991)

Territorial zone	Zoning criteria			
	fallout density on soil, kBq m ⁻² ^(a)			the annual dose mSv year ⁻¹
	radiocaesium	radiostrontium	plutonium	
1 Exclusion zone	—	—	—	—
2 Zone of implicit (mandatory) resettlement	>555	>111	>3.7	>5
3 Zone of guarantee voluntary resettlement	185–555	5.5–111	0.37–3.7	>1
4 Zone of enhanced radiation monitoring	37–185	0.74–5.5	0.18–0.37	>0.5

^(a) — in the text of the Law fallout density is given in units of "Ci·km⁻²"

In Appendix 1 of the Decree of Cabinet of Ukraine № 106 from July 23, 1991 a list of 2172 settlements was given, which were formally assigned to one of four zones of radioactive contamination according to the Law of Ukraine № 791a-XII.

Annual dosimetric certification was held in Ukraine since 1991 until 2008. All dose calculations were based on results of annual measurements of radiocaesium contamination of milk and potatoes produced in those settlements, which were determined by the Decree of Cabinet of Ukraine № 106. In addition, for radiation monitoring of affected areas residents a united WhBC network of Ukraine was set up during the 1995–1997, providing operational control of the levels of radiocaesium in the body of the inhabitants of affected areas (Fig. 1.9).

Medical organization of 12 most affected regions of Ukraine were provided with 57 units of WhBC "Skrynnar-3M" both stationary (40 units) and mobile (17 units) samples produced at the Institute of Human Ecology (Kyiv) with the use of program-methodical and metrology software, developed by the Department of Dosimetry Control of SI "RCRM of AMS of Ukraine". From 1995 to 2008 about 800 thousand WhBC measurements was held within the network of WhBC. The table 1.19 contains the relative distribution of all certified settlements according to established territorial areas as a result of dose certification within 2001–2008.

The dynamics of change in numbers of settlements in different zones in 2001, 2005 and 2008 for selected regions of Ukraine where the largest number of settlements were dose certified is shown in Table 1.19. As it is seen from the table data, since 2001 ~15–19% of all certified settlements were in the 4th zone of "Enhanced radiation monitoring", and 70–80% in general were outside the established territorial zones of radioactive contamination.



Table 1.19. Change in time (2001–2008) distribution of settlements according to value of passport dose

Year	Quantity of certified settlements	Relative number (%) of certified settlements with the passport dose (mSv per year)			
		<0.5	0.5–1	1–5	>5
		-safety-	4 zone	3 zone	2 zone
2001	2163	67	15	18	0.23
2002	2163	68	15	17	0.14
2003	2163	71	16	13	0.09
2004	2163	72	19	9	<0.01
2005	1831	66	14	5	<0.01
2006	1967	82	14	3,5	0.06
2007	1596	81	15	3,6	<0.01
2008	1925	86	12	0,02	—

Table 1.20. A comparative analysis of certification of settlements of Ukraine in 2001, 2005, and 2008

Region	Quantity of inhabited localities with annual doses (mSv year ⁻¹)												
	total	2001				2005				2008			
		≤0.5	0.5–1	1–5	>5	≤0.5	0.5–1	1–5	>5	≤0.5	0.5–1	1–5	>5
Vinnitsa	89	89	—	—	—	88*	—	—	—	87	—	—	—
Volyn	166	—	3	163	—	107	55	3	—	121	40	3	—
Zhytomyr	698	472	135	93	—	524	96	43	—	569	74	14	—
Kyiv	469	441	23	5	—	428	13	3	—	383	9	—	—
Rivne	339	99	109	126	5	151	122	59	—	150	99	25	—
Cherkassy	103	101	2	—	—	100*	2*	—	—	100	2	—	—
Chernihiv	248	205	41	2	—	218	11	—	—	205	11	—	—

*data for 2006 (in 2005 dose certification was not performed)

It should be noted that in the Kiev and Zhytomyr regions in 2001–2008, the number of settlements in 2nd–4th zones continuously decreased in comparison with the official distribution by the Decree of Cabinet of Ukraine № 106 (Table 1.20). Most of the settlements in these areas were “shifted” to “safe” zone, where passport annual dose did not exceed 0.5 mSv. In the Rivne region, according to Decree of Cabinet of Ukraine № 106 the majority (~76%) of settlements was attributed to the third zone of “voluntary resettlement” (passport dose should be between 5.1 mSv). But in 2001 already, in this zone remained ~30% of settlements and in 2008 ~7% of the settlements. Most of the settlements “shifted” in the 4th zone (passport dose — 0.5–1 mSv), or “safe” zone (passport dose <0.5 mSv).

In 2008, almost 82% of the settlements of Kyiv and Zhytomyr regions referred by the Decree of Cabinet of Ukraine № 106 to 2nd — 4th zones actually have a “passport” dose of less than 0.5 mSv/year. That is, the dose criterion of the territory of these settlements have already lost the status of “victims”. At Rivnenshchyna passport doses below 0.5 mSv per year had 44% of settlements.

There is an annual reduction of settlements, which according to the dose criteria can be attributed to 3rd zone ($1\text{--}5\text{ mSv} \cdot \text{year}^{-1}$). For the 2nd zone (dose >5 mSv), since 2000 in Kyiv and Zhytomyr regions there were actually no settlements belonging to that area, and in Rivne region in 2001 in this zone were to be only 5 settlements.

Doses accumulated by the residents of Ukraine for 25 years after the Chernobyl accident

Table 1.21 estimates averages (weighted by number of inhabitants in settlements) effective doses of external, internal and total (external and internal) exposure for all regions of Ukraine. Estimates of the relevant doses in 1986 accrued between 1987–2011 and for the whole post-accident period and the population distribution for each area are presented based on the density of fallout ^{137}Cs in soil. Table 1.22 presents similar data for Ukraine as a whole.

Table 1.23 estimates population-weighted effective doses in different time periods after the Chernobyl accident for all regions of Ukraine, Kyiv, Sevastopol, as well as Ukraine as a whole.

Table 1.24 shows the distribution of rural population in Ukraine, living in areas where the density of ^{137}Cs fallout in 1986 exceeded $37\text{ kBq}\cdot\text{m}^{-2}$, with intervals of doses accumulated during the 1986–2011.

Table 1.21. Effective dose (mSv) external, internal and total irradiation of different regions of Ukraine depending on the density of fallout ^{137}Cs in soil in 1986

Region	Density of ^{137}Cs fallout, kBq/m^2	% of population	Effective dose (mSv) according to years of exposure				
			external		internal		total
			1986	1987–2011	1986	1987–2011	1986–2011
Vinnitsa	<37	94	0.24	0.47	0.51	0.25	1.5
	37–185	6.3	1.7	3.4	3.40	0.31	8.8
Volyn	<37	97	0.19	0.38	0.43	2.4	3.4
	37–185	2.6	1.4	2.9	3.0	13	20.3

Region	Density of ^{137}Cs fallout, kBq/m ²	% of population	Effective dose (mSv) according to years of exposure				
			external		internal		total
			1986	1987–2011	1986	1987–2011	1986–2011
Lugansk	<37	99	0.34	0.68	0.48	0.22	1.7
	37–185	0.69	1.0	2.1	1.7	0.33	5.2
Dnipropetrovsk	<37	100	0.1	0.19	0.18	0.19	0.65
	37–185	0.02	1.29	2.6	2.8	0.40	7.1
Donetsk	<37	94	0.2	0.39	0.29	0.21	1.1
	37–185	5.6	1.1	2.2	1.4	0.27	5.0
Zhytomyr	<37	75	0.2	0.4	0.37	0.58	1.5
	37–185	17	2.5	5.1	1.4	5.9	14.9
	185–555	7.2	6.8	14	1.9	3.4	25.8
	555–1440	0.69	20	39	8.2	12	79
	>1440	0.06	52	103	22	32	208
Transcarpathian	<37	100	0.12	0.25	0.24	0.19	0.80
Zaporizhzhia	<37	100	0.07	0.15	0.15	0.16	0.52
Ivano-Frankivsk	<37	95	0.26	0.52	0.55	0.38	1.7
	37–185	4.6	1.7	3.4	3.5	0.5	9.0
Kyiv	<37	76	0.45	0.89	0.5	0.42	2.3
	37–185	22	1.9	3.8	1.5	1.0	8.2
	185–555	1.1	8.2	16	6.5	2.7	34
	555–1440	0.86	26	52	8.2	1.5	88
	>1440	0.08	92	184	41	57	375
Kirovograd	<37	99	0.20	0.40	0.37	0.15	1.1
	37–185	0.68	1.5	3.2	3.5	0.29	8.7
AR Crimea	<37	100	0.12	0.23	0.20	0.17	0.72
Lviv	<37	100	0.09	0.17	0.17	0.16	0.58
	37–185	0.008	1.2	2.4	2.6	1.5	7.7
Mykolaiv	<37	100	0.12	0.24	0.22	0.15	0.73
	37–185	0.06	2.4	4.8	5.3	0.50	13
Odessa	<37	100	0.19	0.38	0.34	0.15	1.1
	37–185	0.19	1.3	2.7	2.9	1.5	8.5
Poltava	<37	100	0.17	0.33	0.31	0.22	1.0
	<37	78	0.28	0.56	0.45	1.1	2.4
Rivne	37–185	21	2.2	4.3	1.9	14	22
	185–555	0.39	7.2	14	5.9	14	42
Sumy	<37	99	0.21	0.42	0.41	0.32	1.4
	37–185	0.98	1.91	3.8	4.1	1.2	11
Ternopil	<37	97	0.15	0.30	0.35	0.37	1.2
	37–185	3.0	1.6	3.1	3.3	0.77	8.7
Kharkiv	<37	100	0.18	0.36	0.32	0.17	1.0
	37–185	0.01	1.12	2.2	2.4	0.54	8.3

Region	Density of ^{137}Cs fallout, kBq/m ²	% of population	Effective dose (mSv) according to years of exposure				
			external		internal		total
			1986	1987–2011	1986	1987–2011	1986–2011
Kherson	<37	100	0.07	0.14	0.14	0.13	0.49
	<37	98	0.16	0.33	0.35	0.26	1.1
	37–185	1.7	1.6	3.3	3.6	0.29	8.7
Khmelnitsky	185–555	0.003	6.7	13	15	0.15	35
	<37	84	0.30	0.59	0.59	0.27	1.7
	37–185	15	1.9	3.7	3.3	0.54	9.5
Cherkassy	185–555	0.37	7.3	15	15.8	0.12	38
	<37	92	0.36	0.72	0.74	0.35	2.2
	37–185	7.6	1.7	3.4	3.2	0.34	8.6
Chernivtsi	185–555	0.31	5.9	12	13	0.25	31
	<37	97	0.23	0.45	0.41	0.50	1.6
	37–185	3.2	1.8	3.6	2.3	2.2	9.8
Chernihiv	185–555	0.08	7.4	15	8.1	4.0	34
	555–1440	0.01	18	35	35	12	100
Kyiv city	<37	100	0.48	0.96	0.28	0.13	1.9
Sevastopol city	<37	100	0.2	0.40	0.34	0.14	1.1

Table 1.22. Distribution of population-weighted effective doses of total exposure of the inhabitants of Ukraine in general, depending on the density of fallout ^{137}Cs in soil

Fallout density of ^{137}Cs in soil kBq/m ²	% of population	Irradiation, mSv				
		external		internal		total
		1986	1987–2011	1986	1987–2011	1986–2011
<37	96	0.20	0.41	0.33	0.29	1.2
37–185	3.7	1.8	3.7	2.1	3.3	11
185–555	0.29	7.0	14	3.5	3.4	28
555–1440	0.04	23	46	8.4	6.4	84
>1440	0.005	77	154	34	47	313

Table 1.23. Population-weighted effective doses (mSv) of total whole body irradiation of the inhabitants of different regions of Ukraine

Region	Population-weighted effective doses (mSv) of total whole body irradiation according to time intervals (years)			
	1986	1987–1996	1997–2011	1986–2011
Vinnitsa	1.02	0.61	0.30	1.93
Volyn	0.72	2.34	0.81	3.86
Lugansk	0.84	0.60	0.31	1.75
Dnipropetrovsk	0.27	0.25	0.13	0.65
Donetsk	0.60	0.47	0.25	1.32
Zhytomyr	1.95	2.91	1.32	6.19

Region	Population-weighted effective doses (mSv) of total whole body irradiation according to time intervals (years)			
	1986	1987–1996	1997–2011	1986–2011
Transcarpathian	0.37	0.30	0.13	0.80
Zaporizhzhia	0.22	0.20	0.10	0.52
Ivano-Frankivsk	1.01	0.70	0.31	2.03
Kyiv	1.96	1.86	0.94	4.76
Kirovograd	0.61	0.38	0.19	1.18
AR Crimea	0.32	0.27	0.13	0.72
Lviv	0.25	0.22	0.11	0.58
Mykolajiv	0.35	0.26	0.13	0.74
Odessa	0.54	0.35	0.18	1.07
Poltava	0.48	0.38	0.17	1.04
Rivne	1.48	3.77	1.47	6.72
Sumy	0.67	0.53	0.25	1.45
Ternopil	0.63	0.54	0.23	1.40
Kharkiv	0.50	0.36	0.17	1.03
Kherson	0.22	0.19	0.09	0.49
Khmelnytsky	0.59	0.43	0.20	1.22
Cherkassy	1.63	0.95	0.49	3.07
Chernivtsi	1.45	0.88	0.43	2.75
Chernihiv	0.77	0.79	0.34	1.90
Kyiv city	0.76	0.71	0.39	1.86
Sevastopol city	0.54	0.35	0.19	1.08
All Ukraine	0.71	0.69	0.32	1.72

Table 1.24. Relative (%) distribution of citizens of Ukraine living in rural areas with levels of fallout $^{137}\text{Cs} > 37 \text{ kBq}\cdot\text{m}^{-2}$ (in 1986), according to intervals of effective whole body dose accumulated at various times after the Chernobyl accident

Dose intervals (mSv)	Time intervals (years)			
	1986	1987–1996	1997–2011	1986–2011
% of the whole population				
<1	—	—	21	—
1–2	6.2	22	34	—
2–5	60	40	31	9.4
5–10	26	19	11	40
10–20	6.9	15	2.7	31
20–50	0.78	4.2	0.21	18
50–100	0.01	0.14	—	1.5
>100	—	—	—	0.08

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CHAPTER 2 MEDICAL PROTECTION OF VICTIMS IN ACUTE PERIOD OF LIQUIDATION OF CHORNOBYL DISASTER CONSEQUENCES

After the accident at the Three Mile Island NPP TMA (USA, 1979) in November 1981, the report of the meeting of the World Health Organization (WHO) in Brussels, for planning purposes in case of accidents at nuclear power plants recommended scheme of the accident in time, which allocated three consecutive phases: early, intermediate and late. Moreover, this phasing was not dependent on the level of accident (5, 6 or 7) and the scale of contamination of the environment as determined solely by the change in time of emergency radionuclide mixture emissions in accordance with the laws of radioactive decay and a general decline in their total activity [1].

Early phase should be considered as the initial, acute period of the accident, lasting till the stoppage of uncontrolled emissions.

Intermediate (middle) phase — the period of varying lengths when mainly emergency work on the affected unit is accomplished with a warranty for discontinued further pollution. In addition, at the beginning of this phase formation and the final stabilization of the radiation environment completes. It may take several weeks or months, depending on the complexity arisen accident.

Late phase is *per se* post-accident or recovery period of gradual return to normal activity. It will be lasting for many years as medical, social, ecological and economic effects of disaster require long-term research and will determine the number of special peculiarities of population vital activity living in contaminated areas.

However, such gradation of the accident development could not serve as a productive basis for the staged emergency planning, because it does not take into account the radiological characteristics, which identify the main sources of possible harm to population health.

As the experience of Chernobyl showed, early phase lasted from April 26 until May 5, 1986 (9 days after the accident), when basic emissions stopped. Completely emissions stopped and radiation environment got stabilized only in 15 days after the

accident, so all levels of external gamma radiation to compare estimates were reduced to that term [1].

In view of studying the experience of liquidation such a large-scale catastrophe like Chernobyl, the recommended gradation in order to clear criteria for practical planning and carrying out protection activities, we have proposed next stages of this accident:

- a) stage 1 — within 1 day since the accident;
- b) stage 2 — 2–15 days after the accident;
- c) stage 3 — 2–8 weeks after the accident;
- d) stage 4 — 1.5–2 months after the accident.

The first stage is due, on the one hand, to the fact that the first accident emissions are usually most dominant, forming a significant portion of forecasted doses of irradiation, including inhalation dose of radioactive iodine on the thyroid gland (TG), that accumulates very quickly, while iodine prophylaxis efficiency is rapidly decreasing in time.

Terms of the second stage are mainly determined by cessation of uncontrollable accident contaminants emission and stabilization of radioactive situation, as well as by necessity of all completion major evacuation and other urgent anti-radiation protective actions.

The first and second stages cover the initial (acute) period of emergency (early stage within the WHO terminology) and require particularly careful planning and forehanded training of necessary capabilities for operational activities during the liquidation of its consequences. In radiological terms, this is "the period of iodine danger" because of the prevalence and biological significance of iodine radionuclides, as well as for dose formation dynamics with their participation.

The third phase marks the completion of radiologically significant "period of iodine risk", which determines the nature of both protection measures, and medical diagnostics and sanitary measures. Its duration (up to 8 weeks after the accident) is determined with half-value period (T) of iodine-131 (8.1 days), which is the longest of all emergency emissions iodine radionuclides.

The fourth stage completes intermediate phase and marks transition to the last phase (a late one). In the radiological impact it will be presented with radionuclides among which dominate radionuclides of cesium, following after the iodine period — "cesium period" (Table 2.1).

Twenty five years after the Chernobyl catastrophe passed, but still we feel its impact on the health of the population [2–5]. The international community has identified this failure as an environmental and socio-economic disaster of the highest level, which resulted in radioactive contamination of 50.5 thousand square kilometers, inhabited with 2.4 million people in 2,218 locations [6–8].

International Atomic Energy Agency (IAEA) had assigned the Chernobyl accident a highest category and described it as «the biggest nuclear disaster in history» [9].

Are we ready to respond to such radiation accident adequately? Is the Unified Prevention And Emergency Response System serving in Ukraine the perfect one?

Carrying out adequate medico-sanitary measures to protect the population in radiation accidents, ranging from ensuring the effective planning, development of reasonable recommendations on population safety is impossible without estimation of development

Table 2.1. Scheme of large-scale accidents at nuclear power plants and key protection measures

Phase of accident (use the terminology of the IAEA)	Stages of emergency plan	Sources of radiation exposures	Main kinds of irradiation	Radiological characteristics (periods)	Protective measures and their application: + required "applicable, but require assessment of ratio + specific" — basic+
Early	1 (up to 24 hours)	radioactive cloud, precipitation on the body	external (whole body), internal (inhalation)	IODINE	Notification+, shelters+, protection of respiration and body+, iodine prophylaxis+, evacuation+, individual decontamination+
	2 (up to 15 days)	radioactive cloud, precipitation on the body, precipitation on the land, food chain	external (whole body), internal (inhalation, through the gastrointestinal tract), external and internal (adsorption)		Sheltering before evacuation+, protection of respiration and body+, iodine prophylaxis+, evacuation+, control of food and water (individual decontamination+), restrictions on access to controlled zone+
Intermediate	3 (2-18 weeks)	precipitation in the land, food chain	external, internal / through the intestinal tract /	IODINE	Resettlement+, food controls+, area decontamination+, restrictions on access to controlled zone+
Late	4	precipitation on the land, food chain	external, internal (through the intestinal tract)	CAESIUM	Food controls+, area decontamination+

and evaluation of radiation environment, and the sources of possible health injury, volume of medico-sanitary care measures considering possible evacuation of population, districts and in time of relocation.

To answer these questions there is a need to go back in time to those events, called as liquidation of the accident consequences, especially to the organization of health care for population affected by the Chernobyl catastrophe.

As shown by the results of our retrospective study of the Chernobyl catastrophe consequences, in a real environment, any complete official information on all these output data points had been received neither by the Ministry of Public Health of Ukrainian SSR (MPH of UkrSSR) nor by the Ministry of Public Health of Byelorussian SSR, not only timely but also all over first 2 months [10]. Moreover, even information about the real nature of the accident was obtained neither from Government nor from Communist Party officials, including the Government Commission of USSR Council of Ministers. As a result, neither the population nor the authorities of both local and republican levels for several days had no information about what had happened at Chernobyl and what was a scale of the accident.

Republican Committee on accident, which was established already after the 1st May Day demonstration of 1986 kept silence until 7th of May. Population was reported only on the ninth day after the accident, although current information about radiation environment, which consisted of proposals for urgent notification of population with recommendations for mitigation of radiation factor, was supplied to the government of the Republic continuously and without delay.

Alert people about the accident that occurred, should been reported automatically according to a premeditated plan for the radiation protection, but automation has been disabled.

Timely determination and evaluation of radiation situation impeded the following circumstances:

- release of radioactive products from the emergency unit lasted for a long time and forming a radiation environment on specific areas was determined with stuff dispersion and height of emissions of radioactive aerosol, and meteorological conditions that characterized with motions of unstable weak winds in variable directions in the surface atmosphere and dominated by forces at 1 km or more. As a result, radiation situation was complicated and patchy nature contaminations even within one locality and in adjacent sides places without enough clear correlation between the concentration of radioactive clouds and its ground tracks, both in overall radioactivity, and by some isotopes (especially iodine and cesium);
- radiation effects of various degrees of covered large areas that were not only directly adjacent to the accident, but away from it for hundreds and even thousand kilometers, so involving in the accident millions of people.

In this regard, it was necessary to solve complex and diverse tasks to protect people who got into the contamination. Such large-scale problem on radiation protection of huge contingents of the population never have been solved in world practice.

Together, these two factors determined the main difficulties in assessing radiation-ecological situation, the establishing exposure of the affected population, that in turn influenced on the timeliness of medical and preventive measures, primarily for emergency prevention activities.

Lack of concerted action among organizations and institutions particularly evident in the implementation of radiometric control of food, water, environmental objects not only in terms of overall activity, but also in spectrum of radionuclide's content. Paradoxical situation: the ministries and departments responsible for production, harvesting, processing, storage, transportation and sale of food, raw food, and water supply was more than enough. At the same time, they were so poorly equipped and had few trained personnel that at first the main burden of control was laid on the of the MPH of UkrSSR.

The first attempt to join forces and organize a monitoring of radioactive contamination and exposure of people was realized by the initiative of the MPH of UkrSSR, approved by the Decree of Council of Ministers of UkrSSR from 06/13/1986 No 332 pc, about sharing the responsibilities for radiometric and radiation control between ministries and departments in the Ukrainian SSR.

Only with the order of Council of Ministers of the USSR from 10/31/1986, № 2204 pc the State Committee for Hydrometeorology of USSR (Derzhkomhidromet) was assigned as the leading organization to monitor, assess and predict the radiation situation in the country, especially in the contaminated areas around Chernobyl.

From the first days after the accident started a large and meticulously work on the organization of medical care to persons exposed to the disaster. On 3rd of May was issued the first order on organization of health care for population affected by the disaster.

The MPH of UkrSSR in early May established scientific advisory group of the leading specialists of the Republic. The main tasks of the group were identified as the

collection and analysis information related to radioactive contamination of air, water, soil, food and irradiation of population.

Major orders Ministry of Public Health of Ukraine are presented in Tables 2.2–2.4.

The work on the organization of dispensary supervision of exposed persons started for the first time both in the USSR and Ukraine due to the order of Ministry of Public Health of Ukraine from the June 6, 1986 «On the order of prophylactic clinical examination (dispensarisation) of persons who were in a zone of heightened radiation.»

Table 2.2. Major Ministry of Public Health of Ukraine orders dealing with health care of population affected by radiation

Number	Date	Title
21 c*	05/03/1986	On the organization of medical support for population and affected in connection with the Chernobyl accident
27 c	05/15/1986	On medical-sanitary, anti-epidemic ensuring of the population of Kyiv region districts
31 c	05/19/1986	On additional measures to ensure medical organization in connection with the Chernobyl accident
41 c	05/28/1986	On the organization of medical maintenance of population during evacuation from some settlements of Kyiv and Zhytomyr regions and at places of their resettlement

Table 2.3. Major orders Ministry of Public Health of Ukraine on providing healthcare to children and pregnant women

Number	Date	Title
23 c	05/10/1986	On investigations and supplying with medical aid of children and pregnant women
32 c	05/22/1986	On the organization of medical investigation of children population in Zhytomyr and Chernihiv regions
321	05/30/1986	On the organization of healthcare for children and pregnant women from zone of increased radiation

Table 2.4. Major orders Ministry of Public Health of Ukraine for health care of clean-up workers of the Chernobyl accident

Number	Date	Title
24 c	05/11/1986	On the organization of medical support for population and affected in connection with the Chernobyl accident
40 cc*	05/27/1986	On the organization of medical and sanitary care for clean-up workers who participate in the aftermath of the Chernobyl accident

A temporary instruction "Organization of the work of sanitary-epidemiological service of Ukrainian SSR to ensure the radiation safety of the population in connection with the nuclear reactor accident at Chernobyl Nuclear Power Plant", was developed for the Radiological Service of the Republic and approved by the chief sanitary doctor of Ukrainian SSR on 05/22/1986.

All the critical issues of Chernobyl accident were resolved with involving leading scientists and experts of Ukraine.

The worsening of the radiation situation necessitated immediate evacuation. Government Commission had to make a decision on the evacuation of the city of Prypiat and the Chornobyl region. During the period from 04/27/1986 to 05/05/1986 89,400 people were evacuated, including 21,750 children. From the city of Prypiat to Polisske district of Kyiv region were evacuated 20,000 of population, to Ivankiv — 2,500. The population of the Chornobyl area (29,534 persons) was evacuated to the Borodyansky district and part (7,646 persons) to other settlements of the Chornobyl region.

Ministry of Public Health of the Ukrainian Republic was not informed on the decision of the Government Commission of the USSR about the first stage of evacuation from Prypiat and settlements of 10-km zone.

As it was shown by subsequent events — sequential evacuation first from the 30-km zone, and then from the west track and outside the 30-km zone — in the process of discovery and clarification of radiation situation and the deployment of health care means of Ukrainian and Belorussian SSR was performed after the events, not according to the previously worked out plans with assessment of situation and reserves [11].

In the early days after the accident 450 mobile medical teams with ambulatories and 5,960 of personnel (1,240 doctors, 920 nurses, 360 medical assistants and 2,720 operating personnel) were formed and sent to the area of Chornobyl. These teams had made survey of 135,000 people, including 100,000 children, more than 100,000 radiometric investigation of the thyroid gland and blood tests, and thousands of measurements using whole body counters. Medical assistance to population affected by the Chornobyl accident, in addition to 543 doctors and 1068 paramedicals who regularly worked in Polisske, Ivankiv, Makariv and Boryspil district of Kyiv region, was provided by mobile teams from all regions of the country. Only in May 1986 3.4 thousand specialists were attracted additionally, including 840 doctors, 750 nurses, 180 laboratory doctors, 1360 laboratory assistants, 750 students, 120 dosimetrists, 50 researchers, about 300 temporary outpatient clinics were launched [12].

It was important that there was not enough experts in the field of radiation medicine in the country, despite the fact that the subject of radiology has been studied more than any other, problems of radiation medicine really were unknown to broad medical community, except the trainings in Civil Defense.

It should be added that of elimination of large radiation accidents abroad (Wind-scale Great Britain, 1957; NPP "Three Mile Island", USA, 1974) and domestic (South Urals, in 1950 and 1957) (South Ural, 1950 and 1957) was not the achievement nor medical science nor the general public.

Therefore, nobody, including health care system, really was not ready for "Chornobyl": most health professionals and scientists demonstrated their radiation ignorance with very distorted imagination, overly exaggerated with journalism on the waves of emotional anti-nuclear propaganda.

As the damage of such magnitude previously was not expected all medical measures in case of accidents at nuclear power plant were relied on its medical care department without attraction of local forces and involvement of health authorities. In connection with this, both stand-by system and the organizational principles of medical care has not been developed to meet the broad-scale evacuation of population and involvement in the incident both primary, and specialized medical care.

The system of medical care of Civil Defense Service (CDS), capabilities of its forces as well as capabilities of Medical Department of Chernobyl NPP were inadequate to real situation. Therefore, the major burden of health care laid on health care institutions and authorities of Ukrainian and Byelorussian SSR. Acute need for covering of both quantitative and qualitative deficit of necessary forces and means to enhance health and specific local health authorities had to solve in urgently without sufficient justification.

Due to complex picture of fallout over large areas in some localities preventive measures were carried out partially or not performed. As a result, iodine prophylaxis was not applied even to the most young children because areas of vital necessity were not timely marked.

In addition, there was no consensus on the dosage of iodine, and only with the release of "Temporary emergency instructions to prevent destruction by radioactive iodine" on 7th of May, 1986 finally was installed dose of stable iodine.

In this regard, for example, in Zhytomyr region prophylactic intake of iodine started only on 17th of May, 1986, though it is generally known that consumption of stable iodine before the entry of radioactive iodine in the body can reduce the total dose of thyroid by 95%, within 6 hours after radioactive iodine — 50%, and 24 hours after radioactive iodine absorption iodine prophylaxis is practically useless [13].

This definitely shows the delay and incomplete coverage of iodine for all who needed it, especially in areas of Polissia, which are endemic iodine deficiency [14].

The lack of primary medical records violated the sequence of registration of radiation exposure, contamination and medical care, significantly hindered the adoption of balanced and thoughtful decisions. Later it complicated analysis and generalization of medical care results.

Despite the apparent positive results of health care in evacuation, there were many difficulties and unsolved problems, the main of which included:

- CDS medical service plans were not provided with recommendations on the scope and frequency of examination evacuated population at different stages of evacuation, depending on possible dose of radiation absorbed during emergency situations at nuclear power plants;
- ways of evacuation did not provide separate flows of people and cattle, creating a possible risk of secondary contamination;
- places of medical teams formation were not provided with special clothing and other facilities for personal protection from radioactive substances, creating additional difficulties in their ensuring;
- dosimetric instruments from mobilization reserve (ordinance on their depreservation came a day after the evacuation started), had unsuited batteries of 1971–1981 manufacture;
- decontamination–cleaning works were carried out on the road, where dosimetry and medical examination of the evacuated population were conducted. Special areas for cleaning transport have not been equipped by the end of evacuation. This situation contributed to the contamination of additional people and the transport of radioactive substances in adjacent to disaster areas;

- lack even the simplest medical records on evacuees (accounting data of garments and cutaneous dosimetry on stages of evacuation, the amount of sanitization processing, etc.) hindered consecution of medical events;
- temporary cleaning points were overloaded with the sanitary processing of evacuees, especially children, elderly and infirm from the medical point of view people, because of a lack the necessary calculations, and were not adapted to work at night-time and at low temperatures;
- absence during the mass evacuation of allowable pollution standards for body surface and clothing, as well as the failure of institutions and organizations in the Ministries of Communal Services affected Republics for sanitization of the population, despite the fact that it was envisaged by CDS plans; therefore, health authorities engaged in medical activities had to execute other functions not inherent to them;
- lack of sanitization of the population;
- tasks for providing population with exchange clothing funds were solved in a hurry had inflicted more troubles for heads of medical institutions.

The above mentioned circumstances significantly complicated the medical and tactical situation, embarrass health purposeful activity and reduced efficiency of large-scale and laborious activities that were carried out.

Over 135,000 people were evacuated to other areas, resulting in dramatically increased pressure on existing hospitals.

Clinical studies in Post-Chornobyl period confirmed all the previous experimental and clinical experience that blood parameters are valuable diagnostic tests of biological dosimetry only for acute radiation syndrome, but at doses below 0.1 Gy are not informative, because they almost imperceptibly differ from the original indices [15, 16]. However, they clearly respond to physical and psychological overload, and other non-radiation effects, that became a source of inadequate interpretations of the results of blood tests by an overwhelming majority of medical workers who do not have sufficient knowledge of radiation medicine. This was one of the sources of anxiety and increased mental and emotional stress among patients.

A number of therapeutic and diagnostic activities carried out blindly, without the necessary information on radiation doses was focused on revealing radiation injuries.

Involvement of unreasonably large number of health workers, working on shift method of unreasonably frequently changing teams, created a favorable basis for the occurrence of iatrogenic disease.

Frequent change of medical teams, on the one hand, made for quite contradictory results of the studies on health condition monitoring, while, on the other hand, it gave a rise to a sense of irresponsibility for the final results of their activities.

Thus, the shift method itself became a source of anxiety and nervousness of resident population, often considered as another sign of trouble in their radioactive contaminated area.

All this was an evidence of very high psycho-emotional tension among medical workers who arrived for a work in the "contaminated area", which could not but affect the nature of contacts with patients, given the social role of medical officer in overall anxiety.

The key mechanism of growth of psychosocial stress, besides low levels of knowledge and competence in the fields of radiation medicine, psycho-neurology, social and medical psychology of medical staff, who were the main source of inadequate, too controversial, and disturbing information about diseases and defects in the health of the monitored population was excessively low level of knowledge of the population as well as serious defects and lack of official information about the situation. It formed a trust deficit in the acute period of the accident continued for many years, taking the form of crisis of confidence not only against the authorities, including public health, and medical science, as well as foreign experts who worked in the affected areas to objectively assess the medical consequences of the Chernobyl accident [17, 18].

Late and insufficient objective information to the public about the Chernobyl accident by the government created the preconditions for socio-psychological stress in society.

Retrospective evaluation of psychosocial aspects of the Chernobyl disaster shows that the resulting lack of training of social psychology arose situation when emotional tensions could be a source of much greater harm to health than the radiation [19]. The negative consequences associated with stress may be a greater scale than that of the radiation exposure. Persons who have experienced stress, were not just limited by evacuated from the exclusion zone, the direct participants of liquidation of consequences of Chernobyl disaster and those who lived in areas within the zone of radioactive contamination, but covered almost the entire population of Ukraine.

In spite of above mentioned shortcomings from the present position it should be noted that the measures of health protection implemented in the first months after the accident prevented possible acute radiation injury in the population of Ukraine.

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CHAPTER 3 REGISTRIES OF AFFECTED POPULATION

3.1 THE STATE REGISTRY OF UKRAINE OF THE PERSONS AFFECTED BY THE CHORNOBYL CATASTROPHE

One of the major problems arising from large-scale catastrophes and natural disasters is the registration of victims, their identification and formation of registry for future monitoring.

Before the Chernobyl accident, in many countries throughout the world, a number of registries were established and are successfully functioning at the moment. They are aimed monitoring at the population levels the effects of the environmental factors (including radiation one) on human body. These registry data are the basis for investigations of cause and effect relationship between influences and risks for various health changes.

In addition to registries made up to monitor negative impact of environmental and other factors there are population and hospital nosological registries containing information for the persons with one or several diseases. Cancer-registry is one of the most well-known nosological registries in the world; it is functioning in many countries including Ukraine.

To solve the problems registries accumulate identification data (passport-registration information), impact factors characteristics, details of diagnostic and methods of disease treatment (depending on registry type).

First information on negative effects of ionizing radiation (IR) on human health appeared at the beginning of the last century. Further development of IR use in industry and medicine resulted in deeper insight into health effects. Important role here played the registry of persons affected by radiation due to their professional activities and emergency situations. Registries for radiation workers have been successfully functioning over several years in Canada, Japan, France, Great Britain and the USA. There are also various registries of population exposed to radiation due to their location in the districts of mining and processing of uranium ores (Spain and Slovenia), as a result of nuclear weapons tests (the Marshall Islands, Japan) or disaster involving the use of radioactive sources.

Analysis of the consequences of Mayak plant operation (South Urals, Russia) and disaster in 1957 was possible on the data of the registry of exposed persons.

The most complete generalization of medical consequences of Hiroshima and Nagasaki bombings (Japan) also became possible thanks to the monitoring of the popu-

lation-survivors as well as compiling of cancer registries in affected places. The latter enabled to reveal the correlation between exposure to IR and the development of certain tumors, calculate the risks of carcinogenesis for various doses.

Immediately after the disaster at Unit 4 of the Chornobyl NPP on April 1986, during the evacuation and resettlement of exposed population and power plant workers, their accommodation throughout Ukraine, hospitalization emerged a problem of registration and keeping various medical documentation. Radiation monitoring services along all evacuation routes and in the local medical institutions also were keeping medical records. Nevertheless, on the state level such registration documents were not available. The available local registration forms were not standardized for all categories of participants. The scale and specific character of radiation from the Chornobyl disaster in 1986 made it necessary to develop a unified form to register the survivors. Solving this problem was delayed because of departmental dissociation and lack of experience. The first attempt to develop registration documents for evacuees from the Chornobyl area failed as a result of the fact that the majority of scientific investigations persuaded only scientific aims — a complete collection of medical-biological and dosimetric data from the objects and numerous clinical-biochemical analysis data. Such a document was released and adopted by the Ministry of Public Health (MPH) of the USSR, yet it was not executed by the end of 1986. Under coordination of Radiation Hygiene Department, the MPH of the USSR held numerous consultations of employees of the MH of Ukraine, Belarus and scientists of newly organized All-Union Centre for Radiation Medicine, Academy of Medical Sciences of the USSR (AURCRM) in Kyiv and Medical Institute of Radiology in Obninsk were held. Initial registration documents were prepared concerning minimal passport, diagnostic and dosimetric data, same as the results of regular medical check-ups of affected people. Classifiers, instructions and method recommendations on organization, recording and automated registration using modern computers were developed. As a result, an appropriate document (Order No. 640, the MPH of the USSR) was developed and released, it was replicated in all Soviet Republics. Throughout Ukraine, an enormous organizational, methodological and medical diagnostic works were carried out (initial prophylactic medical examination of survivors at the place of residence, registration forms filling, transfer them to the newly created regional and MPH expert committee, following information input and transfer of information on magnetic media in the National Data-Processing (Computer) Centre. Many specialists were involved to do this work within 1987-1988. Those were employees of the MPH, researchers of AURCRM, workers of regional health departments and some research institutes with direct participation of local healthcare personnel. The first results were published and presented at the International Conference held in Kyiv, April, 1988.

Over the next four years the Registry was updated with new case information on population health as a result of targeted clinical examination. With Registry database (DB), the research team of AURCRM, and Medical Institute of Radiology, Academy of Medical Sciences of the USSR, have designed reference books layouts which were further published as a reference material for scientific and practical purposes. To support the Registry, in Ukraine we have developed a system of statistical records of size and health of exposed population in the registry. The system was the basis of the state statistical record on the victims throughout the USSR. It covered all establishments and

was functioning up to the end of 1991. Since independency of Ukraine, the necessity appeared to develop new legal acts to maintain the Registry as a uniform system of monitoring and long-term regular medical check-up of the exposed population.

A new stage of the development of the State Registry of Ukraine of the Persons Affected by the Chernobyl Disaster (SRU) was realized in accordance with the Law of Ukraine "On the status and social protection of citizens affected by the Chernobyl catastrophe", February 2, 1991.

To mitigate the Chernobyl catastrophe effects, to ensure disease diagnostics, treatment and victims rehabilitation as well as to study medical effects in 1991 WHO adopted the International Program on Health Effects of the Chernobyl Accident (IPHECA). Pilot projects were developed to implement them in Belarus, Russian Federation and Ukraine dealing with: (1) "Thyroid Gland", (2) "Hematology", (3) "Brain Damage In Utero", (4) "Epidemiologic Registry", (5) "Dentistry" (only in Belarus). The objective of these projects was to encourage the development and mastering of methods, to accumulate experience for the further long-term period. A special system for DB processing was designed for quality control and updating through all stages.

The SRU kept the three-level distribution nature. Such a system (district, region, country) was the most stable and viable.

The SRU is a special system for detection, collection and storing (on magnetic media), automated processing and analysis of data on health, radiation exposure and medical care of exposed people, for the development of system measures to preserve health and to make scientific and sociological studies.

The SRU goal is: database for medical and social measures on prevention of negative health effects of the Chernobyl disaster.

Main tasks of the SRU are:

- personalized record of the affected persons;
- personalized registration of results of annual clinical examination, including data on doses of external and internal radiation, ¹³¹I thyroid doses;
- annual analysis and evaluation of victims' health (morbidity, disability, mortality with potential risk factors being identified);
- preparation of information and analytical materials for major departments (WHO, Ministry for Emergency Situations, National Academy of Medical Sciences of Ukraine) regarding decision-making in planning of preventive measures, specialized scientific epidemiological, clinical, radiation-biological, social and hygienic, sociological research etc.;
- information support of research projects.

These tasks are provided through the creation and development of automated information systems (AIS) for personal registration of victims in Ukraine.

AIS of the SRU functions under the «Regulations on organization and operation of the State Registry of Ukraine on the Chernobyl Victims» approved by the Cabinet of Ministers of Ukraine on June 9, 1997, No. 571 (hereinafter referred to as Regulations).

SRU functioning is coordinated by the Ministry for Emergency Situations of Ukraine. The MPH of Ukraine is responsible for the list of establishments and institutions that provide the SRU objectives.

Creation of the SRU AIS is an important contribution to the health information technology and research on the medical consequences of the Chernobyl disaster.

Structurally the SRU is a large-scale, geographically distributed or departmental organizational information system that has a three-level structure (state-region-district). The SRU subdivisions are as follows:

- the state level — State Institution «Ukrainian Centre for Information Technology and the National Registry of the MH of Ukraine » (SI "UCIT and NR"), relevant specialized units of the Ministry of Defense, the Ministry of Internal Affairs, Security Service of Ukraine that manage the Ukrainian Military Registry and its subdivisions along with specialized units of research institutes, the National Academy of Medical Sciences and National Academy of Sciences of Ukraine;
- Autonomous Republic of Crimea — the State Registry centre of the MPH of Autonomous Republic of Crimea;
- regional, municipal level — regional or city hospital (centre or dispensary);
- district level — central district hospital.

Functions of state-level SRU which information and communication core is the AIS are executed by SI "UCIT and NR", MPH of Ukraine. It provides centralized management and administration of common distributed SRU DB, automated technology to collect data on victims, secure data storage in distributed info media, collective user access to the SRU DB, data searching and processing in real time. The general scheme of automated data processing technology see in Fig. 3.1.

Regional-level SRU functions on the basis of regional hospitals and basic health facilities that execute registration and examination of the affected persons. They are equipped with specialized software and hardware integrated into the global information network by means of telecommunications.

District-level SRU function on the basis of district hospitals. These are uniform special-purpose program-technical systems on the basis of separate software package integrated into the global information network.

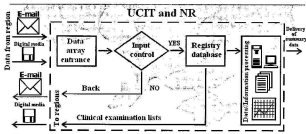


Fig. 3.1. Scheme of data collection and processing

At the regional level, the SRU Centres were established in the Autonomous Republic of Crimea, all 24 regions of Ukraine and also in the cities of Kyiv and Sevastopol. At the district level, data of the SRU are available from 77 contaminated areas.

Scientific subregistries are functioning in the framework of the SRU, among them Clinical-and-Epidemiological Registry(CER) of Research Centre for Radiation Medicine, National Academy of Medical Sciences of Ukraine (RCRM). It was created for in-depth study on the influence of the Chornobyl disaster on victims' health.

As of March 31, 2010, registered in the State Registry of Ukraine were 2 343 798 persons affected by the Chornobyl disaster (Table 3.1).

Table 3.1. Distribution of persons registered in the State Registry of Ukraine by primary registration groups

Primary registration groups	Number of registered persons	Percentage of total number
The Chornobyl clean-up workers**	314 191	13.41
of them		
Clean-up workers 1986-1990*, including	262 818	—
Clean-up workers 1986-1987*	222 807	—
Clean-up workers 1988-1990*	400 11	—
Clean-up workers 1986-1987 (women)*	17 485	—
Clean-up workers according to the Interior Ministry Registry	21 390	—
Evacuees or those who left the area	80 758	3.45
Residents of contaminated areas	1 529 896	65.27
People born to parents of primary registration groups 1-3 (adults)	275 224	11.74
Children born to parents of primary registration groups 1-3	143 731	6.13
TOTAL	2 343 798	100

*excluding the data of the Ministry of Internal Affairs Registry; **including the data of the Ministry of Internal Affairs Registry;

Existing software consists of system software, network, communication software, DB management systems, general-purpose application software, special-purpose application software.

System software includes operating systems and operating system shell. Operating systems used in the SRU: MS-DOS 6.22, Windows NT 4.0, Windows XP.

Network, used in the SRU: network Microsoft Windows.

DB management systems used in the SRU: FoxPro 2.0 and FoxPro 2.5 for MS-DOS, FoxPro 2.5 for Windows.

Special-purpose application software is developed by SI "UCIT and NR" for the Registry.

It should be recognized that the existing information and software AIS of the SRU needs modernization and further development.

Strong necessity for modernization is due to the following factors:

- increased requirements for general scientific and technical level of Registry AIS and its functionality;
- introduction of new registration forms, medical examination and dosimetry data records, containing extended information on indices and relationship between them.

For this purpose the procedures of primary data collection and analysis are to be improved.

Raising of general scientific and technological standards of SRU AIS suggests the replacement of the most outdated technologies and implementation of such measures:

- transition to UNIX platform;
- implementation of Internet technologies;
- analysis and transition to open systems and information interfaces;
- development of SRU telecommunications;
- development and utilization of staff resources.

The SRU AIS operates under the joint interdepartmental programs developed by the Ministry for Emergency Situations and MPH of Ukraine.

To establish the State Medical and Dosimetric Registry of Ukraine and to ensure the long-term automated personal record of victims is one of the priority tasks of "The National Program to Minimize the Consequences of the Chornobyl Catastrophe".

Registry establishment and operation allowed to complete a series of large-scale epidemiological studies of medical consequences of the Chornobyl catastrophe: epidemiology of malignant tumors; epidemiology of non-tumor diseases; the exposed children's health; medical and demographic consequences for population living in contaminated areas. The results are presented in several sections of this monograph.

The SRU and other specialized registries provide DB for epidemiological studies among exposed people. The SRU adequacy and information value are dependent on the amount and completeness of data, quality of data collection, recording and storage. Need for data analysis has been emphasized in UNSCEAR report, UN General Assembly 2000, in the report of Expert Group "Health", UN Chornobyl Forum, 2006, and International Conference "Twenty-five Years after Chornobyl Accident. Safety for the Future", 2011.

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3.2 SPECIAL REGISTRIES

Clinical and Epidemiological Registry

Clinical and Epidemiological Registry (CER) of RCRM is a research subregistry of the State Registry of Ukraine. CER functions in the framework of comprehensive clinical and epidemiological monitoring that is being undertaken since 1992 on the basis of Radiation Registry Polyclinics (RRP) [1].

Over the course of CER operation, an automated medical-information system was created, unique data being collected. This data are unique not only for Ukraine but also for Russia, Belarus and other countries where study of ionizing radiation health effects has been conducted. Herewith, the basis for comprehensive and qualitative assessment of medical and biological consequences of the Chornobyl disaster has been provided [2, 3].

CER is aimed to study, assessment and prognosis of medical and biological consequences of the Chernobyl accident on the basis of long-term health monitoring of victims' contingents, substantiation of measures to improve health monitoring and preventive healthcare measures.

Main tasks of research in the framework of CER are as follows:

- epidemiological analysis and estimated impact of radiation and non-radiation factors on diseases generation among victims in post-accidental period;
- clinical investigations of disease mechanisms in victims of the Chernobyl accident;
- development and improvement of diagnostic techniques, treatment modes, preventive and rehabilitation methods.

A background for complex problems solving is the following — the availability of adequate database size and structure, providing with reliable clinical and dosimetric data, due regard for a set of socio-hygienic, psychological, occupational, environmental, medical, economic and other factors that may affect human health.

To study these problems methods and means of obtaining reliable medical and dosimetric data, software and hardware for mathematical treatment and analysis of CER data, health evaluation and prediction models were developed.

The basic parameters studied within CER system are as follows: non-tumor morbidity, physical and sexual development, mental health, reproductive health, congenital malformations, pattern and pathogenesis of tumor and non-tumor diseases.

The number of survivors examined in Adult Department of RRP, and registered in CER are shown in Table 3.2

Table 3.2. Numerical structure of Clinical and Epidemiological Registry (adults)

Contingents	Registered			With individual doses from external whole-body exposure		
	cohort	men	women	cohort	men	women
Total	21609	11934	9675	7936	4687	3249
Clean-up workers, including	10092	8214	1878	4724	3862	862
– clean-up workers o 1986–1987	8943	7168	1775	4410	3555	845
Evacuees, including	6717	2036	4681	3243	867	2376
– adults at the time of the accident	2809	188	2621	1420	61	1359
– children and adolescents at the moment of the accident	3908	1848	2060	1823	806	1017
Residents of contaminated areas, including	4292	1435	2857	—	—	—
– adults at the time of the accident	2207	645	1562	—	—	—
– children and adolescents at the moment of the accident	2085	790	1295	—	—	—
The rest, including those exposed “in utero” and mixed contingents	508	—	—	—	—	—

Table 3.3. Presents information by primary registration groups of children and adolescents examined in Children's Department of RRP within 1992–2009 and registered in CER.

Table 3.3. Numerical structure of Clinical and Epidemiological Registry (children and adolescents)

Primary registration groups	Number	Total number
Children-evacuees from 30-km zone, including those exposed "in utero" (720 persons)	4354	4354
Children born to the Chernobyl clean-up workers of 1986–1987	2614	2662
Children born to the Chernobyl clean-up workers of 1988	48	
Children born to evacuees from Prypiat and 30-km zone	2079	2079
Children born in zone 2	33	6598
Children born in zone 3	2709	
Children born in zone 4	3856	
Children from Kyiv – the control group, including those exposed "in utero"	957	957
Children born to evacuees from controlled areas	204	204
Total	16854	16854

CER structure was not formed simultaneously. Table 3.4 presents number of patients registered within 1992–2009 and first examined under CER program, as well as total number of examined persons (re-examined ones included) by years.

Table 3.4. Number of patients annually examined under Clinical and Epidemiological Registry program.

Year	CER (Adult Department)		CER (Children's Department)	
	registered (step 1)	examined	registered (step 1)	examined
1992	512	512	705	705
1993	2873	2873	1520	1528
1994	2183	2293	1574	2334
1995	1444	2804	1084	2166
1996	1657	3256	744	2554
1997	1924	3488	712	2559
1998	1302	3660	847	2645
1999	2360	4130	1541	2907
2000	1774	4003	903	2706
2001	1147	4165	879	3028
2002	758	4218	228	2959
2003	562	4304	609	2874
2004	812	4264	1031	2959
2005	672	4527	945	3014
2006	440	4220	1017	3013
2007	367	4124	655	2976
2008	498	4352	959	3013
2009	324	4152	901	3240
Total	21609	65343	16854	47180

Three-quarters of the adult CER size (74.3%) was formed during 1992-2000. People who wished to be registered in CER and to be screened every 2 years did not respond simultaneously and provided incomplete data. That resulted in differing number of examined persons by years. There is also an early dropout from monitoring.

CER structure for children and adolescents in dynamics is variable. As to the persons who are to be followed-up within CER, their medical cards will be automatically transferred to adult Radiation Registry Polyclinic when they reach the age of 18. Though some contingents (evacuees, children and adolescents, exposed "in utero") dropped out of CER, their records are being stored in CER database.

Medical examinations in CER system are unified by standardized protocols. The results obtained are submitted to CER database, both adults and children and adolescents directly from workplace of medical specialist. For this purpose a local computer network for data collecting and processing was set up.

Dosimetric section contains dose-calculation protocols of clean-up workers, evacuees, residents of contaminated areas. Radiation monitoring laboratory estimates body content – caesium and strontium. Socio-sanitary protocols provide data on demographic, social, occupational, behavioural and other characteristics.

Mandatory clinical trials of CER patients include physical examination by specialists, instrumental and laboratory study. In accordance with standardized protocols, adults are examined by neurologist, endocrinologist, cardiologist, gastroenterologist, hematologist, ophthalmologist, pulmonologist, psychiatrist, urologist, dermatologist, gynecologist, otorinolaryngologist; children and adolescents are subjected to additional examination by pediatrician, orthopedic surgeon, cardioreumatologist. In case of necessity, along with the mandatory program, patients undergo advisory examination by surgeon-oncologist, gynecologist-geneticist, dentist; children and adolescents — by pediatrician-geneticist.

Compulsory instrumental and diagnostic study involves ultrasound investigation of abdominal cavity, thyroid, heart, small pelvic, electrocardiography. Electroencephalography, rheoencephalography, reography, spirometry, ultrasound investigation of female genitals, breast, bladder, lymph nodes, vessels of the neck, upper and lower limbs, kidney, prostate; fibrocolonoscopy, X-ray, radiography, audiometry, colposcopy, gastroscopy, bronchoscopy are used if needed.

In the complex of laboratory and diagnostic investigations, general blood analysis, blood biochemistry, general analysis of urine are obligatory. There are additional cytological study of various materials, special analysis of urine, coprogram, bacteriological testing of different materials, hormonal and immunodetection, etc.

If necessary, patients are referred to the Clinic of the Institute of Clinical Radiology of RCRM. Usually 50-70% of children examined in RRP are referred to the Clinic.

The data are being accumulated in CER system provide an opportunity to obtain important information on medical and biological consequences of the Chernobyl accident, they are used in particular to determine mechanisms of non-tumor diseases influenced by radiation and non-radiation factors [4, 5]. Lack of funding, especially concerning devices retrofitting, disposable materials, reagents etc. is a main problem of this unique system for health monitoring of persons affected by the Chernobyl accident.

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3.3 NATIONAL CANCER REGISTRY OF UKRAINE

Reforming of public health in Ukraine is impossible without radical changes in its organization and administration. Implementation of information technologies is an important part of it. Computerized information technologies are the most effective tools for obtaining the reliable information, which serves optimising both health care control as a whole and activity of medical institutions. They also become a significant toolkit for changing the habitual way of analysis and interpreting of data and their quality, a basis of evidence-based medicine as well. A special place in the process of implementing the computerized information technologies in health care system takes creation of registries, which is possible mainly in branches with state system of registration of cases of disease, cancer including.

Creation of the computerized registry of cancer patients in Ukraine was conditioned by the need in reliable information for both scientific research and reasonable decision-making in oncology service. State system of cancer registration, which existed in Ukraine, allowed National Cancer Institute to commence on creating common information medium in oncology in 1989. In 1996 this initiative has received the legislative approval in the Order of Ministry of Public Health of Ukraine from 12.30.1996 No 10 "On Establishment of the National Cancer Registry of Ukraine", and the Order from 11.09.1998 No 318 "On Approval of Registration Card of Patients with Malignant Neoplasm". The main feature of this Registry is data accumulation about all cancer cases in Ukrainian population.

Implementation of modern information technology of National Cancer Registry of Ukraine (NCRU) in epidemiological research in oncology created potentialities for resolving following tasks: computerized registration of detailed personal data of all cancer patients in Ukraine; control on cancer treatment quality and dispensary follow-up. It, also, made easy:

- monitoring the level of cancer incidence and mortality in Ukrainian population; analysis of their dynamics;
- making forecast estimates;
- determination of cancer risk for different population groups;
- exploration of impact of the Chornobyl accident on the Ukrainian population;
- data assistance in cooperative randomised research of clinical oncology;
- cancer survival studies;
- information and analytical support for the authorities of public health and oncology service.

Nowadays NCRU contains personal information of all cancer cases and all cancer patients registered in Ukraine since 2002, but for most of regions these data are available since 1993–1997. At the beginning of 2010, its database contains records of 3 million cancer patients.

Computerized information system of NCRU enables receiving adequate information about dynamics of cancer epidemiological process in Ukrainian population. The system allows analysis of the process on the level of population, cohorts or separate groups of persons depending on administrative territory (regions, districts), age, gender, cancer nosologic form, etc. Further, much attention is paid to assessing the basic health care rates: percentage of cases detected in screening, level of morphological verification of cancer, level of patients that received special treatment, stage of the tumour disease in terms of Tumour – Node – Metastasis (TNM) Classification of Malignant Tumours. It is also possible to study cancer incidence, mortality, prevalence etc. of persons suffered from the Chornobyl accident (liquidators, evacuees, residents of contaminated areas and their children).

NCRU was created as a distributed network of data collection. Central cancer registry (CR) unit is a department of the National Institute of Cancer; it accumulates all personal data from regional cancer registries (RCR). RCR gather information on each cancer case or death, detected in this region (region mainly, but districts CRs also exist). The network of population-based RCRs covers the whole territory of Ukraine. Besides, the computerized hospital-based cancer registries are implemented in 33 oncological dispensaries and centres of Ukraine. They register all cases of hospitalisation of patients with tumors and accumulate information about their diagnoses, treatment and dispensary follow-up that is more detailed than in population-based CRs.

Commonality and uniformity of information technologies of hospital-based and population-based registries and facility developed for data exchange between them are particularly relevant for improving the quality and completeness of cancer data and are a major prerequisite for creating common information medium in Ukrainian oncology. One of the main features of modern registry is a scrupulous quality control of data registered provided by means of information technology. The NCRU procedures, which provide data quality control, are mainly based on recommendations of International Agency for Research of Cancer (IARC) [1, 2]. They include checks of accuracy, consistency and integrity of data, automatic coding of certain data attributes (ICD and ICD-O codes are derived from the site and morphology of the tumour registered), checks for stage of the disease based on the TNM indices registered, checks for correct registration of multiple primary cancers. The procedures of quality control also enable calculation of

some indices that describe data quality, correctness and features of data registration in RCRs. Special attention is paid to avoiding and eliminating duplicate records of the same patient in the RCRs database. To organize the user-friendly input and attendant data quality control, the standardized codifiers having intelligent interface for registration of tumour site, its morphology, types of medical treatment (surgery, chemotherapy, radiation therapy and their combinations), records of treatment courses, etc. are developed, that helps to decrease mistakes in coding of information.

The procedures of automated data linkage are also developed and implemented. This enables exchange of data both between hospital-based and population-based cancer registries, and with any other database that contain personal information. In turn, this opens broad way to cooperative studies, which formerly were impossible or extremely time-consuming to conduct.

Thus, we can state that oncology branch of health care service of Ukraine has functioning on the national level branch-wide cancer registry, which enables good use of present technical and information possibilities to obtain adequate data about epidemiological situation as well as the state of anti-cancer activity in Ukraine. NCRU technology and database are also used for calculation and preparing for publication the annual "Bulletin of the National Cancer Registry: Cancer in Ukraine". The Bulletin is also available on the NCRU website (<http://www.i.com.ua/~ucr>).

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CHAPTER 4 ACUTE RADIATION SYNDROME

Acute radiation syndrome (ARS) because of the accidental radiation exposure at Chernobyl NPP (ChNPP) was diagnosed at first in 237 persons. After the retrospective analysis of case histories in 1989 the clinical and hematological symptoms of ARS have been revealed in 134 patients only, including 28 persons deceased due to severe radiation damages of organs and systems. The rest of 103 patients without signs of radiation bone marrow syndrome (BMS) were supposed to have been exposed to ionizing radiation in doses below 1 Gy. Together with ARS survivors this group of patients with non-confirmed ARS (ARS NC) constituted the cohort of priority follow-up to study probable early and long-term consequences of radiation impact.

As far back as in 1986–1987, when RCRM researchers planned long-term research, they understood that these investigations would resemble the Life Span Study for A-bombing victims in Hiroshima and Nagasaki since 1945. The main tasks for studying negative effects of radiation exposure have to contain the assessment of (1) stochastic consequences (leukemia and cancer development); (2) skin changes after local acute radiation burns; (3) radiation cataract and other eye pathology development and (4) how radiation contribute to somatic diseases occurrence. The last task had to be the most difficult because of the integral test of patient's health status should be done, and wide spectrum of medical investigations should be applied as well as numerous non-radiation disturbing factors are needed to be analyzed.

At the beginning of October 1986, in the list of patients under follow-up there were 91 ARS survivors and 99 persons with ARS NC living in Ukraine (Table 4.1).

The groups of patients did not differ significantly by age at the moment of irradiation. On the contrary, average values of absorbed doses differed significantly ($p < 0.001$).

The absorbed doses of external irradiation were determined in 1986 by analyses of chromosomal aberration in peripheral blood lymphocytes (number of dicentric per 100 cells) [1].

During 25 post-accidental years every time having admission into the RCRM Clinic patients were examined by specialist as therapist, hematologist, cardiologist, gastroenterologist, pulmonologist, ophthalmologist, endocrinologist, neurologist, psychiatrist, dermatologist, urologist and gynecologist (women). Clinical-laboratory investigation included obligatory complex of blood and urine analyses, studying of cellular

and humoral immunity, hormonal status, ultrasound examination (USE) of abdominal and small pelvis organs, as well as thyroid gland and heart. Electrocardiogram (ECG) and electroencephalogram (EEG) recording, X-ray examination, endoscopy of gastro-intestinal tract (GIT), bronchoscopy and other special methods were used for patients' examination as well.

Table 4.1. Age-gender and dosimetric characteristics of ARS survivors and ARS NC patients

Indices	ARS NC	ARS grade 1	ARS grade 2	ARS grade 3	ARS grade 1-3
Gender: f/m	10/89	2/35	1/40	0/12	3/88
Age at irradiation, years					
M±SDa	35.9±10.3	33.2±8.2	38.5±13.8	40.8±16.5	36.6±12.5
min-max	18.4-60.3	17.6-56.3	17.9-79.3	20.4-72.6	17.6-79.3
95% CI	33.8-38.2	30.5-35.8	34.1-42.9	30.4-51.3	34.0-39.2
Absorbed radiation dose, Gy					
n ^c	n=15	n=30	n=37	n=11	n=78
M±SD	0.4±0.3	1.0±0.6	2.4±0.9	4.5±1.4	2.2±1.5
min-max	0.1-1.0	0.1-3.3	0.5-4.9	2.9-7.1	0.1-7.1
95% CI	0.2-0.5	0.8-1.3	2.1-2.7	3.6-5.5	1.9-2.5

a — mean ± standard deviation; b — confidence interval; c — number of patients for whom the absorbed radiation dose was determined

The proceedings of patient's examination have the certificate of conformity to national and international medical standards though certain methods of examination were improved during follow-up period, and exploited laboratory and diagnostic facilities were upgraded with new analogs.

To increase effectiveness and validity of statistical analysis the patients' data from case histories since 1986 were input in computer database, which structure corresponded to chapters and items of special questionnaire that had been worked out by German, Russian and Ukrainian specialists and was oriented on irradiated persons under follow-up [2]. Microsoft Access program was used for this purpose.

Statistical analysis was done by computer program SPSS 16. Entire follow-up period was divided on 5 years stages for analysis. Thus, the 1st stage corresponded to time passed since 26.04.1986 to 25.04.1991, the 2nd stage included the same dates of 1991-1996, the 3rd of 1996-2001, 4th of 2001-2006 and the 5th stage of 2006-2011.

4.1 THE MAIN REASONS OF PATIENTS POST-ACCIDENTAL DEATH

The number of patients naturally reduces during any longitudinal follow-up caused by two reasons as a rule: (1) deaths of cohort members or (2) their dropping out of follow-up, for example, owing to resettlement in another city or refusing from medical examination.

At the end of 1986 RCRM medical staff had medical documentation delivered from hospitals where patients included in the group of primary follow-up were treated right away after irradiation and where ARS was diagnosed for them. At the 1st stage 86 patients of 91 ARS survivors and 93 patients of 99 persons of ARS NC group were examined because non-examined men were living in rural regions or other cities than Kyiv and refused from visit in RCRM Clinic. Several patients died on this stage due to

diseases and accidents. The number of examined and deceased patients on every stage is represented in the Table 4.2.

Table 4.2. Number of patients in follow-up groups and its changing during post-accidental period

Number of patients	Stages*									
	1		2		3		4		5	
	ARS NC	ARS grade 1-3	ARS NC	ARS grade 1-3	ARS NC	ARS grade 1-3	ARS NC	ARS grade 1-3	ARS NC	ARS grade 1-3
examined	93	86	81	84	63	63	54	51	37	32
deceased	3	2	3	9	1	3	8	4	2	4

*The 1st stage corresponds to time passed since 26.04.1986 to 23.04.1991, the 2nd stage includes the same dates of 1991–1996, the 3rd of 1996–2001, 4th of 2001–2006 and the 5th stage of 2006–2011.

Within 25 years 20 patients of ARS NC group, 3 survivors of ARS grade 1 group, and 7 survivors of ARS grade 2 were lost for follow-up. These persons skipped medical examination at three consecutive stages or more. The most of the patients, when they had been called up by phone, refused from examination and treatment because they feel well.

During post-accidental period 39 patients died. All lethal cases could be divided on four classes in dependence on the main reason of death: (1) cardiovascular diseases, (2) oncological and oncohematological pathology, (3) somatic, neurological diseases, and infections, (4) trauma and accidents (Table 4.3).

Table 4.3. The main reasons of death during post-accidental period amongst ARS and ARS NC survivors

Reasons of death	Quantity of deceased amongst				
	ARS NC	ARS grade 1	ARS grade 2	ARS grade 3	total
Cardiovascular diseases	5	3	2	2	12
Malignant blood diseases and solid tumors	8	2	3	2	15
Somatic and neurological diseases, infections	2	1	3	1	7
Trauma, accidents	2	2		1	5
Total	17	8	8	6	39

Cardiovascular and oncological diseases brought 26 (66.4% of all deceased) patients to fatal outcome. More detailed characteristics of these persons and every death case will be submitted below. However, it is a fact that the number of patients have died due to cardiovascular diseases lesser than oncological pathology (ratio 0.9:1). Even if we exclude from the first class three cases of death owing to cerebrovascular disease (AICC) and from the second class five cases because of oncohematological pathology, the ratio will be 1:1. Though, in accordance with WHO publication [3] in 2003 the frequency of death from all cardiovascular diseases consisted of 60% and from cancers 12% amongst men in Ukraine. In 2006 this relation remained the same: 62% and 12% for CIS countries. Looking over WHO statistic since 1986 till the present time, we found that during the last 20–23 years the number of men, who died from cardiovascular diseases, has always exceeded by 4–5 times the number of men, who died from cancer. Such stable indices, which are typical for a country population, let to suppose that the ratio of death cases

will be the same in any restricted cohort (not replenished by the new persons) if outside factors such as ionizing radiation do not change the existing ratio.

Today it is too early to say about any increase of oncological pathology caused by radiation because it is hardly proved by statistics on such small group of follow-up. Equal number of people died from cardiovascular diseases and cancers is typical for highly developed European countries (relatively 35–37% and 30–31% in 2006). However, in Ukraine the morbidity and mortality indices as well as life expectancy, always have differed from European countries of Eur-A group¹, therefore it is not unlikely to suppose the outside reason for so high today's level of deceased from cancer. The further study of lethality dynamics in the group of priority follow-up probably let to come to more definite conclusion.

Somatic diseases ranked the third as a reason of death (2 patients with liver cirrhosis and a patient with lung disease complicated by fatal lung gangrene; all patients survived ARS grade 2) together with neurological diseases (encephalitis with bulbar paralysis, ARS NC group) and infections (lung tuberculosis in ARS NC, ARS grade 1 and ARS grade 3).

Five persons (13%) died as a result of accidents that is corresponded to WHO statistic on CIS [3].

4.2 BLOOD MALIGNANT DISEASES AND SOLID TUMORS

Studying the consequences of Chernobyl accident several authors came to conclusion that during post-accidental period oncological morbidity increased in victims [4] and experimental animals inhabited round the Chernobyl NPP [5]. Other researchers concluded that the accident at the ChNPP did not result in essential increase of cancer development and mortality from malignant tumors correspondingly in irradiated persons in comparison with the rest of population [6]. Such different research results most likely were connected with substantial variation of dose ranges that was typical for ChNPP clean-up staff. There is an opinion that dose 0.1 Gy and higher could lead to statistically significant increase of cancer risk [7, 8], and 0.4 Gy to leukemia increase on 100% between 1988 and 1998 [9]. As far as radiation doses in ARS survivors were higher than these levels it is the topical task to find oncological and oncohematological pathology in this cohort.

The first case of malignant hypoplasia of hemopoiesis was revealed on August 1986 in a patient who was working in the ChNPP zone on May and July. Hematological status was characterized by essential leukopenia and slight anemia. The debut of oncohematological pathology was mistakably assessed as ARS grade 1. However, further disease development eliminated any doubts concerning hematopoietic tissue pathology that was proved by bone marrow analysis.

During following years myelodysplastic syndrome (MDS) developed in two ARS grade 3 and one ARS grade 1 survivors. All cases brought these three patients to death (Table 4.4). One more ARS grade 2 survivor fell ill with myelomonoblastic leukemia in 11.8 years after irradiation and died in spite of treatment.

All patients with oncohematological pathology were male of mean age 46.3 ± 7.5 years at the moment of radiation exposure. At the time of diagnosis they were 54.0 ± 8.1

years old and at the moment of death 55.5 ± 6.8 years old. The latent period since irradiation to the first signs of disease was 9.6 ± 2.1 years. Today the relative number of persons, who fell ill and died from malignant blood diseases, is 2.6% of 190 patients of the group of primary follow-up and 4.4% amongst ARS survivors.

Table 4.4. Cases of malignant blood diseases in ARS survivors and persons of ARS NC group

No	Diagnosis	ARS	Firstly diagnosed	Age (years) at		Outcome
				diagnosis	death	
1	Hypoplasia of hematopoiesis	NC	August 1986	48.6	49.3	died on April 1987
2	MDS	grade 1	September 1986	45.0	50.7	died on May 2002
3	MDS	grade 3	March 1993	52.1	52.2	died on April 1993
4	MDS	grade 3	October 1995	64.6	64.7	died on December 1995
5	Acute myelomonoblastic leukemia	grade 2	February 1998	58.9	60.7	died on December 1998

The first case of malignant tumor was revealed in ARS NC survivor at 1992 (Table 4.5). A subcutaneous, dense, and painful formation having size as a pigeon egg was found on right hip of 34 years old patient. The patient was suggested to visit city oncological center, however due to unknown reason he refused from examination. In a year, 08.04.1993, when this patient was admitted to RCRM hospital, several dense subcutaneous nodes had been found on the right hip. The patient again ignored the visit to oncologist. At the end of 1993 he died. An autopsy revealed sarcoma of soft tissues of the hip.

Table 4.5. Cases of oncological pathology in ARS survivors and persons of ARS NC group

No	Diagnosis	ARS grade	Firstly diagnosed	Age (years) at		Outcome
				diagnosis	death	
1	Sarcoma of hip soft tissues	NC	April 1992	34.1	34.8	died on 1993
2	Cancer of colon	1	March 1997	54.5		operated on June 1997
3	Leiomyosarcoma of shin	NC	August 1998	72.5	75.9	died on 2001
	Cancer of colon		June 1999	73.4		
4	Cancer of throat	NC	July 2000	59.3	59.9	died on February 2001
5	Cancer of thyroid gland	2	November 2000	37.6		operated on November 2000
6	Cancer of thyroid gland	2	December 2000	42.9		operated on January 2001
7	Cancer of kidney	NC	December 2000	44.4		operated on January 2001
8	Cancer of colon	NC	November 2001	63.6	67.5	died on October 2005
9	Cancer of prostate	NC	May 2001	71.7	73.3	died on 2003
10	Cancer of lung	NC	February 2001	46.1		operated on June 2003
11	Neurinoma of lower jaw	2	October 2003	52.7	53.9	died on December 2004
12	Cancer of stomach	NC	May 2004	77.5	78.1	died on January 2006
13	Cancer of stomach	NC	July 2004	65.7	65.9	died on September 2004
14	Basal cell carcinoma of head	1	November 2006	51.9		irradiation
15	Cancer of urinary bladder	2	June 2008	66.8	66.9	died on August 2008
16	Cancer of maxillary sinus	1	January 2009	51.0	52.2	died on April 2010

The second case of oncological pathology, notably sigmoid colon cancer, was diagnosed in 1997. Adenocarcinoma in situ had been revealed during colonoscopy and proved by histological investigation.

It is necessary to note that all following cases of solid tumors either developed without any clinical symptoms and were revealed by chance during routine examination (endoscopy, ultrasound) or were attended with minimum non-specific compliances (so called "syndrome of minor signs"). In the last case, a tumor diagnosis should be attributed to oncologic alarm of physicians. However, the situations took place when patients ignored specialists' opinion and refused from informative diagnostic procedures and treatment. Cancer of stomach with evident clinical symptoms and clear endoscopic picture was found in a patient. There were no contra-indications to surgical intervention but he declined the operation and died in three month after the day of diagnosis.

Totally during 18 years after the first diagnosis of sarcoma 15 cancers were revealed more with different localization: 3 cases — colon, 2 cases — stomach, 2 cases — thyroid gland, each case — throat, lung, kidney, urinary bladder, maxillary sinus, and prostate, as well as neurinoma of lower jaw, and basal cell carcinoma of head. In a patient of ARS NC group cancer of stomach followed leiomyosarcoma of the left shin which was operated and fractionated radiation expose on the shin was applied in a dose of 52 Gy.

In two patients of ARS NC group malignant tumors (stomach and throat cancer) had rapid development. The time since diagnosis to lethal outcome was 3 and 6 months, respectively. A gastroscopy did not reveal any changes of stomach mucous membrane on February 2004 but when this patient was admitted in the hospital in July 2004 clinical and endoscopic picture of cancer was evident. Cancer of throat $T_3N_1M_0$ was diagnosed in the second patient during annual follow-up. The patient had not any complains. An extended surgical intervention could not stop metastases in distant organs.

All patients with cancer and sarcoma were male of 40.1 ± 12.1 years at irradiation. At the moment of firstly diagnosed oncological pathology and death they were 55.8 ± 13.1 and 61.4 ± 13.0 years old, respectively. The latent period from irradiation to the first signs of the disease was 15.7 ± 4.2 years. The relative number of patients with oncological pathology was 8.4%. Amongst all ARS survivors malignant tumors were revealed in 7.7% of patients, and in ARS NC group in 9.1% of persons.

Statistical analysis showed that tumor development did not depend on ARS grade or the fact that a patient survived ARS (Table 4.6).

Table 4.6. Dependence of solid tumors development on radiation factor

Radiation factor	Spearman's rank correlation		χ^2 -test	
	r	p	F	p
ARS grade	-0.07	0.36	2.69	0.44
ARS existing	-0.083	0.28	1.2	0.27

The malignant blood diseases in ARS survivors preceded solid tumors development. They were not characterized by diversity of nosological forms (3 MDS cases, acute myelomonoblastic leukemia and hypoplasia of hemopoiesis) whereas in A-bombing victims in Hiroshima and Nagasaki from 1950 to 1987 different forms of acute and

chronic leukemia, lymphomas (Hodgkin and non-Hodgkin), and multiple myelomas were revealed [10, 11]. The leukemia highest frequency was observed on the 5–8 year after irradiation [10].

Analyzing leukemia data in hibakusha and persons who was medically irradiated Hall [12] came to conclusion that leukemia latent period was 5 years. Other authors concluded it shorter, notably 2–3 years, or longer — 7–10 years [13]. Our data corresponds to the last opinion because four cases of oncohematological pathology excluding the first patient are kept within this term. Hypoplasia of hematopoiesis in the first patient (Table 4.4) developed in unlikely short term after the first contact with ionizing radiation to conclude this disease induced by radiation. Most likely the disease had started earlier but the first clinical signs appeared during the patient's stay in ChNPP zone.

After the Chernobyl accident MDS was diagnosed in 6 patients, including ARS survivors, of 110645 “liquidators” [14]. All these people were working during 1986–1990 in zone of radiation and makes up 46% of all clean-up staff living in Ukraine. According to I. Sekine [15], amongst 93700 victims of A-bombing MDS developed in 13 persons during 50 years that is absolutely and relatively higher than in ChNPP accident victims. However the duration of observation period in Chernobyl victims is twice lesser than in Hiroshima and Nagasaki inhabitants. Today no one argument was submitted to prove MDS dependence from radiation.

Cancers developed in “liquidators” during the first years after the accident could be the result of promotion not induction of existing tumors. The maximal frequency of radiation induced cancer should be expected in 15–25 years after irradiation. The first case of cancer in survivor of ARS grade I was revealed in 11 years after the radiation impact. This fact together with following cases of malignant tumors development proves Kato et al. [16] opinion that minimal latent period for solid tumors is 10 years and more. After this time a frequency of cancer will increase.

Cancers are the nonthreshold radiation effect. After short-term total body irradiation with low energy transfer in dose up to 0.1 Sv the risk of cancer death increases by 0.5–1.4% in excess of existing level, and in dose 1 Sv it consists of 9% for male and 13% for female. On the basis of this data a higher number of tumors should be expected in ARS survivors in comparison with persons, who have been exposed to radiation in doses below 1 Gy. However, in our research cancer was diagnosed insignificantly more frequent (9.1%) in patients of ARS NC group in comparison with ARS survivors (7.7%). No difference was revealed in frequency of oncological pathology between ARS grade 1–3 survivors.

Amongst all cases of oncological pathology in ARS survivors and patients with dose of irradiation less than 1 Gy it is impossible to determine what tumor is induced by radiation or spontaneously developed. Patients' groups are too small for applying epidemiological analysis. Data of studying Hiroshima and Nagasaki A-bombing victims showed that during 1950–1990 from the total number of 86572 people in the cohort of “life span study” 7578 patients died of solid tumors from which 334 persons (4.4%) could be attributed to radiation. Evidently this data is not possible to extrapolate on relatively small group of ARS survivors together with ARS NC group.

In ARS and ARS NC survivors cancer of colon (3 cases), stomach (2 cases) and thyroid gland (2 cases) occurred more frequent. Based on follow-up results (1958–1987)

on A-bombing survivors it was revealed the significant risk excess for cancer development and death for the first two types of tumor. The same conclusion concerns cancer of lung and urinary bladder. It was found the significant excess of skin cancer (not melanoma) in Japan, however their lethality did not exceed population level. Cancer of throat, prostate and kidney was not connected with radiation impact.

Sarcoma is a malignant tumor of tissues originated from mesenchyme, whereas cancer appears in epithelial tissue. No one research of A-bombing victims in Hiroshima and Nagasaki described increase of sarcoma appearance after radiation exposure. In our research two sarcoma cases were diagnosed in followed-up patients what is not enough for statistical analysis.

Mean age of ARS and ARS NC survivors was 56.2 ± 12.2 years at diagnosis of malignant blood diseases and tumors, moreover 4 patients belonged to young and mature age group (20–44 years), 9 ones were middle age persons (45–59 years) and 8 ones reached old age (60 and more years). The lifetime of 14 people who died from onco-hematological pathology and tumors was 59.3 ± 11.3 years and in 8 patients it was less than life expectancy for male in Ukraine, which in 2001–2003 was 62.3 year, in 2005 decreased to 61.6 and in 2008 “elevated” to 62.3 year as WHO had reported [3].

The fact that more than a half of the patients, who had died, did not reach this extremely low life expectancy in Ukraine (for example, this index in Eur-A was 77.8 years for male in 2008) let to consider the oncological situation for ARS survivors and persons with radiation doses lower 1 Gy as very alarming. A way out from this situation is to diagnose tumors at the early stage of their development and to apply radical cure. All neoplasm cases were diagnosed in the cohort of priority follow-up by routine methods of examination (blood test, X-ray examination, USE, endoscopy etc.), which are used in RCRM Clinic.

Our research work has showed that malignant tumors appeared in exposed people against the background of chronic non-specific diseases of GIT, hepatobiliary system, and lungs, which did not threaten patient's life but as any pathological state increased the probability of cell mutagenesis. Therefore, the treatment of chronic diseases is one more way to decrease a risk of malignant tumor appearance.

4.3 SKIN STATUS DURING FOLLOW-UP IN PATIENTS WHO HAD ACUTE RADIATION SKIN LESIONS

There were 38 patients under RCRM follow-up for whom local radiation skin lesions were diagnosed in 1986, including 5 patients of ARS NC group, 8 of ARS grade 1, 15 of ARS grade 2 and 10 of ARS grade 3. The main damaging agents consisted of β - and γ - β -emitting radionuclides, which fell on skin together with dust particles and combustion products of 4th reactor constructions and building as well. Patients' distribution in dependence on it represented in Table 4.7.

Local radiation damages of grade 1 were found in 29 patients, of grade 2 in 14 and grade 3 in 4 patients. Amongst all patients with radiation burns 147 zones of skin damages were revealed, including 83 regions with lesion of grade 1, 51 of grade 2 and 13 of grade 3. The most often local skin damages localized on lower extremities (30 patients), including shins frontal (23 persons) and foot back (10 persons) surfaces. Ra-

diation damages of upper extremities (19 patients) notably hands (17 patients) ranked second followed by lesions of face and neck (12 patients), chest frontal surface, back and buttocks (11 patients).

Table 4.7. Distribution of patients in dependence on severity of radiation skin lesion

Grade of radiation skin lesion severity	Patient's groups			
	ARS NC	ARS grade 1	ARS grade 2	ARS grade 3
1	3	6	12	2
2	2	1	1	2
3				1
1 and 2		1	2	2
2 and 3				2
1, 2 and 3				1

Local radiation lesions grade 1 and 2 resulted in complete recovery of skin during the first months after irradiation whereas epithelium of damaged surface recovered only in one of 4 patients with skin burns of grade 3. The second patients had erosion on the place of skin burns, the third had unrecovered ulcer, and the fourth could not overcome necrosis of the left lower extremity. It was amputated till the upper third part of the shin in 10 months after irradiation.

On the first and further follow-up stages it was not revealed any signs of skin changes at the places of acute radiation burns in 15 (38.5%) patients (3 of ARS NC, 5 of ARS grade 1, 5 of ARS grade 2 and 2 of ARS grade 3). Amongst these patients acute radiation skin lesions grade 1 had been diagnosed in 14 persons and grade 2-in one patient. Acute radiation skin lesions grade 1 completely recovered in 4 patients of 6 ones who had skin regions with different severity of damage. The same situation concerned 2 patients with skin lesions grade 2. During post-accidental period the rest of patients had following pathological skin changes at the places of former radiation skin burns.

- Atrophy. Skin became dry and thin with well visible veins; skin turgor decreased, peeling appeared (Fig. 4.1). Histological research showed thin epidermis, flatness of epidermodermal boundary, rarefaction of elastic fibers, atrophy of skin appendages.
- Hyper- and hypopigmentation that caused by changes of melanin content in cells of epidermis basal layer (Fig. 4.2).
- Peeling. It is the result of cells rejection in corneal layer.
- Telangiectasia. It is the steady non-inflammatory dilation of superficial capillaries (Fig. 4.3, 4.4).
- Hyperkeratosis is the thickening of epidermis corneal layer (Fig. 4.5).
- Erosions are the surface skin defects within epidermis (Fig. 4.6), ulcers are the deep damages of epidermis, derma, and sometimes of underlying tissues (Fig. 4.7).

During 25 years period all aforementioned skin changes have been developed and progressed in three patients with radiation grade 3 local skin lesions (Table 4.8). The frequency of pathological skin changes was significantly lesser in persons with grade 1–2 radiation burns.



Fig. 4.1. Patient E, case history No 1579, ARS grade 2 and radiation skin damages of shins grade 1 (regions of thin skin are visible)



Fig. 4.3. The same patient as on the Fig. 4.1. (evidence of hyperkeratosis on the foot plantar surface)



Fig. 4.2. Patient K, case history No 545, ARS grade 3 and local radiation skin damages of shin grade 3 (on the frontal surface of both shins there are visible regions of hyper- and hypopigmentation, and thin skin)



Fig. 4.3. Patient N, case history No 548, ARS grade 3 and radiation skin damage of shin and hip grade 2

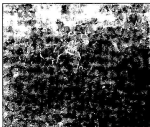


Fig. 4.6. Patient K, case history No 878, ARS grade 2 and radiation skin damages grade 2 (there is the erosion 3-5 mm under apthalization, too of hypo- and hyperpigmentation)

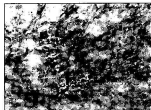


Fig. 4.4. Focus of hip lateral surface of the same patient as on the Fig. 4.3.



Fig. 4.7. Patient S, case history No 2684/2770, ARS grade 3. Radiation skin damages of shins grade 3 (on the lateral surface of right shin there are two ulcerous defects having linear 3x1 cm and oval 5x4 cm shape; both ulcers have undermined edges and are filled with paravenous contents; there are bloody crust on the edge of greater ulcer)

Table 4.8. Pathological skin changes in the follow-up period after radiation exposure in patients with radiation burns

Grade of radiation skin lesions	Number of patients with pathological changes of skin						
	atrophy	hyperpigmentation	hypopigmentation	peeling	telangiectasia	hyperkeratosis	ulcers
1 (n=10)	10	7	4	7	5		
2 (n=13)	12	10	7	9	11	4	5
3 (n=3)	3	3	3	3	3	3	3

n – number of patients with radiation burns of the same grade

All pathological skin changes had high and significant correlation with grades of radiation skin lesions that was showed by χ^2 -square test and Spearman's rank correlation (Table 4.9). Telangiectasia, hyperkeratosis and ulcers were significantly correlated with grades of ARS as well.

Table 4.9. Correlation of skin pathological changes with grades of local and total radiation exposure

Skin pathological changes	Grade of radiation skin lesion				Grade of ARS			
	χ^2 -test		Spearman's correlation		χ^2 -test		Spearman's correlation	
	F	p	r	p	F	p	r	p
Ulcers	21.15	<0.001	0.66	<0.001	17.01	<0.01	0.56	<0.001
Hyperkeratosis	21.43	<0.001	0.62	<0.001	21.00	<0.001	0.58	<0.001
Telangiectasia	15.33	<0.001	0.62	<0.001	12.26	<0.05	0.32	<0.05
Atrophy	9.98	<0.01	0.49	<0.01	5.63	>0.05	0.33	<0.05
Hyperpigmentation	10.34	<0.01	0.49	<0.01	4.55	>0.05	0.32	<0.05
Hypopigmentation	11.13	<0.01	0.49	<0.01	8.96	>0.05	0.42	<0.01
Peeling	7.34	<0.05	0.41	<0.01	7.00	>0.05	0.25	>0.05

The analysis of latent period mean values for such pathological skin changes as atrophy, hyper- and hypopigmentation, hyperkeratosis and ulcers showed that it was shorter if the grade of radiation skin lesion had been higher (Table 4.10). Peeling and partly telangiectasia were the exclusion from this rule because of they developed rapidly in patients with radiation burns grade 1 in comparison with grade 2. Individual meanings of latent period of various pathological skin changes differed greatly therefore the value of standard deviation even exceeded the mean in any Table cells. However in spite of these sample peculiarities the significant difference in Student's t-test was found between various indices.

The skin changes during follow-up period after local radiation exposure are conditional on pathological processes, which had been developing in epidermis and derma during acute phase of radiation burn. The most radiosensitive skin structures are the basal layer epithelium, sebaceous glands and hair follicles. The deepness of skin lesions increases with elevation of dose absorbed by tissue and recovery of damaged skin elements becomes defective. Therefore during the follow-up the effect of doses, which caused acute radiation dermatitis (radiation burn grade 1), are exhibited by decrease of epidermis thickness up to several cellular layers, papillary layer flattening, appearance of melanin foci in superficial derma. A dose increase leads to loss of hair follicles,

dysfunction of oil and sudoriferous glands, appearance of telangiectasia and fibrosis of derma with underlying tissues that caused a decrease of capillary and blood small vessels quantity, and obliteration of arteries. The impairment of regional capillary circulation is a precondition to development of trophic ulcers during follow-up period.

Table 4.10. Latent period mean values (M±SD, years) of skin pathological changes during follow-up period after irradiation in patients with radiation burns

Grade of radiation skin lesions	Latent period mean values (M±SD, years) in patients with pathological changes of skin						
	atrophy	hyperpigmentation	hypopigmentation	peeling	telangiectasia	hyperkeratosis	ulcers
1 n=10	5.8±4.3	6.9±5.1	4.4±5.7	2.6±3.1	4.7±3.3		
2 n=13	4.2±3.8	3.6±4.2	2.9±2.2	1.9±1.5	6.6±4.8	6.9±3.3	5.6±3.8
3 n=3	0.8±0.2	1.1±0.5	1.1±0.5	5.1±3.6	1.5±0.8	5.1±4.4	4.4±6.4
ANOVA P	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
F-TEST	P ₁₋₂	>0.05	>0.05	>0.05	>0.05		
	P ₁₋₃	<0.01	>0.05	<0.01	>0.05		
	P ₂₋₃	<0.01	>0.05	<0.01	>0.05	<0.01	>0.05

Our researches showed that after mild local skin radiation lesion as dry epidermitis many but not all pathological skin changes of epidermis and derma could be revealed in patients. So we observed dermoepidermal atrophy, telangiectasia, pigmentation disorders, but did not detect hyperkeratosis, skin erosions and ulcers, which are typical for radiation burns grade 1–2.

Chronic atrophic changes of skin and ulcers needs to be under medical control because they are the pre-cancer states. It was found that after radiation exposure of skin malignant tumors developed more often on altered skin (fibrosis, telangiectasia, late radiation ulcers, and keratosis).

During 25 years after the Chernobyl accident no one tumor on the places of former radiation skin burns was revealed. Only one malignant neoplasm notably basaloma developed on non-injured part of head in ARS grade 1 survivor. Nevertheless all patients with late radiation skin lesions were included in the subgroup of high oncological risk.

4.4 CHANGES OF EYE LENSES

During the post-accidental period radiation (posterior subcapsular) cataract was diagnosed in 24 patients and involutional cataract in 118 ones (Table 4.11). In order to discriminate these types of cataracts we use criterion described by Choshi [17]. Combination of radiation and involutional cataracts was revealed in 12 ARS survivors and a patient of ARS NC.

Radiation cataracts significantly depended on ARS grade whereas a frequency of involutional lens opacification didn't differ in patients of ARS NC and ARS grade 1. The lesser frequency of involutional cataracts in ARS grade 2–3 survivors in comparison with other patients could be explained by the fact of more radiation cataract cases. In all groups the radiation cataract latent period was shorter than involutional ones (Table 4.12). The minimal latent period mean value for radiation and involutional cataracts was

revealed in ARS grade 3 survivors, however any differences between group of ARS survivors and patients of ARS NC group were insignificant.

Table 4.11. Frequency of radiation and involutional cataract in ARS grade 1-3 and ARS NC survivors

ARS	Radiation (abs. /%)	Involutional (abs. /%)
NC (n=74) ^a	3 / 4.1	61 / 82.4
grade 1 (n=34)	3 / 8.8	28 / 82.4
grade 2 (n=35)	8 / 22.9	24 / 68.6
grade 3 (n=11)	10 / 90.9	5 / 45.5
χ^2 -test	F 66.7	8.18
	p <0.001	>0.05

a — total number of followed up patients

Table 4.12. Latent period (M $\pm$ SD years) of radiation and involutional cataracts

ARS	Latent period (M $\pm$ SD years) of cataract	
	radiation	involutional
NC	7.2 $\pm$ 4.1	10.4 $\pm$ 4.1
grade 1	9.9 $\pm$ 5.2	11.5 $\pm$ 3.7
grade 2	8.1 $\pm$ 3.3	10.5 $\pm$ 4.6
grade 3	5.1 $\pm$ 3.4	9.0 $\pm$ 5.0

During the first 5 years of follow-up the radiation cataract had been diagnosed in 10 patients, whereas the rest 14 cataracts appeared between the 5th and the 15th post-accidental years (Fig. 4.8). There was not revealed any radiation induced lens opacities on the 4th and 5th stage of follow-up. This fact lets us to suppose that radiation cataract develops not later the first 15 years after irradiation. The appearance of lens opacification under the back capsule later this term should be considered as cases of spontaneous not radiation cataract.

Amongst all radiation cataract cases only minimal lens changes in 21 patients were revealed that consisted in dot opacities and single vacuoles. These changes corresponded to the 1st stage of cataract development [17]. The rest three ARS grade 3 survivors demonstrated cataract of 2nd, 3rd and 4th stages. During 25 years radiation cataracts had progressed in one patient of ARS NC group, three ARS grade 2 and three ARS grade 3 survivors (Fig. 4.9).

According to opinion of some authors [18] a dose of ex-

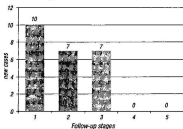


Fig. 4.8. Appearance of the new radiation cataracts on the follow-up stages

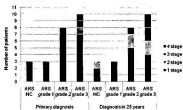


Fig. 4.9. Radiation cataracts progressing during follow-up in ARS survivors and patients of ARS NC group

ternal irradiation has to be not less than 4 Sv to cause the radiation cataract. However another researchers consider that minimal dose for cataract development should be 0.7–0.75 Gy [19] or less [20]. In our research the posterior subcapsular cataracts were diagnosed in persons with external radiation dose below 1 Gy.

According to traditional conceptions about radiation cataracts they are the deterministic effects of irradiation and probability of their appearance approximates to 100% if dose threshold was exceeded. ARS is the classical example of deterministic effect. It develops in every person if dose of external irradiation exceeds 1 Gy. The second distinctive feature of these effects is the increase of its severity with increment of above threshold dose. Long-term examination of ARS survivors had shown that radiation cataract did not develop in the most of patients whose radiation doses exceeded the known cataract threshold. The high severity cataract had been diagnosed only in several ARS grade 3 survivors during prolonged follow-up. Cataract afterripening was also found in ARS grade 2 survivors and patients of ARS NC group. We excluded from the analysis those patients having local radiation skin damage of face, neck and head which probably could influence on cataract progressing. We also did not find that radiation cataract development correlates with radiation burns of such location (χ^2 -test: $F=1.212$, $p>0.05$, Spearman's rank correlation $r=0.085$, $p>0.05$).

Therefore our analysis showed that radiation cataract does not fit in with classical features of deterministic effects (Table 4.13) but much better corresponds to stochastic radiation effects. If we accept this conception as basis it becomes clear that absence of dose threshold causes cataract even in persons irradiated by small dose. Here is why Fedirko [21] found typical radiation cataract signs in clean-up staff, who had been exposed to radiation in dose near 0.02 Gy, but he considered this dose level as cataract threshold on the assumption of classical idea on deterministic effects. Radiation cataract progressing was observed more often in ARS grade 3 survivors and this fact does not allow without any doubts to think radiation induced lens opacification as stochastic consequence. Perhaps the following examination of these patients together with analysis of all non-radiation factors influenced on the lens can answer what kind of phenomenon radiation cataract is.

Table 4.13. Comparison of factors that determine radiation cataract property to stochastic and deterministic effects of radiation

Effect features	Expected consequences	Effect approval
Deterministic		
Threshold availability	Cataract develops in all patients if a threshold is exceeded	No
The effect severity increase with dose increment	Higher dose stipulates more severe cataract development	Partially
Stochastic		
Absence of a threshold. Probability of the effect increase with dose increment	Cataract develops in persons irradiated in minimal dose and high dose as well but in the last case more frequently	Yes
Severity of the effect does not depend on dose	Intensity of lens opacification is similar in persons irradiated in different doses	Partially

During 25 years of follow-up involutional cataracts were revealed in 65.8% of examined patients. Puchkovskaya et al. [22] consider that under ordinary conditions a senile cataract begin to develop after 45 years and in persons older than 65 years its frequency increases from 7% to 60%. In our researches the relative number of patients with cataracts were essentially higher than aforementioned authors reported and 46.4% of patients were young and mature age. This fact let to suppose a predominant contribution of radiation in involutional cataract forming. As far back as Chernobyl catastrophe it was reported more early and frequent senile lens changes in employers who have been working under conditions of fixed irradiation that did not exceeded limits for acceptable doses [23]. In favor of radiation influence on lens changes the fact says that in Chernobyl NPP clean-up staff the frequency of involutional cataracts was significantly greater but in group exposed to radiation in higher doses. It is the opinion that as a result of radiation impact premature aging applies to eyes.

When we assess the contribution of ionizing radiation in lens pathology it is necessary to take in account another risk factors for involutional cataract development. Thus research data of Filatov's Institution of Eye Diseases and Tissue Therapy of National Academy of Medical Sciences of Ukraine showed that frequency rate and terms of involutional cataracts are closely connected with such widespread somatic pathology in patients as hypertensive heart disease (HHD) of 2–3 stages, liver and GIT diseases. In presence of these diseases the cataract appears 10 years earlier. Our investigations showed that every patient under follow-up suffered with these somatic diseases, which could also influence on age-related lens opacities.

Thus the data of other researchers and our investigations showed that joint influence of radiation and non-radiation factors can result in the increase of involution cataract frequency rate during long-term period after radiation exposure in ARS survivors and persons who had undergone to irradiation in doses below 1 Gy.

4.5 CHANGES OF HEMATOPOIESIS BY PERIPHERAL BLOOD COUNT

In the post-accidental period ARS survivors and patients of ARS NC showed various hematological syndromes generally connected with decrease of mature peripheral blood cells number. Erythrocytopenia was the most frequent (57.8% of all cases amongst patients), then lymphocytopenia (40%), thrombocytopenia (32.4%), and granulocytopenia (24.9%) followed. In order to study these syndromes correlation with radiation exposure we changed approaches to assessment of cytopenia in comparison with those that was used before and reflected in several publications [24, 25]. First, the patients of ARS NC group were excluded from follow-up if they had been examined on the initial stage only but not later. Second, we excluded from statistical analysis those patients with transitory cytopenia if the last one had a duration several days only and never repeated.

During all follow-up period the cytopenia frequency in ARS survivors was higher than in patients of ARS NC group (Fig. 4.10).

During all five stages of follow-up it was not revealed by t-test the significant difference in cytopenia frequency amongst patients who survived ARS of grade 1, 2 and 3 but on the 1st stage the frequency rate of aforementioned hematological syn-

dromes was higher in ARS survivors in comparison with ARS NC patients (Fig. 4.11) and these difference was significant according to χ^2 -test excluding granulocytopenia (Table 4.14).

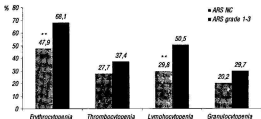


Fig. 4.10. Frequency rate of hematological syndromes during follow-up period in ARS survivors and patients of ARS NC group (** — significant difference, $p < 0.01$)

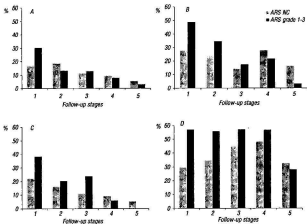


Fig. 4.11. Frequency of granulocytopenia (A), lymphocytopenia (B), thrombocytopenia (C) and erythrocytopenia (D) on the follow-up stages in ARS survivors and ARS NC patients. A —

Table 4.14. Correlation (by χ^2 -test) of radiation factor and hematological syndromes, which were revealed during the first five years after irradiation in examined cohort

Hematological syndromes	ARS grade		ARS presence or absence	
	F	p	F	p
Granulocytopenia	3.0	>0.05	2.3	>0.05
Lymphocytopenia	12.7	<0.01	6.8	<0.01
Thrombocytopenia	14.1	<0.01	4.4	<0.05
Erythrocytopenia	13.9	<0.01	10.1	<0.01

Note: for 2×2 tables the data is represented with Yates' correction for continuity

After culminating the cytopenia frequency level on the 1st stage of follow-up it was found a tendency to frequency decrease during subsequent years. On the 5th stage the frequency of all hematological syndromes was significantly lower than on the 1st stage in ARS survivors. The same situation was in patients of ARS NC group excluding erythrocytopenia and lymphocytopenia (Table 4.15).

Table 4.15. Comparison of frequency rate (%) of hematological syndromes in ARS survivors and ARS NC patients on the first and the last follow-up stages

Hematological syndrome	1 st stage (1985–1991)		p1	5 th stage (2006–2011)		p5	pNC	pARS
	ARS NC	ARS grade 1–3		ARS NC	ARS grade 1–3			
Granulocytopenia	16.5	30.2	<0.05	5.4	3.1	>0.05	<0.05	<0.001
Lymphocytopenia	27.5	48.6	<0.01	16.5	3.1	>0.05	>0.05	<0.001
Thrombocytopenia	22.0	38.4	<0.05	5.4	0	>0.05	<0.01	<0.001
Erythrocytopenia	29.7	57.0	<0.001	32.4	28.1	>0.05	>0.05	<0.01

Note: p1 – significance of difference between ARS NC and ARS grade 1–3 on the 1st stage

p5 – significance of difference between ARS NC and ARS grade 1–3 on the 5th stage

pNC – significance of difference in ARS NC between the 1st and the 5th stages

pARS – significance of difference in ARS grade 1–3 between the 1st and the 5th stages

The dynamics of erythrocytopenia frequency differed from other hematological syndromes both in ARS survivors and ARS NC patients. Amongst the first ones the relative number of patients with this syndromes was sufficiently constant (56–57.1%) during 20 years and decreased on the 5th stage. In ARS NC group the erythrocytopenia frequency increased from the 1st to the 4th stage (from 29.7 to 48.1%) and decreased on the 5th stage but remaining above the initial level on 2.7%.

Transitory short-time and prolonged (during several years) cytopenia in fact is inevitable consequences of middle and high doses irradiation of man that can bring to ARS. It was described by Guskova and Bayisogolov [26] as a result of reparation processes finishing in hematopoietic system, which has been taking place during long time. Leukopenia, thrombocytopenia and anemia of long time duration were revealed in A-bombing Japanese victims [27] and sufferers as a result of various accidents [28, 29]. Prolonged cytopenia was also observed in clean-up staff of ChNPP whose radiation doses were lower than in several ARS NC patients.

Bone marrow investigation in patients, who had survived ARS and demonstrated cytopenia in the long-term period, revealed such changes as focal hypo- and hyperplasia

of different severity together with damage of marrow vascular bed and stroma sclerosis. Transient cytopenia in the peripheral blood continued during several years [30].

Bebeshko et al. [30] studied the state of hematopoiesis in patients, who survived ARS in a result of the accident at ChNPP, and analyzed scientific publications devoted to this field of knowledge. They came to conclusion that depending from proportion of foci with hypoplasia and active regeneration in bone marrow the following ways of hematopoiesis recovery can exist: (1) complete compensation with normalization of peripheral blood cell quantity; (2) impairment of hematopoiesis with residual cytopenia; (3) reticular-plasmatic proliferation with cytopenia or cytopenia phase in the development of hematological proliferative disease. The second choice is more often and has in its own basis the post-radiation deficit of stem cells that resulted in the decrease of mature cells content of erythroid and myeloid lineages. In 10 years after the accident at ChNPP clean-up staff having steady changes of peripheral blood count demonstrated in bone marrow dysplasia of granulocytic elements, hypocellularity, disorders of circulation and damage of vascular wall, edema of stroma, and substitution of hemopoietic tissue by fibroreticular fibers [30].

Submitted data proves the cytopenic state in persons, who exposed to ionizing radiation impact, including ARS survivors, and also explains it nature. Transitory cytopenia does not agree with classic frames for stochastic and deterministic radiation effects but its relationship with the last ones is confirmed by direct correlation of the all hematological syndromes frequency rate, excluding granulocytopenia, with ARS grade and ARS occurrence as well. We consider cytopenia as "the effects of uncompleted recovery" in bone marrow after radiation exposure. Since radiation BMS is a typical deterministic effect, so its own distinctive features have continued some time during recovery period.

4.6 SOMATIC DISEASES DURING LONG-TERM PERIOD AFTER THE IRRADIATION

At the moment of irradiation, the majority of people owned to cohort of priority follow-up were healthy persons in fact. According to their professional membership, more than 80 per cent of patients were ChNPP employers and guards, builders, fire-fighters, and police officers. The ChNPP guards, firefighters and police officers actually served in military force whose staff should be healthy what was proved by annual medical commission. ChNPP employers also had their regular medical examination. However, the analysis of medical documents had shown that diseases of internal organ and systems were revealed before the accident in several patients of priority follow-up cohort (Tables 4.16 and 4.17). Diseases of GIT and hepatobiliary system were the most frequent pathology amongst ARS grade 1–3 survivors and ARS NC patients. There were chronic inflammatory diseases of stomach, duodenum and gall bladder, as well as virus B hepatitis, which had been diagnosed in six ARS survivors and nine ARS NC patients. Diseases of cardiovascular, bronchopulmonary, endocrine and urogenital systems had been diagnosed rarely whereas lens pathology in nobody. One convalescent of ARS grade 2 in 1979 suffered from arachnoiditis and a survivor of ARS grade 3 had the cerebral contusion in his 17 years old.

Table 4.16. Frequency (%) of various organ and systems diseases in ARS survivors before the irradiation and its growth during follow-up stages

Diseases of	Before the accident	Growth					
		In					within 25 years
		5 years	10 years	15 years	20 years	25 years	
Cardiovascular system	5.5	47.8	9.6	8.4	17.4	0.0	89.7
Bronchopulmonary system	7.7	12.6	8.8	28.1	25.3	5.8	88.3
Endocrine system	1.1	16.1	16.7	29.2	22.4	3.6	80.1
Urogenital system	4.4	28.6	15.6	11.6	9.9	23.9	94.0
Nervous system	5.5	78.9	13.7	1.9	0.0	0.0	100
Hepatobiliary system	14.3	56.8	14.5	7.2	7.2	0.0	100
GIT	9.9	68.3	10.6	6.9	2.1	2.2	100
Eye	0	44.6	35.2	17.3	2.9	0.0	100

Table 4.17. Frequency (%) of various organ and systems diseases in ARS NC patients before the irradiation and its growth during follow-up stages

Diseases of	Before the accident	Growth					
		In					within 25 years
		5 years	10 years	15 years	20 years	25 years	
Cardiovascular system	6.1	62.0	7.8	3.6	7.9	1.6	89.0
Bronchopulmonary system	3.0	17.9	13.5	15.5	25.5	0.0	75.4
Endocrine system	1.0	5.6	6.3	11.8	45.5	14.9	85.1
Urogenital system	3.0	29.0	21.8	18.6	10.3	5.8	88.3
Nervous system	0.0	82.8	10.7	3.9	1.3	0.0	98.7
Hepatobiliary system	16.2	61.6	8.7	8.1	2.7	2.7	100
GIT	27.3	54.7	4.3	1.8	4.0	2.0	94.1
Eye	0.0	48.4	27.6	15.1	6.4	2.5	100

During analysis of different organ and system diseases several peculiarities of their development were found. The first was that during the initial five years after the irradiation ARS grade 1–3 and ARS NC survivors gave the essential rise of diseases of cardiovascular, nervous and hepatobiliary systems, GIT and eye (Table 4.16, 4.17 and Fig. 4.12). During subsequent years, this increase of frequency growth became slower. At initial follow-up stage the diseases of bronchopulmonary, endocrine and urogenital systems occurred infrequently but at the every following stage their relative quota steadily rose and in 25 years was the same as diseases of cardiovascular, nervous and other systems notably 85–100%. So high level of prevalence of different organ and systems pathology was the second peculiarity of health status in this cohort of the Chernobyl accident victims. It essentially exceeded corresponding population indices. In 25 years after irradiation the frequency of all organ and systems diseases, excluding hepatobiliary system and eye, was higher in ARS survivors in comparison with patients of ARS NC. However, these differences were insignificant under t-test.

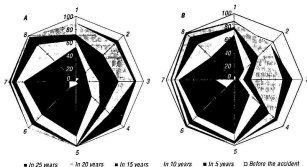


Fig. 4.12. The frequency rate of different organ and systems diseases that developed during long-term period in ARS survivors (A) and patients of ARS NC (B) (1 – cardiovascular, 2 – bronchopulmonary, 3 – endocrine, 4 – argentinal, 5 – nervous, 6 – hepatobiliary, 7 – GIT, and 8 – eye)

4.7 CARDIOVASCULAR SYSTEM

Before the accident, different diseases of cardiovascular system were revealed in six patients of ARS NC and five ARS survivors. Amongst ARS NC patients two ones had diagnosis of rheumatic carditis with heart valves damages in their childhood, three persons suffered from HHD (I10, I11, according to ICD-10) and one patient from IHD (I20.1, I25.0–I25.2, I25.6). In the group of ARS survivors four patients suffered from IHD, and one man had acute myocardial infarction ten years before the accident at that, whereas in another one varicose veins of lower extremities was diagnosed. These people were excluded from the statistical analysis of radiation factors influence on cardiovascular diseases development.

During the first five years after irradiation, such diseases as HHD and neurovascular dystonia (NVD) dominated in all groups (Fig. 4.13). The NVD frequency rose with increase of radiation damage severity, in other words, from patients of ARS NC to ARS grade 2 and 3 (χ^2 -test, $p=0.042$), whereas HHD frequency decreased ($p=0.167$). This phenomenon could be explained by an occurrence in a patient the same pathological process — hypertension. NVD was diagnosed in a case of short-time transitory BP boost (very often it had a characteristic of sympathoadrenal crisis) that decreased to normal level usually without constant hypotensive therapy. If AH had a stable character, what is demanded the treatment with drugs as β -adrenoreceptor blocking agent, diuretics, and reserpine comprising medicaments used in that years, it was considered HHD developed in a patient but not NVD.

Therefore, if we assessed the total number of patients with AH (NVD and HHD) on the 1st follow-up stage its frequency in ARS NC group was 74.2% and in ARS grade 1, 2 and 3, 71.1, 83.8 $\approx$ 81.8%, respectively. It is seen that the dependence of the disease

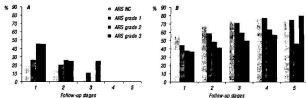


Fig. 4.13. Frequency of neurovascular dystonia (A) and hypertensive heart disease (B) in ARS survivors and patients of ARS NC on different follow-up stages

occurrence from radiation exposure disappeared. The same situation was observed on the 2nd and 3rd stages. The HHD frequency increased from 1st to 5th stages, and NVD frequency decreased, respectively, and this pathological state was not revealed in the late terms of follow-up.

On whole stages HHD frequency in ARS NC patients was higher in comparison with ARS survivors (Fig. 4.13, B). That estimate the significance of these difference during 25 years period we used the Kaplan-Meier survival analysis and logrank criterion (Fig. 4.14). In 2 years after irradiation and later the quota of patients with HHD was higher in ARS NC group at every timeline segment. The median of survival rate, that is duration of period when the half of group population did not fall in illness, was 5.8 years for ARS NC group, and 7.9 years for ARS survivors. Nevertheless, logrank test showed that this difference was insignificant ($p=0.166$).

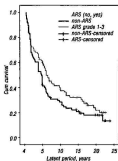


Fig. 4.14. Survival curve of HHD (in our case it indicates the term "not fallen ill with HHD") in ARS grade 1-3 survivors and patients of ARS NC (non-ARS on the picture) during 25 years of medical examination

IHD ranked third according to frequency of occurrence after HHD and NVD. At every stage the relative number of patients with IHD was higher in ARS NC group than in ARS grade 1-3. During 25 years after the accident at ChNPP the IHD frequency steadily increased in whole cohort of primary follow-up (Fig. 4.15) but if at any stage it

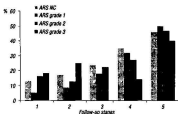


Fig. 4.15. Ischemic heart disease frequency in ARS survivors and patients of ARS NC at the follow-up stages

decreased in comparison with previous stage this fact was explained by loss of patients due to their death or migration.

During post-accidental period acute myocardial infarction had two patients of ARS NC group and one ARS grade 2 survivor.

At the end of 3rd follow-up stage we used the analysis of variance to determine HHD and IHD dependence from different risk factors, including radiation exposure. It had shown that IHD developing was influenced by combination of factors including AH, hypertriglyceridemia and excessive body mass ($F=2.406$, $p<0.05$, $RR=1.5$), whereas HHD occurrence correlated with excessive body mass only ($F=14.188$, $p<0.001$) [24]. Ten years later we applied the survival analysis for the same purpose, notably Cox regression or the model of proportional risks. As risk factors, which could influence on HHD and IHD appearance, we estimated (1) ARS grade, (2) the gender, (3) an age at the moment of radiation exposure, (4) smoking, (5) total cholesterol, (6) body mass index and (7) AH that preceded IHD.

As it is shown in Table 4.18, only the age at irradiation, BMI and total cholesterol were the indices, which influenced on HHD developing in both ARS survivors and ARS NC patients. Odds ratio shows if patient's age at the moment of radiation exposure increased on a year probability to fall in ill with HHD increased by 2.3% (confidence interval 0.5–4.2%). The risk of HHD occurrence increased by 4.8% if BMI (ratio of body mass in kg to squared height in m) rose on a unit. The growth of total cholesterol by 1 mmol per liter increased HHD risk by 27.2%.

Table 4.18. Results of Cox regression for hypertensive heart disease (the original table of SPSS program)

	B ^a	SE ^b	Wald	df	Sig ^c	Exp(B) ^d	95.0% CI for Exp(B) ^e	
							Lower	Upper
ARS grade	-0.123	0.097	1.609	1	0.205	0.884	0.731	1.069
Gender	0.118	0.354	0.111	1	0.739	1.125	0.582	2.254
Age at the irradiation	0.023	0.009	6.248	1	0.012	1.023	1.005	1.042
Smoking	0.196	0.196	1.006	1	0.316	1.217	0.829	1.787
BMI	0.109	0.032	11.915	1	0.001	1.115	1.048	1.187
Total cholesterol	0.240	0.089	7.341	1	0.007	1.272	1.089	1.513

^aa — regression coefficient; ^bb — standard error of regression coefficient; ^cc — significance of regression; ^dd — odds ratio; ^ee — 95%-confidence interval for odds ratio

Concerning IHD, the risk of its occurrence was significantly increased by more older patient's age and greater BMI but decreased by AH (Table 4.19). The last fact contradicts multicentered investigations including Framingham Heart Study. It is explained by the fact that patients, whom AH had been diagnosed at first, started to be treated by hypotensive therapy including β -adrenoreceptor blocking agent and the drugs for atherosclerosis prophylactics. If these medical prescriptions was used not entirely and regularly by the patients they postponed developed coronary atherosclerosis in any case.

During post-accidental period sudden cardiac death was the most frequent reason of patients' death (Table 4.20). Although all dead persons suffered from HHD and / or IHD autopsy did not revealed the signs of coronary syndrome or other diseases that could bring them to lethal outcome. Three patients had 2nd stage HHD that complicated

by AICC ended with death. One patient, who survived ARS grade 1, had died as the result of progressing HF developing due to IHD.

Table 4.19. Results of Cox regression for Ischemic heart disease (the original table of SPSS program)

	B ^a	SE ^b	Wald	df	Sig. ^c	Exp(B) ^d	95.0% CI for Exp(B) ^e	
							Lower	Upper
ARS grade	0.041	0.153	0.072	1	0.788	1.042	0.771	1.408
Gender	0.863	0.507	1.708	1	0.191	1.940	0.718	5.240
Age at the irradiation	0.113	0.015	53.380	1	0.000	1.120	1.088	1.154
AH	-0.664	0.297	4.977	1	0.026	0.515	0.287	0.923
Smoking	-0.208	0.317	0.430	1	0.512	0.812	0.436	1.512
BMI	0.120	0.048	6.233	1	0.013	1.127	1.026	1.238
Total cholesterol	0.259	0.163	2.527	1	0.112	1.295	0.941	1.785

^aB — regression coefficient; ^bSE — standard error of regression coefficient; ^cSig — significance of regression; ^dExp(B) — odds ratio; ^eCI — 95%-confidence interval for odds ratio

Patients' age (all of them was male) at the moment of their death was 61.1 ± 16.8 years. In seven men the age was lower than 62.3 years, notably the mean life span of man in Ukraine [3].

Table 4.20. Cardiovascular diseases and death reasons in ARS survivors and patients of ARS NC during follow-up period

No	ARS grade	Diagnosis	Death reason	First diagnosed	Date of death	Age at the moment of death
1	NC	HHD 2 nd stage	AICC	1991	2007	57.7
2	NC	HHD 2 nd stage IHD	SCD	1991 1992	2004	61.6
3	NC	HHD 3 rd stage IHD	AICC	1987 1990	2006	57.7
4	NC	IHD HHD 2 nd stage	AICC	1990 1992	2008	54.0
5	NC	IHD HHD 2 nd stage	SCD	1989 1989	1993	55.9
6	1	IHD* HHD 2 nd stage	HF	1985 1987	2007	77.3
7	3	IHD	SCD	Before 1986	2001	87.3
8	1	HHD 1 st stage	SCD	1987	1993	41.2
9	1	HHD 2 nd stage	SCD	1988	1995	26.3
10	2	IHD*	SCD	1976	1998	80.5
11	2	IHD	SCD	1987	1990	67.8
12	3	HHD 1 st stage IHD	SCD	1987 1990	1992	66.5

*The disease was diagnosed before the irradiation; AICC — acute impairment of cerebral circulation; HHD — hypertensive heart disease; HF — heart failure; IHD — ischemic heart disease; SCD — sudden cardiac death.

Myocardium is essentially radioresistent tissue and damaged in human by lethal radiation doses only, but vessel endothelium has showed the high sensitivity to radiation exposure. A death of endothelial cells in laboratory animals was observed in early terms after external X-ray irradiation under dose of 0.5 Gy and remains during long follow-up period [31]. It is considered that the direct damaging radiation effects on endothelium start to appear since doses, which induced ARS. Endothelium injury due to indirect radiation impact can develop under substantially smaller doses that are considered "low" in radiation medicine. One of the injuring factors is prolonged activation of lipid peroxidation processes, which started after irradiation [32]. Steady changes of oxidative homeostasis form the state in cells, which is called of "oxidative stress". Thus, the cells of radiosensitive tissues are undergone to maximal structural and functional reorganization.

Today it is the opinion that the reason of endothelial dysfunction is just an oxidative stress [33]. The nature of this stress plays no essential role in onset of endothelial dysfunction. The direct and indirect radiation impact on endothelial cells starts up a cascade of biochemical reactions, which finally leads to endothelial dysfunction and AH development [34, 35].

Therefore, the theoretical preconditions existe for seeking a connection between cardiovascular pathology and radiation. However, in our investigation the severity of radiation exposure or absorbed radiation dose did not significantly influenced on HHD and IHD occurrence. This fact results in two assumption: first, radiation impact had too short exposure that was enough for changing biochemical processes in a cell but not enough to give them the steady pathological character. The increase of dose value could not compensate this lack of radiation impact duration. By other words, the energy of ionizing radiation, what high it could be (in the frames of organism survivability), is impossible to compete with conventional risk factors, which start to affect the human organism actually since his birth. The second assumption is oppositely to the first: radiation exposure, even it was short-term, having influence on endothelial cell metabolism can lead to appearance of cardiovascular pathology but the level of this influence lays below that doses, which the patients of priority cohort (clinical and subclinical form of ARS) had. Perhaps, in the range of "low" doses cardiovascular morbidity and mortality depend from dose value, at least this fact was proved by the data of Russian scientists, Ivanov et al. [36], who conducted the epidemiological investigations on the voluminous cohorts of ChNPP clean-up staff living in Russia and victims of the accident at Mayak industrial association.

4.8 ENDOCRINE SYSTEM

Taking into account that at the moment of explosion the majority of persons amongst the cohort of priority follow-up was close to destroyed 4th block it is possible to suppose they accumulated radioactive iodine into the thyroid gland. This thesis was proved by other authors' data [37] according to which thyroid gland doses varied from 0.1 to 1.8 Gy in ARS survivors.

In post-accidental years goiter and diabetes mellitus were the most often pathology. Their frequency differed insignificantly in groups of ARS survivors and ARS NC

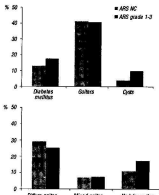


Fig. 4.16. Frequency rate of endocrine system diseases in ARS survivors and ARS NC patients during follow-up period

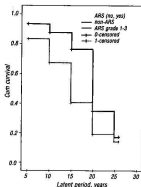


Fig. 4.17. Survival curve of endocrine pathology in ARS grade 1-3 survivors and patients of ARS NC (non-ARS on the picture) during 25 years of medical examination

patients (Fig. 4.16). Amongst whole cases of thyroid gland enlargement the diffuse or nodular goiters were diagnosed frequently but their combination rarely.

The analysis of entire endocrine pathology has shown that during 20 years its frequency in ARS NC patients was lower than in ARS survivors (Fig. 4.12) and only on the 5th stage approached to the level of the last ones. The survival analysis let to reveal that ARS survivors has been falling ill "faster" than ARS NC patients (logrank test, $p < 0.001$). So, the half of the group ARS grade 1-3 fell ill in 15 ± 1.1 years, and the half of ARS NC group in 20 ± 0.7 years (Fig. 4.17). At the end of 5th stage of follow-up endocrine pathology was revealed in 89.1% of ARS survivors and in 85.1% of ARS NC.

The significant difference of goiters frequency (diffuse, nodular and mixed) in ARS survivors and ARS NC patients was absent. It is connected most likely with the fact that the majority of patients of both groups spent their time equally in the accident zone and therefore were underwent to risk of Iodine-131 intake in equal parts.

4.9 OTHER ORGAN AND SYSTEMS

Morbidity of bronchopulmonary, hepatobiliary, urogenital, and nervous systems and GIT did not reveal their dependence from ARS grade or ARS existing in a patient. The correlation with radiation absorbed dose was absent too.

The most widely-distributed disease of bronchopulmonary system was chronic bronchitis (Fig. 4.18). It did not depend on severity of radiation exposure. During 25 years of follow-up its frequency steadily increased both in ARS survivors and ARS NC

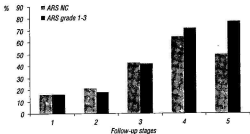


Fig. 4.18. Frequency rate of chronic bronchitis in ARS survivors and patients of ARS NC during follow-up period

patients. Bronchial asthma was diagnosed in three ARS NC patients and a patient of ARS grade 3.

In post-accidental period chronic non-specific diseases of GIT (gastritis and gastroduodenitis) and hepatobiliary system (cholecystitis) were the most widely-distributed pathology of digestive system (Fig. 4.19). Their frequency did not differ according to follow-up groups in fact. Erosive and ulcerous processes ranked as second diseases according to prevalence of GIT pathology and hepatitis as pathology of hepatobiliary system.

During entire follow-up period inflammatory kidney diseases (chronic pyelonephritis) were revealed in a patient of ARS NC group and five ARS survivors (Fig. 4.20). Kidney cysts and calculi were the more frequent pathology revealed by USE. Chronic prostatitis was diagnosed on the base of a patient's complaints, rectal examination, USE and analysis of prostate secretion. Adenoma of prostate was found in five ARS NC patients and three ARS survivors.

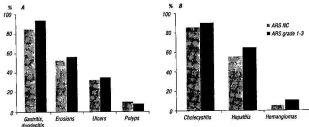


Fig. 4.19. Frequency rate of gastrointestinal tract (A) and hepatobiliary system (B) diseases in ARS survivors and patients of ARS NC during follow-up period

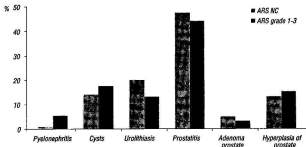


Fig. 4.20. Frequency rate of urogenital system diseases in ARS survivors and patients of ARS NC during follow-up period

During entire follow-up period the native feature of nervous system diseases was a predominance of functional disorders as asthenic syndrome and vegetovascular dystonia of the first stages. Starting from the second stage the frequency rate of organic pathology as dyscirculatory encephalopathy and cerebral atherosclerosis increased (Fig. 4.21). On the 25th year of follow-up the quota of these nosological forms reached 100% in ARS survivors and 98.7% in ARS NC group.

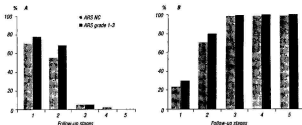


Fig. 4.21. Frequency of functional (A) and organic (B) disease of nervous system diseases in ARS survivors and patients of ARS NC during follow-up period

Conclusions

Our investigations that had been fulfilled during 25 years let us come to following conclusions concerning «restricted» cohort of patients, who survived ARS, and persons irradiated in doses below 1 Gy.

1. Mortality rate from tumors and blood malignant diseases in the cohort of primary follow-up exceeds the same index from cardiovascular system diseases and this fact could not be explained except the radiation stochastic effects occurrence.

2. The age of half patients, who died from oncological and cardiovascular diseases was lower the mean life span of men in Ukraine (62.3 years).

3. Deepness and evidence of long-term skin damages on the foci of former skin burns are directly and proportionally depended on their severity.

4. The latent period of radiation cataracts did not exceed 15 years and was shorter in ARS grade 3 patients. Radiation cataracts are closer to stochastic effects than to deterministic ones.

5. In early and long-term period after irradiation various cytopenia in the peripheral blood were revealed amongst the most frequent erythrocytopenia and lymphocytopenia followed by thrombocytopenia and granulocytopenia. The frequency rate of cytopenia, excluding granulocytopenia, during the first five years of follow-up significantly correlated with severity of radiation exposure and ARS occurrence in a patient.

6. During the first five years after irradiation the essential rise of diseases of nervous, digestive, cardiovascular diseases (45–84%), and eyes as well was found. The frequency of bronchopulmonary, endocrine and urogenital system increased insignificantly (7–33%). In 25 years the frequency of aforementioned diseases was 85–100%. No one disease was connected with radiation factor.

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CHAPTER 5 LEUKEMIA

5.1. EPIDEMIOLOGY

Hemopoietic tissue is one of the most sensitive to ionizing radiation human organs and systems. In the remote period after exposure ionizing radiation causes the appearance of stochastic effects, especially leukemia.

Excessive risk of leukemia is proven in studies of those who survived after the atomic bombing in Japan in 1945 [1, 2], of Marshall Islands residents, patients who received different doses of irradiation due to the diagnostic and therapeutic procedures [3], as well as professionals-radiologists [4].

According to Japanese researchers data [5] the excess relative risk of leukemia per 1 Sv of exposure among people who survived the atomic bombing (hibakushi), in average was from 3.3 to 9.1 depending on the type of leukemia.

One hundred and three of 310 recorded deaths from leukemia in the hibakushi cohort during 1950–2000 (LSS study) were rated as caused by radiation exposure [2]. The largest share of excess deaths from leukemia among the survivors of the atomic bombing was registered in the first 15 years after exposure [1]. However, presented estimates were obtained for persons affected by a high dose for a very short time.

Study of long or protracted (fraction) exposure to small or large doses on leukemia risk caused considerable interest. In studies conducted among employees of the nuclear industry in North America and Europe the statistically unreliable results were obtained that require further analysis [6, 7].

Statistically significant dose-dependent increase of mortality from leukemia was found in the cohort of workers of nuclear plant Mayak in Russia [8] that received significant external radiation dose (on average 0.8 Gy) for the prolonged time. According to these data a significant dependence of leukemia risk excess from the period of irradiation was determined. For doses received in the period from 3 to 5 years before death, excess relative risk was approximately 7 per 1 Gy of irradiation ($p < 0.001$), but this figure was only 0.45 per 1 Gy ($p = 0.02$) for doses received from 5 to 45 years to death.

Because of the unique conditions and the heterogeneity of exposure following the Chernobyl accident the study of leukemia risk in exposed populations deserves high priority and is of a great applied and scientific interest.

The study of leukemia and related diseases among the Chernobyl clean-up workers (liquidators) was initiated by RCRM and the U.S. National Cancer Institute in 1997. The study was conditionally divided into 2 phases of execution.

During the first phase the cases of hematopoietic system cancer diagnosed in 1986–2000 were analyzed and during the second one — the cases diagnosed in 2001–2006.

Studied population group was restricted to clean-up workers (liquidators) registered in the SRU. Cohort consisted of 110 645 male liquidators who were residents in Dnipropetrovsk, Kyiv, Kharkiv, Cherkassy and Chernihiv regions and Kyiv city at the time of registration. Survey period included the years 1986–2006. All forms of acute and chronic leukemia, multiple myeloma and in addition to the first phase myelodysplastic syndrome were studied. Doses to the bone marrow were estimated retrospectively using a new time-and-motion method called RADRUE. It bases on interviewing of study subjects and further analysis of the nature, timing and location of works during period of 1986–1990, and the related radiation dose [9].

Cases of leukemia, multiple myeloma and myelodysplastic syndrome diagnosed in 1986–2000, were identified by linkage between created computerized registry of leukemia and related hematological disorders among the male population of corresponding age and regions, and the cohort file. Cases of leukemia and multiple myeloma diagnosed in 2001–2006 were identified by automated linkage of database of NCRU with the cohort file. The tasks of the first phase of the study were fulfilled. As a result calculations of dose-dependent leukemia risk among liquidators were performed.

A significant association between Chernobyl-related radiation dose and increased total risk of leukemia was observed. The excess relative risk (ERR) of total leukemia was 3.44 Gy [95% CI 0.47–9.78, $p < 0.01$] [10]. Similar estimates were obtained for chronic lymphoid leukemia (CLL) (Table 5.1), but values did not achieve statistical significance. Other specific forms of leukemia were analyzed together because of few cases. To further clarify all unclear in phase I issues and hypothesis, it was decided to extend the period of observation for another 6 years (2001–2006). This allowed to cover the whole 20-year post-accident period with the potential possibility to give a more precise estimates for separate forms of leukemia and above all chronic lymphoid leukemia. Finding an excess risk of this form of leukemia in the first phase is a new significant contribution to the radiobiological knowledge, taking into account previous research experience in this area. Thus, there was apparent need for increased monitoring to obtain statistically significant estimates.

Table 5.1. Excess relative risk of leukemia per unit dose of external irradiation of red bone marrow (ERR/Gy) in the liquidators cohort (1986–2000)*

Leukemia type	Excess relative risk per 1 Gy of irradiation (ERR/Gy)		p – value
	value	95 % confidence interval	
All leukemias, including:	3.44	0.47–9.78	<0.01
CLL	4.09	<0–14.41	0.75
Non-CLL	2.73	<0–13.50	0.75

*two year lag period is considered

Basic methodological approaches to the 1st and 2nd phase of the project were identical [11]. This ensures comparability of data and the possibility of their association and further joint analysis.

The only significant difference compared with the previous methodology was the method of cases identification, which was first thoroughly tested in order to ensure completeness of data. During the 1st phase writing out data was conducted for each case of leukemia or related diseases diagnosed in 29 hematological departments among the male population of corresponding age (birth years 1926–1972). These data were introduced in a special form, and then all filling forms were entered into a database. Thus, the Provisional Leukemia Registry was formed. Then linkage of the study cohort file with the Leukemia Registry was accomplished using computerized probabilistic record linkage. As a result records on two separate files were compared and the probability was estimated that a pair refers to the same person. Scores above a certain threshold were accepted as true matches. Thus, the cases in the cohort were identified.

To identify cases on the phase 2 the linkage of the cohort file with fragment of NCRU database corresponding to region, sex, age and cancer site was performed.

This technique could not be applied at the 1st phase, because the NCRU began operating in automatic mode in the studied regions only in 1993–1997's, and the study period included 1986–2000.

A search for all available medical records (case histories, clinical and laboratory records, autopsy protocols, individual cases in specialized expert Interdepartmental Commission for the Disease Linked to the Chernobyl Accident) together with peripheral blood smears and slides of bone marrow aspirates preserved in the clinical and pathological-morphological laboratories was undertaken for each identified case. Prerequisite was to obtain the most complete information at the time of diagnosis. Based on the collected data a paper and electronic "Brief Clinical Forms" were filled for each case. This document was accompanied by marked biological material preparations and it was referred to final case validation directly to an International Hematology Panel which was held in several sessions. Smears or biopsy sections (or a detailed description of biological material) were available for 75% of cases submitted for the review. Diagnostic data were presented only by medical records for 25% of cases.

The International Hematology Panel (both for the 1st [12] and 2nd phase diagnostic review sessions) was consisted of five expert hematologists and hematopathologists from the U.S., UK and Ukraine. They were: Dr. Barbara Bain, Dr. Lo-Ann Petersen, Dr. Peter MakFedran, Dr. Svetlana Gaydukova and Dr. Daniel Gluzman.

According to the previously developed procedure, the experts considered the set of 5 cases. Each expert on the Panel independently reviewed a clinical abstract of each medical record together with available bone marrow aspiration smears or sections of each case and brought its opinion to the special form. In cases with a disparity of opinion, each case was discussed at length until a consensus diagnosis was reached. Consensus results were recorded on a blackboard and fixed by camera. Then the experts considered the next block of cases. The experts studied 20–25 cases each working day.

Leukemia, MDS, multiple myeloma, Hodgkin malignant lymphoma 252 cases in total, and 21 control cases were referred to the International Hematology Panel (Fig. 9.1). This approach with verification of the diagnosis by the international expert group significantly increased the level of the data reliability.

The inclusion of non-Hodgkin's malignant lymphoma in this sample was due to finding cases of lymphoma with small lymphocytes (SLL), which according to modern classification of lymphoid neoplasms, is regarded as identical with CLL (CLL / SLL).

One hundred fifty eight of 195 cases of leukemia referred to the panel were confirmed. In addition, one case of MDS (form RAEB-t), 3 cases of non-Hodgkin's malignant lymphoma of the 15 submitted for examination were reclassified to leukemia. Thus, for the period of 1986 — 2006 162 confirmed cases of leukemia were identified in the study cohort (Table 5.2).

Table 5.2. Structure of leukemia cases identified in the liquidators cohort (1986–2006) and confirmed by the International Hematology Panel

Type of Leukemia	Number of cases #
Acute Myeloblastic (AML)	20
Acute Lymphoblastic (ALL)	8
Acute Leukemia non-classified (AL)	11
Chronic Lymphoid (CLL)	90
Chronic Myeloid (CML)	28
Chronic Leukemia from Large Granular Lymphocytes (T-cells) (LGL-T-cells)	5
All Types	162

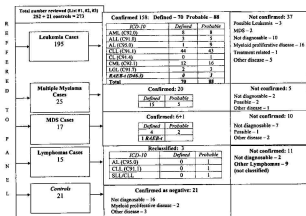


Fig. 5.1. Scheme of the International Hematology Panel results

The number of identified leukemia cases corresponded with calculated expected number that suggesting a satisfactory completeness of collected data.

A case-control study method of epidemiological research was used. According to the strategy for each confirmed case of leukemia 9 controls were randomly selected: 5 major and 4 — to replace if necessary. The controls were matched to the case on region of residence and year of birth. Further the study subjects were traced to contact with them and conduct interviews.

Search was conducted using the data of medical institutions (clinics, regional dispensaries of specialized radiation protection, NCRU), information of reference and information bureau. A tracing procedure included written application to the study subject address of residence, telephone and personal contacts. Alive study subjects were interviewed personally. In a case of deceased subject his relative and colleague (so-called "proxy") were interviewed to obtain the detail information on his clean-up activities in the Chernobyl zone. In some cases the attempts to apply a contact were not successful. The main reasons for this were lack of reliable information on a subject location and the subject's refusal to participate in the study (Table 5.3). This led to a significant increase in required resources to obtain the necessary information. Particular concerns have caused losses among the cases that were impossible to replace with randomly selected subject from the cohort members. The loss of each case could potentially be displayed on the accuracy of analytical estimates.

Table 5.3. Characteristics of losses to follow up

Type of the Study Subject	Cause of the loss		
	refused	not traced	total
Alive Leukemia Cases	3	0	3
Deceased Leukemia Cases			
Proxy Relatives	12	6	18
Proxy Colleagues	1	20	21
Alive Controls	160	130	290
Deceased Controls			
Proxy Relatives	25	34	59
Proxy Colleagues	0	6	6

These results identified the response rate that is the identifier of the efficiency of interaction with the study subjects. Rather high response rate for all categories of the study subjects was reached, approximately the same for both phases (Table 5.4).

Interviews were conducted by experienced interviewers, former liquidators, and familiar with details of work after accident. In a case of consent to participate in the study before the interview liquidator or "proxy" signed informed consent. Without this interview was not conducted. This requirement of ethical standards in research was strictly followed.

Completed questionnaires were registered and processed in the data coordinating center (DCC) and then transferred to the expert dosimetrist for dose reconstruction with RADRUE method.

Now all data have been processed in dosimetry group, and twice entered into the DCC database. Total for the entire study period (1986–2006) 1003 doses of external irradiation to the red bone marrow were reconstructed. Summary results of this work are presented in Table 5.5

Table 5.4. Response rate by study subject types.

Type of the Study Subjects	Response Rate (%)	
	Phase 1	Phase 2
Alive Leukemia Cases	100	93
Deceased Leukemia Cases		
Proxy Relatives	78	82
Proxy Colleagues	79	82
Alive Controls	72	68
Deceased Controls		
Proxy Relatives	60	29
Proxy Colleagues	Not defined	Not defined

Table 5.5. Number of reconstructed doses for leukemia cases and their controls.

Type of the Study Subjects	Number of reconstructed doses		
	Phase 1	Phase 2	Total
Alive Leukemia Cases	29	39	68
Deceased Leukemia Cases	43	26	69
Alive Controls	465	358	823
Deceased Controls	36	7	43
TOTAL	573	430	1003

It should be emphasized that although the study subjects represented a wide range of categories, about two-thirds of them were military liquidators (36%) and those defined as sent to mission to the 30-km zone (32%). Place and duration of clean-up activities were substantially different in various groups. As a result, subjects dose could vary by 7 orders of magnitude: from the natural background to almost lethal.

It should be noted that at this stage of research only part of the error, so-called internal uncertainty of the data could be evaluated. At the same time component of uncertainty associated with the human factor could only be defined as "significant." The main source of such errors are data received not from the liquidator personally, but from his deputies, "proxy". Deviations from the true dose values can be quite large, but in general estimates are not biased. Each task of the Project, including dose estimates, was performed with the mandatory use of quality control procedures to ensure a high level of research in general and analytical assessments based on the results.

Summarizing the above data we can conclude that:

1. As a result of the research the information basis for leukemia risk analysis in a 20 years' post-accident period was formed.
2. Analysis of the leukemia risk in the studied liquidators cohort of 1986–2000 completed. Based on that the excess relative risk of leukemia in total was defined to be 3.44 (95% CI 0.47–9.78, $p < 0.01$).

3. Cases identified and verified at 2nd stage of research (2001–2006 study period) will increase study power and allow obtaining statistically significant risk estimates of certain forms of leukemia.

4. Current approaches to radiation support of the project provided an opportunity to obtain dose-dependent estimates of excess risk of leukemia among liquidators of the Chernobyl disaster.

5. Using quality assurance procedures at every stage of the task performance allows getting risk estimates of the high level of trust that meets the requirements to analytical epidemiological studies.

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5.2 MOLECULAR BIOLOGICAL FEATURES OF ACUTE LEUKEMIA IN PATIENTS EXPOSED TO IONIZING RADIATION DUE TO THE CHORNOBYL ACCIDENT

The results of epidemiological studies among clean-up workers at the Chernobyl NPP established a causative link of leukemia with preceding radiation exposure. However, the question remains debatable as to the existence of associations between specific molecular abnormalities in acute leukemia (AL) and previous exposure of human to ionizing radiation (IR). The main problem that hampers determination of radiation leukemogenesis is the uncertainty about radiation-induced nature of specific cases that developed in the exposed human population.

As the most promising for further study in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) in persons exposed to IR due to the Chernobyl catastrophe, we recognized the rearrangements of chromosome 21q22 with involvement of *AML1* gene, and chromosome 11q23 with involvement of the *MLL* gene. The data obtained in several published studies demonstrated the probable relationship between the formation of the fusion transcripts between *AML1* gene and unusual partners on chromosomes 1, 18 and 19 and radiation-induced AML [1]. Japanese researchers reported a high frequency of point mutations of *AML1* gene in MDS in victims of the atomic bombing of Hiroshima [2]. The rearrangements of *MLL* gene are common in secondary leukemias induced by DNA topoisomerase II inhibitors [3]. The fact, however, that secondary AML shares some clinical features irrespective of the causative agent as IR or cytostatics makes it likely that leukemias developing after exogenous genotoxic agents have some common molecular alterations.

A number of facts encouraged to study leukemia-specific translocations. First, AL in humans *per se* is often associated with translocation of genes [4]. Second, the dose-effect dependence for additional cases of leukemia after exposure to IR is characterized by the curvilinear function. And, thirdly, IR is able to form dicentrics and translocations in irradiated cells with a frequency described by curvilinear dependence on dose [5].

Fifty-three AML samples at diagnosis were analyzed in our study for the presence of *AML1* abnormalities using fluorescent *in situ* hybridization (FISH) and reverse transcription polymerase chain reaction (RT-PCR). Selection bias can be excluded in this analysis because all available AML patients were enrolled in the study and blindly checked for the presence of *AML1* rearrangements. In contrast to the study of Hromas et al. [1], we did not find any unusual alteration of the *AML1* locus accompanied by involvement of other than gene *ETO* translocation partners in patients with radiation-associated AML. The observed patterns of FISH signals are shown in Fig. 5.2. An example of *AML1/ETO* fusion gene amplification products electrophoresis is shown in Fig. 5.3.

By FISH and the RT-PCR assay, *AML1/ETO* translocation was found in only 1 of 24 radiation-associated AML. In contrast, *AML1/ETO* translocations were identified in 9 (31%) of 29 spontaneous AML cases ($p < 0.05$). Noteworthy, that the frequency of *AML1/ETO* translocation registered in the control group was similar to that observed in AML among residents of the western regions of Russia and was higher than that among the AML patients in Scandinavia and North America [4]. The higher level of transloca-

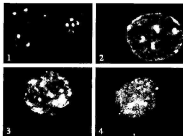


Fig. 5.2. The observed patterns of hybridization of AML cells with *AML1/ETO* probe, $\times 100$: 1 — interphase cells showing the normal pattern (two green and two red signals located separately); 2 — *AML1/ETO* translocation in case 29 (one red, one green and two fused (red and green) signals); 3 — an interphase cell from patient 34 showing three red and three green signals located apart; 4 — in patient 45 three red and two green non-fused signals are observed.

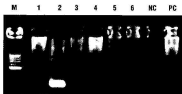


Fig. 5.3. The results of electrophoresis of amplification products after RT-PCR for *AML1/ETO* gene: M — molecular weight marker lane, 100 bp; 1-6 — bone marrow samples of patients with AML, which were analyzed for the presence of *AML1/ETO* translocation; NC — negative control; PC — positive control.

severely limits their therapy. Treatment of AML is largely based on the use of high doses cytarabine (Ara-C) in consolidation of the disease remission. It is most successful if *AML1/ETO* translocation is present. The fewer portions of patients expected to respond to high doses Ara-C reduces the chances of exposed patients to be cured by conventional chemotherapy. This fact can be considered as an important negative prognostic factor in radiation-associated AML and convincing evidence in favour to appropriate use of intensive therapeutic, based on hemopoietic stem cells (HSC) transplantation protocols and clinical trials of innovative treatment.

However, there are several discords with other studies on *AML1* gene to comment. First, *AML1* gene translocations were detected among atomic bomb survivors with AML in Japan [6]. Though, it is impossible to exclude the spontaneous nature of the disease

tions of *AML1/ETO* in spontaneous AML. In Russian and Ukrainian cohorts reflects the features of Eastern European population in general.

In the identical spectrum of morphological variants, frequency of large rearrangements in the *AML1* locus in study groups differs significantly, indicating peculiarities of radiation-associated leukemia. Chromosomal translocations of the *AML1* gene, characteristic for the disease *de novo* and therapy-related AML after treatment with topoisomerase II inhibitors, are not common among the AML patients exposed to IR due to the Chornobyl accident. These patients rarely belong to the category of favorable AML characterized by translocation of *AML1/ETO*. It is likely that because of the peculiarities of the pathogenesis of leukemic process with the more frequent gains and losses of chromosomal material in radiation-associated AML, there is some redistribution of cases by cytogenetic categories accompanied by relative decreased frequency of translocational events. This feature of patients with radiation-associated leukemia severely

in those cases, and this fact could only reflect the heterogeneity of the cohort of patients in terms of etiology. Moreover, other mechanisms of leukemia development could be involved in some atomic bomb survivors received much higher radiation dose than most of the patients in our study. Second, the formation of AML1/ETO fusion gene in cells exposed to radiation was reported in experimental systems [7]. The discrepancy with this observation could be interpreted by relatively low radiation dose of AML patients in our study. Higher doses are needed to cause chromosomal translocations resulting from radiation-induced double-strand DNA breaks. Other molecular mechanisms, rather than gene translocations could contribute to radiation leukemogenesis at low doses range.

In general, the heterogeneity of group of radiation-associated AML patients in terms of etiology complicated the analysis in our study. The assignment of patients to a group of patients with radiation-associated leukemia certainly did not mean that IR doubtless contributed to the disease development. The mean absorbed dose of radiation for the entire cohort of clean-up workers participated in recovery operations at the Chernobyl NPP in 1986–1987 is 17 cSv [8]. Based on calculations of Mettler et al. [9] on excess relative risk, we can assume that only 30–40% of the clean-up workers with leukaemia developed the radiation-induced disease. The existence in the main study group significant portion of patients with spontaneous AML makes the big study group necessary to draw the solid conclusions.

During the FISH experiments, which were primarily aimed to study the abnormalities at AML1 gene, trisomy of chromosome 8 was revealed in 3 patients. Trisomy 8 is one of recurrent chromosomal anomalies in myeloid tissue neoplasms. Anomaly occurs as sole abnormality in 5% of cases with abnormal karyotype AML and as a second aberration in approximately 15% of patients identified as cytogenetically aberrant [10]. The pathogenic role of trisomy 8 in leukemogenesis is under discussion. The anomaly is often described as secondary in the process of clonal evolution of AML. Trisomy 8 is recorded in 30% of all cases of acute promyelocytic leukaemia (APL) with t (15;17) as the second anomaly. The frequency of trisomy 8 in AML and MDS developed following chemotherapy and radiotherapy is much lower compared to the leukemic process *de novo* [10]. Thus, there is some association between trisomy 8 and spontaneous nature of AML. In our study, in the absence of statistical differences between groups, trisomy 8 registered not only among patients with spontaneous (1 out of 16 cases) but also in radiation-associated AML (2 of 21 cases). This fact supports the assumption of heterogeneity of patient groups with radiation-associated AML, which contains both radiation-induced and spontaneous cases. Other investigations have indicated that trisomy 8 may be related to exposure to benzene or other organic solvents [10]. Davico et al. [11] found a correlation between smoking and chromosome 8 aberrations. Thus, even more pronounced heterogeneity of our groups in terms of AML causality could be assumed.

To determine the mechanisms of radiation leukemogenesis, AML1 point mutation analysis was performed in 6 patients with MDS, or AML following MDS, by direct sequencing of DNA. There were 2 cases of refractory anemia, 1 case of refractory anemia with excess of blasts in transformation and 3 cases of AML post MDS. All patients participated in the Chernobyl clean-up work in 1986. One of the patients survived acute radiation syndrome (ARS) (case 263). No single nucleotide changes in the gene were found in any of the investigated cases. However, in patient, who had survived ARS

10 years before (case 263), an in frame hexanucleotide duplication of CGGCAT in exon 8 (after position 1502) was found, that causes an extra Glycine and Isoleucine inserted into the protein. The sequencing of PCR products of *AML1* gene exon 8 cloned in TOPO-TA vector confirmed the presence of the mutation (Fig. 5.4).

In spite of some differences to the data of Harada et al., our study does not contradict the model that radiation may contribute to leukemia development through *AML1* gene mutation [2]. *AML1* point mutations in MDS arising among atomic bomb survivors

were found in the DNA-binding Runt homology domain, whereas mutations in the carboxyl(C)-terminal region were considered to be strongly correlated with a spontaneous etiology [2]. The *AML1* mutation in our study was localized to the C-terminal transcriptional activation domain after residue 368, but was present in the MDS case with the highest probability of being radiation-induced. This patient survived ARS caused by whole-body exposure to a dose of 1.7 Gy. Epidemiological data on radiation-associated leukemias in clean-up workers, and derived excess relative leukemia risk [12], indicate that the MDS in this patient has at least a 90% probability of being radiation-induced.

There are several issues that may explain these somehow controversial findings on *AML1* gene sequencing in victims of Chernobyl accident and victims of A-bomb in Japan. First, the different patient populations were examined (Asian and European). Second, the differences in the frequencies of point mutations of a single nucleotide, which are on the border of statistical significance (6 out of 13 cases in the study of Harada et al. [2] versus 0 of 6 cases in our work, $p=0.1$), disappear in the analysis of mutations of all types. A small number of patients in all studied groups make any quantitative assessment of the mentioned results being controversial. Third, screening for mutations of the *AML1* gene was done by different methodological approaches. Harada et al. screened with PCR single-strand conformation polymorphism of genomic DNA with conformation cDNA sequencing, and we used genomic DNA sequencing. And fourthly, there is the difference in terms of MDS manifestation after radiation action. We believe that this factor is the most important because the average latency period for manifestation of the disease was 45–55 years in the cohort of Japanese patients and only 12 years in the group of the Chernobyl liquidators.

There is no doubt in the existence of different pathogenic ways of spontaneous and secondary MDS development. However, uncertainty remains whether the found alterations reflect the existence of the distinctive features of radiation-induced pathology or

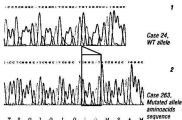


Fig. 5.4. Parts of the *AML1* genomic sequences of PCR products of *AML1* gene exon 8 cloned in TOPO-TA vector: 1 — wild-type sequence in AML post MDS patient (case 24); 2 — sequence with a hexanucleotide duplication of CGGCAT inserted in frame into exon 8 of the *AML1* gene in patient survived ARS in 1988 (case 263). The additional Glycine and Isoleucine coded by mutated allele are shown in red in the aminoacids sequence.

are coincidental. Further research will eventually clarify the significance of changes of *AML1* gene in radiation leukemia etiology. Our data confirm the hypothesis of Harada et al. [2] that *AML1* point mutation can be considered the most likely distinct molecular feature of the leukemic process induced by IR.

The results of our further studies show that translocations of *MLL* gene, which are typical for spontaneous and DNA topoisomerase II inhibitors therapy-induced AML [3] are not common in radiation-associated leukemia. The presence of *MLL* gene abnormalities was examined by FISH in 41 AML patients. Of these, there were 11 clean-up workers, 18 inhabitants of contaminated areas, and 18 control cases. An abnormal pattern of hybridization was found in two cases. Variants of the signals recorded during the AML cells hybridization with *MLL* break apart probe are presented in Fig. 5.5.

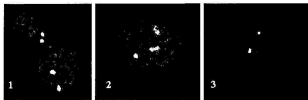


Fig. 5.5. The patterns of hybridization of AML cells with *MLL* probe. 1 — two interphase cells from patient 29 showing the normal pattern (2 fusion signals, each of which appears to consist of two contiguous red and green signals). No break apart signals suggestive of *MLL* rearrangement were detected; 2 — in patient 12 three copies of the *MLL* gene are present (three fused (red and green) signals); 3 — *MLL* translocation with deletion in case 239 (one yellow signal fused from red and green and one separate green signal).

Statistical analysis showed no differences between patients with radiation-associated and spontaneous AML patients in terms of translocations (0 of 23 vs. 1 in 18 cases, $p=0.44$) and any rearrangement (1 of 23 vs. 1 in 18 cases, $p=1.0$) of *MLL* gene, identified by FISH experiments. The frequency of *MLL* translocations in the whole cohort of patients in the study of AML was 2.4%, which corresponds to data of literature on the prevalence of such anomalies in unselected patient groups [13]. There is no evidence that *MLL* translocations are essential in leukemogenesis in those exposed to IR as a result of the accident. Moreover, radiation-induced leukemia is cytogenetically different from those that occur after therapeutic application of topoisomerase II inhibitors, where non-random rearrangements involving the *MLL* locus on chromosome 11 were observed. Nevertheless, our data support the hypothesis of Smith et al. [3], postulated that radiation may act as many other DNA-damaging agents and similar to alkylating agents. Indeed, the only *MLL* gene rearrangement found in radiation-associated AML cases in our study was the gene duplication in a patient evacuated from the Chernobyl exclusion zone (case 12). In patients with an additional copy of the *MLL* gene, only 2 signals of centromeres 11 were observed in all analyzed cells, which allowed us to exclude trisomy 11 in this case. However, the number of copies of the gene *Cyclin D1* varied from 3 to 5 in 17% of the cells (Fig. 5.6).

The genomic amplification of oncogene is a frequent event in solid tumours, but is rarely observed in haematological malignancies. The estimated incidence of cytogenetically detectable gene amplification in AML is approximately 1%. A rough estimate of the incidence of 11q23 segmental amplifications in all myeloid disorders, including not only AML and MDS but also chronic myeloproliferative disorders was given by Reddy et al. [14] as 4/690 (0.57%). This is

dramatically different to leukaemia cases diagnosed after mutagen exposure. Here duplication or amplification of chromosome band 11q23 with 3–7 copies of the *MLL* gene was detected by FISH in 12 out of 70 unselected patients (17%) who developed MDS or AML following a preceding chemotherapy with alkylating agents [13].

Our findings exclude *MLL* gene translocations as an important factor in the etiology of radiation-induced leukemia, but support the assumption that loss and gain of chromosomal material could be of importance in the leukemogenesis of AML patients with the history of radiation exposure due to the Chernobyl accident.

Cytogenetic studies of recent decades have made a significant contribution to the understanding the mechanisms of leukemogenesis. Karyotype analysis helps to distinguish AML into three main categories. The first category includes AML with balanced chromosome aberrations and a favorable clinical prognosis. This category includes cases with *AML1/ETO* translocation. AML patients exposed to IR due to the Chernobyl accident are rarely characterized by such abnormalities. The samples of leukemic cells of Chernobyl victims were not checked for the presence of other major anomalies of this category such as *PML/RAR- α* and *CBF β /MYH11* translocations, which are closely associated with specific morphological subtypes of AML namely M3 and M4eo respectively. However, as it is described below, AML M4eo is more often registered in spontaneous disease compared to radiation-associated AML ($p < 0.005$), whilst, it is vice versa for AML M3 ($p < 0.05$). We believe that AML M3 characterized by balanced translocation could be induced by IR. This hypothesis is supported by the results obtained in the University of Chicago [3].

The second category of AML includes patients with normal karyotype without significant prognostic cytogenetic markers, which belong to the intermediate risk subgroup. The third category includes adverse prognosis AML with unbalanced chromosomal abnormalities, such as losses/deletions of chromosomes and complex karyotypes [4]. According to our data, the spectrum of molecular genetic alterations of substrate cells in radiation-associated AML is shifted towards such anomalies. This hypothesis was confirmed by a chromosomes copy number study with using of Affymetrix high-density single-nucleotide polymorphism (SNP) arrays carried out on samples of leukemic cells

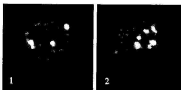


Fig. 5.6. Patterns of hybridization with *CyclinD1/CEP11* probe (patient 12). $\times 100$: 1 — the normal pattern with two green signals of centromere 11 and two red signals of *Cyclin D1* gene are observed; 2 — an interphase cell showing two green and four red signals corresponding to two centromere 11 and 4 copies of *Cyclin D1* gene respectively.

of a set of 19 AML cases that developed in patients exposed to IR from the Chernobyl nuclear accident. We examined 13 Chernobyl liquidators and 6 residents of territories contaminated with radionuclides fallout. We studied the losses and gains of chromosomal material that could by size be identified by karyotyping of G-painted chromosomes. In 16 of the cases we detected at least one large region of allelic loss. Eight cases (42%) demonstrated loss of heterozygosity (LOH) at 5q and 7, which are known to characterize the AML subgroup with complex karyotypic alterations and poor prognosis. The frequency of deletions of chromosome 5 and/or 7 in patients with radiation-associated AML appeared significantly frequently ($p < 0.05$) compared with the expected according to the literature data in spontaneous AML. Arguably, the consequence of genotoxic effect of IR is similar to alkylating agents that trigger AML with high frequency of deletions at chromosomes 5 and 7 [3].

Recently, mutations of FLT3 receptor have acquired a great interest in the context of mechanisms of leukemogenesis and improvement of therapeutic management. In large cohorts of patients suffering from AML *de novo*, FLT3 mutations occur in 30–33% of cases and mainly among patients with normal karyotype and promyelocytic subtype of the disease [15]. Implications of FLT3 receptor mutations are still unknown. The changes in FLT3-signaling pathway may impair the apoptosis, promote or at least assist the reparatory processes in leukemic cells after cytostatic damage. Any of these mechanisms may provide tumor cells resistance to chemotherapy. Given the prevalence of FLT3 gene mutations, the use of receptor inhibitors is promising for the treatment of leukemia.

The frequency of FLT3 internal tandem duplications (ITD) at AML in our study appeared to be lower compared to the expected by the literature, as in the group of radiation-associated cases (7 out of 69 cases, 10%) and as in the group of spontaneous cases (9 out of 48 cases, 19%). Mutations in codon D835 of FLT3 gene activation loop was found in 1 of 69 patients with radiation-associated AML and in 3 out of 48 spontaneous AML cases. FLT3 abnormalities were not found in patients with MDS. Among the 35 patients with ALL, the sole gene mutation was internal tandem duplication in the resident of territories contaminated with radionuclides. There was statistical difference in the presence of FLT3 abnormalities between radiation-associated and spontaneous disease group neither in AML, nor in ALL. Moreover, the frequency of FLT3 mutations of all types in radiation-associated AML appeared significantly lower compared with the frequency in large groups of not-selected AML patients in other studies [16]. The results suggest that FLT3 gene mutations are insignificant in radiation leukemogenesis. Christiansen and Pedersen-Bjergaard [17] also noted the low frequency of FLT3 mutations in the secondary, therapy-related AML.

Alignment of all inserted residues revealed the inclusion of a common tetrapeptide (Arg-Glu-Tyr-Glu) in all cases. The FLT3 ITD alterations in the radiation-associated AML exhibited the longest duplication events, each having a duplication of more than 60 nucleotides.

The difference in average size of longest duplicated fragments in patients with radiation-associated and spontaneous AML was statistically significant ($p < 0.05$). Questions about the significance of the presence of repeated motifs of amino acids and greater length of duplicated fragments of the gene for radiation-associated leukemia require the further study.

The main conclusion obtained at the study of *FLT3* gene is that because of low prevalence of *FLT3* receptor changes, the use of its inhibitors for the therapy of AML has limited perspectives in persons exposed to IR. The frequency of *FLT3* mutations in the case of AML, as defined in this chapter has been less than expected, according to data of literature. It is possible that in many published studies based on the use of DNA banks, somewhat overestimated actual frequency of mutations in *FLT3*. There may be a predisposition to having in bank the DNA samples from patients with higher levels of initial white blood cell count, which is associated with increased frequency of anomalies. In addition, only 9% of patients in our study had AML M3 subtype of the disease, which is usually characterized by the highest *FLT3* mutation frequency [15]. For the full cohort's compatibility, M3 variant of the disease should account 15% of all cases of AML.

But the most important is that the presence of *FLT3* mutations are usually closely correlated with normal karyotype of leukemic cells [16]. In this regard, the low frequency of *FLT3* mutations in radiation-associated AML suggests that the studied liquidators and residents of contaminated areas are characterized by the presence of other unidentified chromosomal aberrations. This confirms the hypothesis put forward in the study and made conclusions about the shift of the spectrum of cytogenetic and molecular characteristics of radiation-associated and radiation-induced cases of AML toward losses and gains of chromosomal material. At this scenario the low frequency of *FLT3* mutations should be observed in patients exposed to IR due to the Chernobyl accident.

The frequency of *FLT3* mutations was also lower in the group of spontaneous AML cases in our study than expected according to data of literature. However, if the radiation-associated leukemia is more often belongs to the category of AML with unbalanced chromosome abnormalities and has smaller portion of patients with normal karyotype, which could cause the lower frequency of abnormality, the control group has excessive *AML1/ETO* translocation frequency characteristic for spontaneous AML in Eastern European populations. To confirm, we cite data Kotaridis et al. [15] who showed internal tandem duplication of *FLT3* gene in only 9% of patients with translocation of *AML1/ETO*.

However, if duplicated, the *FLT3* gene in our study in radiation-associated cases of AML was disproportionately more often characterized by the presence of multiple mutants (Fig. 5.7). Among the patients with ITD, multiple alleles were found significantly more often in radiation-associated AML compared to spontaneous cases (5 of 7 vs. 1 of 9 cases, $p < 0.05$). This fact provides the evidence that AML in patients exposed to IR due to the Chernobyl accident is characterized by clonal heterogeneity already at the time of initial diagnosis of leukemia and supports the hypothesis that radiation-associated AML more often than spontaneous AML has signs of genetic instability. The presence of several leukemic clones at the time of manifestation, which are usually characterized by slightly different biological characteristics such as proliferative capacity and resistance to chemotherapeutic drugs, or their quick acquisition during the evolution of the disease reduces the chances of exposed patients for successful treatment results after conventional chemotherapy.

It is proved that classification scheme based on the quantitative characteristics of *FLT3* mutants with categories such as "not mutant", "single mutant", "more than one



Fig. 5.7. The results of electrophoresis of *FLT3-JM* domain amplification products in 18 AML patients: 1-9, 11, 13, 15, 16, 18 — bone marrow samples of patients without mutation, 10, 12 — samples with multiple mutant allele in cases 45 and 62 respectively, 17 — sample with one mutant allele; NC — negative control; VII — molecular weight marker.

mutant" can successfully replace the prognostic classification scheme such as "mutation present" and "not mutant" as the more powerful predictor for AML prognosis in terms of relapse and relapse-free survival. Patients with AML with more than one mutation of *FLT3* are characterized by significantly higher mortality in remission and shorter overall survival [15].

The results of monitoring for the Chernobyl clean-up

workers and residents of contaminated areas indicate that molecular genetic features of AL in persons exposed to IR are translated into clinical behaviour, which makes them somehow different from spontaneous leukemia. This supports the need to allocate the radiation-induced AL cases into a separate classification category.

After we compared the clinical course of 82 AML cases developed in Chernobyl victims with 70 sporadic AML cases, we concluded that the patients with radiation-associated AML frequently manifested with factors of unfavourable prognosis of the disease. The clean-up workers and residents of contaminated areas in our study had more often AML preceded by MDS than sporadic AML cases (18 of 82 cases vs. 6 of 70 cases respectively, $p < 0.05$). For high frequency of myelodysplastic prephase, radiation-associated leukemia resembles AML induced by alkylating drugs. The presence of lymphadenopathy, splenomegaly, hepatomegaly, extramedullary lesions such as involvement of skin and central nervous system, severe hemorrhagic syndrome at presentation was recorded in patients with spontaneous and radiation-associated AML with equal frequency. However, clinical course in patients exposed to IR, more often complicated by infections at the onset of the disease. The performance status of patients with radiation-associated AML was generally lower. A significantly greater part of the radiation-associated AML cases had the performance score estimated by the WHO scale as more than 2 points. Every fifth patient with AML among Chernobyl victims had contraindication to intensive chemotherapy for remission induction (16 of 82 cases vs. 2 of 70 in the control group, $p < 0.05$). Significant difference between groups of patients with radiation-associated and spontaneous AML in the number of cases, which can be assigned to the adequate chemotherapy, reduces the chances of Chernobyl patients for the long-term survival without relapse.

The lactate dehydrogenase (LDH) level more often exceeded the value of 500 units/L in Chernobyl AML and ALL patients compared to sporadic leukaemia cases ($p < 0.05$). The patients with radiation-associated AML initially had lower number of red blood cells and hemoglobin level in blood. Significantly more patients of this group compared to the control group required red blood cells transfusions at the time of diagnosis judged by the hemoglobin level lower than 80 g/L. AML in clean-up workers and

inhabitants of contaminated areas more often than AML in the control group were represented with promyelocytic morphology and rarely had M4eo variant of the disease.

The analysis of patients with radiation-associated AL treatment results provides the convincing argument on the need to refine the existing classification of AL in terms of extraction of this entity into the separate category of leukemia. The effectiveness of AML and ALL treatment in clean-up workers and inhabitants of contaminated areas of Ukraine was substantially different from those for spontaneous cases for overall survival and response to therapy. Patients with radiation-associated AL both myeloid and lymphoid nature rarely reach complete remission and have a shorter overall survival. Median survival for patients with radiation-associated and spontaneous AML was 99 and 407 days respectively ($p<0.0001$), and 293 and 807 days respectively for patients with ALL ($p<0.05$). Overall survival curves are shown in Fig. 5.8.

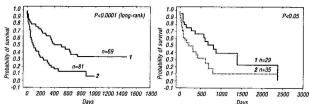


Fig. 5.8. Overall survival of patients with spontaneous and radiation-associated AML (left) and ALL (right) in individuals exposed to IR due to the Chernobyl accident: 1 — group of patients with spontaneous leukemia, 2 — group of patients with radiation-associated leukemia.

Complete remission was achieved in only 4 of 64 patients with radiation-associated AML in our study whereas in 31 of 64 people of control group $p<0.0001$, and in 14 of 30 patients with radiation-associated ALL compared with 22 of 28 spontaneous ALL cases, $p<0.05$. Moreover, Chernobyl AML patients more frequently than the patients with spontaneous leukemia were considered as resistant to standard chemotherapy (43 of 64 vs. 23 of 64 cases respectively, $p<0.001$).

Besides the clinical features, we have studied the phenotypic characteristics, including expression of multi-drug resistance determinants (MDR) in radiation-associated AML patients. Drug resistance has multiple causes and has been described in membrane transport, drug metabolism, drug targets, cellular repair mechanisms, and in the ability of cells to recognize a toxic insult and engage apoptosis. We examined the expression of drug transporters such as P-glycoprotein (P-gp), lung resistance protein (LRP) and multidrug resistance associated protein (MRP), apoptosis-related proteins such as BCL-2, Bax, Bcl-XL, p53 and Fas and a target of anticancer drugs such as topoisomerase II α in AML patients exposed to IR due to the Chernobyl accident.

Most researchers believe that the most significant role in the formation of MDR plays P-gp [18]. There is the hope that pharmacological blocking of P-gp function might significantly improve the results of standard chemotherapy. According to our experi-

ence, leukemic cells in patients with radiation-associated AML were more often P-gp positive than in patients with spontaneous disease (12 out of 20 cases compared to 9 of the 32 cases respectively, $p < 0.05$). Indicative results of flow cytometric analysis of leukemic cells are shown in Fig. 5.9. There was an association of overexpression of P-gp by leukemic clone with resistance to chemotherapy.

However, there was no difference found in overall survival of P-gp positive and negative patients both in all studied cases and at separate analysis of radiation-associated and sporadic AML cases. This could be explained in the way that P-gp negativity might not be translated into the overall survival advantage over the patients with P-gp overexpression because of early mortality if the supportive therapy was not adequate. It is likely that the greater accumulation of cytostatics in leukemic cells at P-gp negativity, which is accompanied by the more intense tumor destruction compared to antigen positivity, leads to pronounced and life threatening intoxication and electrolytic disturbances, which causes increased death risk. As a consequence, in case of insufficiency of supportive care, the advantage of P-gp negative patients in complete remission achievement might be balanced by early lethality.

There was not found any difference between radiation-associated and sporadic AML in terms of LRP, MRP, p53, Bax, and Bcl-XL expression. In general, the low expression of MRP, which we observed in studied cases, could reflect the peculiarities of Eastern European populations of patients with AML. We found also that the expression of P-gp, MRP and LRP in leukemic cells of patients with ALL, who were exposed to IR due to the Chernobyl accident, is not a significant factor determining the unsatisfactory results of their treatment, and the modulation of transport proteins function should not be considered for treatment strategy of radiation-associated ALL. Our studies on P-gp, MRP and LRP overexpression could provides the information to assess the relative impact of transport proteins in the formation of MDR in radiation-associated and spontaneous AML and ALL (Table. 5.6).

Table. 5.6. Overexpression of transport proteins on leukemic cells in patients with radiation-associated and spontaneous AML and ALL

Determinants of multi drug resistance	AML		ALL	
	radiation-associated	spontaneous	radiation-associated	spontaneous
P-gp overexpression	+++	++	—	—
MRP overexpression	—	—	—	—
LRP overexpression	+	+	—	—

The relative degree of overexpression is marked by scale from (-) to (+++), where (-) means the lowest degree or absence of sign, and (+++) means the largest degree.

The important reason of MDR in radiation-associated AML in victims of the Chernobyl accident might be the alteration of the apoptotic process. The leukemic cells in AML patients exposed to IR statistically more often expressed anti-apoptotic protein BCL-2 (12 of 21 vs. 6 of 35 cases respectively, $p < 0.005$) and rarely expressed pro-apoptotic receptor Fas (9 of 21 vs. 5 of 35 cases respectively, $p < 0.05$). The difference of tumor cells in radiation-associated compared to spontaneous leukemia in presenting as either BCL-2 positive and/or Fas negative reached even higher significance ($p < 0.001$). Moreover, radiation-associated nature of the disease frequently associated

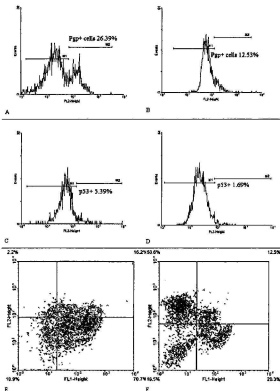


Fig. 5.9. An increase of P-gp positive cells number (M2-region) is radiation-associated (A) in comparison with spontaneous (B) AML after staining with MRK-16 primary and Anti-Mouse-kappa-PE secondary antibody. An increase of the number of cells reacting with Pab-240 antibody to p53 (M2-region) in radiation-associated (C) in comparison with spontaneous (D) AML after staining with Anti-Mouse-kappa-PE secondary antibody. At dot-plots representing staining with Anti-BCL-2 FITC (FL-1) and Anti-Fas PE (FL-2) antibodies a significant increase was shown of BCL-2 positive cells with moderate Fas expression in radiation-associated AML (E), and substantial Fas expression in spontaneous AML (F).

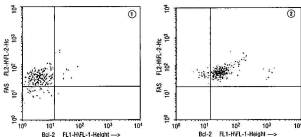


Fig. 5.10. Histograms of FAS and BCL-2 expression on blast cells of the patient R, who participated in the clean-up work at the Chernobyl NPP, at different stages of evolution of the tumor clone: 1 — at the time when diagnosis of MDS, refractory anemia with excess of blasts was verified, 2 — during the transformation of MDS to AML, M2.

with asynchronism of BCL-2 and Fas expression. BCL-2 positivity combined with Fas negativity was found in 4 out of 21 exposed patients, whereas in none of 35 spontaneous AML cases ($p < 0.05$).

The patients with AML post MDS more often were BCL-2 positive ($r = 0.3$, $p < 0.05$) compared to those without myelodysplastic prephase. We believe that the preceding MDS is a specific feature of leukemic process in persons exposed to IR. Acquiring by the MDS tumor cells of BCL-2 overexpression might be the further stage in the evolution of the tumor clone. Radiation-induced nature provides leukemia with pathogenetic difference and AML in patients irradiated due to the Chernobyl accident is often characterized by myelodysplastic prephase in the disease development. The further transformation of MDS in overt AML could be driven by BCL-2 mediated apoptosis alteration. The histograms on Fig. 5.10 show how the transformation of MDS, refractory anemia with excess of blasts in patient participated in Chernobyl clean-up work into AML M2 was accompanied by changes of BCL-2 negativity of tumor cells into BCL-2 positivity.

Our data demonstrated that, the expression of programmed cell death regulators such as FAS, p53, BAX, BCL-2 and BCL-XL does not discriminate between the radiation-associated and spontaneous cases of ALL. However, the expression of inhibitors of apoptosis such as BCL-2 and BCL-XL could be the significant reason for the drug resistance of leukemic processes in ALL in general. Thus, the modulation of these proteins function represents a promising direction to improve treatment outcome in ALL universally. According to our experience, the overexpression of BCL-2 and BCL-XL is extremely common for ALL both of radiation-associated and spontaneous aetiology. Other researchers have shown that BCL-2 and BCL-XL cells determine resistance to the action of cytostatic agents. Leukemic cells with high levels of BCL-2 and BCL-XL are characterized by autonomous growth. In addition, BCL-2 and BCL-XL makes cells unable to undergo FAS mediated apoptosis. It is shown that leukemic cells of patients with minimal residual disease express high levels of anti-apoptotic proteins BCL-2 and BCL-XL. The assumptions were made about their crucial role in the survival of

the tumor clone at chemotherapy. Given the aforementioned, the drug that can overcome the inhibitory effect of BCL-2 and BCL-XL to the tumor death will be the useful supplement to therapeutic applications being used to treat ALL in patients affected by the Chernobyl disaster.

The performed FISH experiments identified the high frequency of BCR/ABL translocation in blast cell of clean-up workers and inhabitants of contaminated areas with AL. The liquidators with AML, who experienced the greatest impact of IR, were found to have BCR/ABL translocation significantly more frequently than the patients of control group with sporadic AML (3 out of 6 vs. 1 out of 20 cases respectively, $p < 0.05$). While we found that radiation-associated leukemia is characterized by translocation of BCR/ABL disproportionately more often than spontaneous, considering this abnormality as specific feature of radiation-induced tumour would be too ambiguous. Given the high frequency of BCR/ABL positivity in AML and ALL in patients exposed to ionizing radiation due to the Chernobyl accident, the differential diagnosis between AL and chronic myelogenous leukemia might be challenging in some cases and will make it necessary to use the broad spectrum of clinical, cytological, cytogenetic, and molecular genetic parameters. This data provides the background for the concept of treatment of secondary AML and ALL. Expression of BCR/ABL is an essential factor of drug resistance in case of leukemia. It provides independence of the tumor cells from growth factors and disrupts the implementation of apoptosis. The fact that Chernobyl patients are often characterized by BCR/ABL translocation opens the perspectives for the use of tyrosine kinase inhibitors like imatinib. Unfortunately, there are none ongoing clinical trials testing the effectiveness of imatinib in Ph positive AML and publications on its use in AL are limited to date.

The data obtained in our study demonstrating the likely contribution of pro-apoptotic (FAS, BAX, p53) and anti-apoptotic (BCL-2, BCL-XL, BCR/ABL) regulators to MDR in radiation-associated and spontaneous AML and ALL are presented in Table 5.7. We believe that the primary diagnostic work-up for radiation-associated AL should include the tests for MDR markers. Given their prevalence and clinical significance, the expression of P-gp, BCL-2, and FAS for AML; BCL-2 and BCL-XL for ALL, and the presence of translocation BCR/ABL both for AML and ALL should be determined.

Table 5.7. The likely contribution of proapoptotic and antiapoptotic regulators to MDR in radiation-associated and spontaneous AML and ALL

MDR determinants	AML		ALL	
	radiation-associated	spontaneous	radiation-associated	spontaneous
FAS	++	+	—	—
BAX	—	—	—	—
P53	+	—	—	—
BCL-2	+++	+	++	+
BCL-X _L	+	+	+	++
BCR/ABL	++	—	—	—

The relative degree of overexpression is marked by scale from (-) to (+++), where (-) means the lowest degree or absence of signs, and (+++) means the largest degree. For proapoptotic regulators (FAS, BAX, p53) the lowest degree of contribution means that their function is implemented fully and vice versa.

Series of experiments we aimed to identify potential targets for chemotherapeutic drugs for the treatment of radiation-associated AML and ALL. Many researchers have conducted studies of DNA topoisomerase II, and primarily their α forms in the context of the features of the clinical course and prognosis, and to response to cytostatic treatment of human cancers. Expression of this enzyme was associated with prognostic markers, such as Ki67 in non-Hodgkin's lymphomas and gastric cancer, fraction of cells in phase of synthesis in breast cancer, and with cyclin A in leukemia.

Regarding the relationship between expression of DNA topoisomerase II α and sensitivity of tumor cells to DNA topoisomerase II inhibitors, most of the work published in the literature were performed on the cell lines. Some of them allowed concluding that cells that express more enzyme are more sensitive to inhibitors of topoisomerase II, and the way you can reduce resistance to treatment is to increase the level of topoisomerase II [19].

The data of our study for the levels of topoisomerase II α expressing by substrate cells in AML and ALL patients go along with the published data of other researchers. We found no benefit in patients with positive expression of the enzyme on the overall survival and believe that the high level of expression of the enzyme is not a favourable prognostic factor in terms of AL treatment results. The method evaluating the levels of the enzyme has limited prospects for application in clinical practice. There was no difference between the frequencies of DNA topoisomerase II α positivity in patients with spontaneous and radiation-associated AML (0 out of 21 vs. 2 out of 34 cases respectively, $p=0.52$) and ALL (2 out of 10 vs. 0 out of 9 cases respectively, $p=0.47$).

Thus, acute leukemia in patients exposed to IR due to the Chernobyl accident is characterized by drug resistance resulting from its pathogenesis and pronounced MDR phenomenon with the lack of targets for directed tumor therapy. The tumor cells in radiation-associated leukaemia are likely not responding to conventional chemotherapy. Therefore, if we can not stratify patients with radiation-associated AL according to the results of karyotype analysis and molecular-biological characteristics of substrate cells to certain risk group, it should be considered as a patient of group with unfavourable prognosis of the disease.

The formation of drug resistance, its multifactor nature, molecular characteristics of substrate cells in radiation-associated leukemia forces using the transplantation of HSC as a necessary component of therapeutic strategies. Given the unfavourable prospects for achieving the complete remission by conventional chemotherapy, the initial chemotherapy can be positioned as cytoreductive before making HSC transplantation and may not have a goal of achieving complete remission during treatment of radiation-associated AL. The proposal for treatment strategy for clean-up workers at the Chernobyl accident and inhabitants of contaminated areas with AL is graphically depicted in Fig. 5.11 and Fig. 5.12.

In the absence of prognostic markers of favourable response to conventional chemotherapy, such as translocation of *AML1/ETO*, *PML/RAR-alpha* and *CBFb/MYH11* in AML, and the impossibility of performing allogeneic HSC transplantation, patients with radiation-associated AL should be considered as priority candidates for inclusion into clinical trials of new treatments. The promising directions are modulation of the function of apoptotic molecules and P-gp, tyrosine kinase receptor inhibitors, and inhibitors of

angiogenesis. High frequency of *BCR/ABL* translocation in radiation-associated AML and ALL and its adverse prognostic significance forces to prove the effectiveness of imatinib in their treatment.

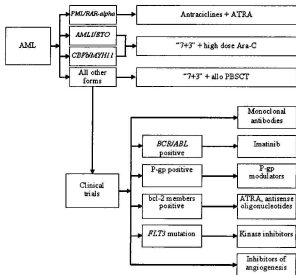


Fig. 5.11. Treatment strategy of radiation-associated AML in Chernobyl victims

The clinical course, outcomes, clinical-hematological features, molecular biological characteristics of AML and ALL that developed in persons exposed to IR due to the Chernobyl accident significantly distinguish radiation-associated leukemic process from spontaneous one. There are many factors that can determine the drug resistance, which are characteristic for radiation-associated leukemia and should be considered in planning the therapy of Chernobyl victims. The study of multistage pathogenesis of radiation-associated AML and ALL, events that change the balance between the cell proliferation and death, and form the drug resistance of radiation-induced diseases should be continued. The accumulation of knowledge about the pathological process will allow modifying the therapeutic goals and treatment strategies, to refine diagnostic markers and useful indicators of prognosis in radiation-induced leukemia.

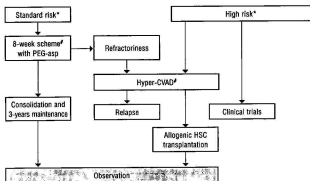


Fig. 5.12. Treatment strategy of radiation-associated ALL in Chernobyl victims.

1. (*) — ALL of common type and pre-B type, T-ALL if WBC count is less than $30 \times 10^9/L$, no translocations t(9;22), t(4;11), 2. (**) — early pre-B, bilinear ALL, early T-ALL if the WBC count exceeds $30 \times 10^9/L$, translocation t(9;22) or t(4;11) is present, 3. (#) — program details are in [20].

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5.3. RADIATION AND MYELOPROLIFERATIVE DISEASE

Monitoring of the blood disorders and diseases within 24 years after accident allowed to select a number of peculiarities of hematologic malignancies, including chronic myeloproliferative: chronic myelogenous leukemia, osteomyelofibrosis among persons exposed to negative factors of the Chernobyl accident [1].

Along with many unresolved problems further study of the peculiarities of the pathogenesis of chronic myeloproliferative diseases (CMPD) in individuals affected by the Chernobyl accident in remote post-accident period remains as urgent one.

Important reduction of the apoptosis intensity, which leads to expansion of the pathological clone cells plays an essential role in the pathogenesis of CMPD. In this regard the study of regulatory mechanisms responsible for apoptosis in hematopoiesis system is extremely important.

At present it is well known that the apoptosis processes are under the influence of affecting the functioning of apoptosis genes-inducers and suppressor of apoptosis — *p53* and *bcl-2*. Activation of the gene *p53*, located on the short arm of chromosome 17, occurs when DNA is damaged, and because of many other processes in the cell (hypoxia, aging, etc.) [2]. Running process of apoptosis occurs by activating *p53* family genes proapoptotic *BAX* or *BID-bcl-2*. Mutations in *p53* are found in the case of tumor progression [3].

The function of genes *bcl-2* family is the inhibition of apoptosis. In norm this protooncogen localized on chromosome 18q21, a chromosomal translocation as a result of t (14; 18) moves in 14q32 [4]. This leads to gene transcription dysregulation and hyperexpression of *bcl-2* protein that is missing on primitive hematopoietic predecessor, but is expressed on immature precursors of T-and B-cells and its expression increases with their maturation [5]. Peripheral blood lymphocytes at rest in most express *bcl-2* and CD95 antigen. In activated lymphocytes in vitro and in vivo T-expression of *bcl-2* decreased.

One of the mechanisms regulating the process of apoptosis in normal and malignant transformed hematopoietic cells is receptor-ligand system CD95/FasL. This system is induced through the CD3 T-cell antigen receptor. Binding of T-cell receptor antigen with monoclonal antibodies anti-CD3 leads to activation of CD95-ligand, which interacts with its own receptor and initiates apoptosis. CD95-receptor, which mediates apoptosis, was detected in malignant transformed hematopoietic cells in chronic myeloid leukemia, myelodysplastic syndrome, acute myeloid leukemia [6]. Th1⁺ CD4⁺ killer cells, which transmit the signal to cells to apoptosis, are characterized by a pronounced expression of CD95-ligand.

Cytostatic drugs that are prescribed for the treatment of chronic myeloproliferative diseases act on malignant transformed cells through CD95-receptor-ligand system. Thus, cells that express CD95-receptor are more sensitive to entry into Fas-mediated apoptosis and this makes it possible to predict the further course of the disease and the effectiveness of therapy.

The literature presents data on study characteristics of apoptosis in patients with chronic myelogenous leukemia and osteomyelofibrosis [6]. However, only a few works devoted to the diagnosis of disorders of apoptosis processes and molecular genetic

changes in the genome affected by the Chernobyl accident [7]. These aspects require further study.

Significant progress recently achieved in the treatment of chronic myelogenous leukemia — a single oncohematological disease with characteristic cytogenetic aberration Ph⁻-chromosome, which is formed by translocation between 9q34 and 22q11 — t (9; 22) and is a marker for 90–96% of patients with the disease. Due to the gap in the M-BCR region (b1-b5) of chromosome 9 and the gap in the a2 exon of the gene *c-ABL*, which is localized on chromosome 22, is formed chimerical gene *BCR/ABL*, located on the shortened 22 chromosome.

Protein p210, which encodes this gene has a higher protein kinase activity than p145 by which *ABL* gene is normally synthesized [7]. The consequence of gene activation *ABL* in gene *BCR/ABL* is a lengthening of granulocytic cells life expectancy sprout in connection with reduction processes of apoptosis and increased proliferative capacity of cellular elements. Imatinib is a drug that effectively inhibits *BCR/ABL*-tyrosine kinase at the cellular level, selectively suppresses proliferation and restores apoptosis of cell lines positive for *BCR/ABL*. However, the effect of the drug also has limitations, because at some stage of treatment effectiveness decreases or levels out and the disease enters the terminal phase, caused by the development of resistance to imatinib.

Some authors observed statistically unreliable increasing of CD38 antigen in CML patients treated by imatinib in cases of the absence of adequate response to therapy. If initial level of CD38 antigen is high in primary CML patients, it becomes lower with the achievement of remission and increase in the absence of response to therapy. There is also an idea that the basis of resistance to the action of tyrosine kinase inhibitors is the accumulation in the subpopulation of cells CD34⁺CD38⁺ different mutations that affect the effectiveness of treatment [8].

The osteomyelofibrosis (OMF) is among radiation-associated hematological diseases, but [9] questions on the dose dependency remain unsolved.

It is known that the sensitivity of precursor megakaryocytes to ionizing radiation is higher in comparison with predecessors of leuko- and erythropoiesis. In the process of megakaryocytes maturation change their functional properties, metabolic activity and ability to accumulate from various environmental compounds.

Mature megakaryocytes have a higher content of ATP, more intensely include nucleotides, adenine, arachidonic acid, they synthesise proteins, phospholipids, cholesterol several times more intensively than immature. The composition of granules content in megakaryocytes depends on the processes of protein synthesis. Most of biochemical characteristics and functional properties of platelets are determined by megakaryocytes, depending largely on the degree of maturity them [10]. The activity of acid phosphatase contained in lysosomes and glycogen — energetic substance accumulated in the cytoplasm reflect the intensity of metabolic processes in megakaryocytes.

Thus, summarizing current information about the effects of ionizing radiation on the hematopoietic system clear signs of dependence of malignant diseases of hematopoietic and lymphoid systems on inducing effects of ionizing radiation were revealed. If the influence of ionizing radiation in high doses and effects of such actions are widely covered by domestic and foreign literature, the impact of IR in the range of low-dose and long-term effects of such actions are contradictory. To date, no systematic data on the

morphological and functional abnormalities in cells of hematopoietic system at various doses are available. So, it is known that as a result of IR among the first from hematological diseases appears acute leukemia, chronic myeloid leukemia, and then — solid tumors [1]. All the above indicates the need for research to obtain new knowledge in radiation hematology.

Incidence of chronic myeloproliferative disease among residents of Ukraine is about 1.5 per 100 000. Among the sufferers of the Chernobyl accident, this figure is higher, but there are certain features of the course CMPD in this category of patients. Under the supervision of the hematologists of RCRM for 25 years after accident were more than 700 patients with chronic myeloproliferative diseases among them — 132 patients who were exposed to a complex of negative factors of the Chernobyl accident.

CMPD course, both in affected by the Chernobyl accident with radiation dose in the range 17–21.5 cSv in March and in the opposite group of people, due to the general condition of the patient, the depth of hemopoietic disorders, pathological stage process, timely diagnosis and therapeutic adequacy measures. For most patients in the clinical sense CMPD were characterized by organomegaly with increased size of the spleen and liver, increase the number of leukocytes with a shift to immature forms of granulocytes, thrombocytosis, gradual development of anemia.

The analysis of peripheral blood of patients with chronic myeloid leukemia showed that the number of red blood cells and hemoglobin level in affected by the Chernobyl accident patients was significantly higher (4.19 ± 0.005 T/L and 140.66 ± 0.29 g/L, respectively, compared with opposite group — 3.28 ± 0.01 T/L and 110.06 ± 0.45 g/L), and the number of white blood cells — significantly lower (14.5 ± 0.11 g/L compared with 48.01 ± 0.10 g/L). The same trend was observed for platelet indices (207.33 ± 1.20 g/L and 396.3 ± 6.65 g/L, respectively).

The peripheral blood of affected patients have significant reduction in both immature (promyelocytes, myelocytes, metamyelocytes) and mature granulocytes (band and segment). The same pattern was found for lymphocytes and monocytes ($p < 0.05$).

For bone aspirate patients — sufferers of the Chernobyl accident is characterized significant reduction in myelokaryocytes against increasing the number of megakaryocytes compared with the opposite group.

The number of mature granulocytes (band and segment) in the liquidator's group exceeded similar values in the group of untreated patients. They also found probable decrease eosinophilic granulocytes, while the percentage of basophilic granulocytes was significantly higher ($p < 0.05$). There were also reduction of lymphocytes, monocytes and plasma cells ($3.3 \pm 0.04\%$, $1.8 \pm 0.19\%$ and 0.1 ± 0.001 , respectively).

Erythroid lineage of this group are characterized by reliable reduction of the percentage proerythroblasts, basophilic erythroblasts with a gradual alignment of content and oxyphilic polymorphic erythroblasts.

For the peripheral blood of chronic phase increase of the number of mature and immature (myelocytes, promyelocytes) as neutrophilic and eosinophilic granulocytes, basophilic CML was characterized. A number of qualitative differences in CML were registered in hematopoietic cells.

In a small proportion of CML patients (10–12%) in the bone marrow dominated by immature megakaryocytes without detaching cytoplasm and isolated mikromega-

kariocytes indicating the presence of dysplastic disorders in megakaryocytes lineage of hematopoiesis.

Because CML is based on the proliferation of granulocytic lineage, the cells affected by the accident showed dysgranulocytopenia phenomenon with varying degrees of severity. Qualitative changes included: nuclei of neutrophils fragmentation, toxic grain in eosinophilic and neutrophilic granulocytes cytoplasm, vacuolization their nuclei and cytoplasm and degranulation of the cytoplasm. The presence of granulocyte cell with toxic, clearly formed grain, and cells with few azurophilic granules and vacuolization of cytoplasm and a complete absence of granules (degranulation), and the presence Pelger anomaly attracted attention (Fig. 5.13, 5.14).

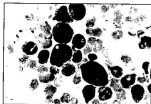


Fig. 5.13. Peripheral blood smear photomicrograph of patient with CML. Nucleus and cytoplasm vacuolization of myelocyte and granulocytes. Staining by Romanovsky, $\times 1000$.

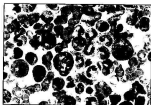


Fig. 5.14. Peripheral blood smear photomicrograph of CML patients. Hyper- and hypogranular neutrophils. Staining by Romanovsky-Gimza, $\times 1000$.

A feedback was established between the fragmentation of the mentioned cells and exposure ($r = -0.87$, $p < 0.05$). Revealed that there is a direct relationship between these parameters ($r = 0.39$, $p < 0.05$), but reverse with toxic granularity in the cytoplasm of neutrophils ($r = -0.61$, $p < 0.05$) were revealed and no such connection in degranulated neutrophils ($r = -0.05$, $p < 0.05$).

In CML patients from the radiation affected group a significant increase of cells with nuclei fragmentation, toxic grain of cytoplasm, nucleus and cytoplasm vacuolization and degranulation of the cytoplasm were seen compared with a group of non-irradiated individuals ($p < 0.05$).

Lymphocytes in chronic myeloid leukemia patients were characterized by morphological features, namely: wide cytoplasm, hairy and basophilic cytoplasm, nucleus and cytoplasm vacuolization. The percentage of lymphocytes with changes in the cytoplasm (basophilia, and hairy cytoplasm) did not differ in both groups of investigated patients. At the same time significantly increased levels of lymphocytes with vacuolization of the cytoplasm and nucleus in a group of sufferers were observed as compared to the opposite group of patients.

Erythroid lineage of CML patients in both groups was characterized by moderate anisocytosis: erythrocytes of different forms from microcytes to megalocytes against the background of increased number of normocytes.

Enzyme system in CML irradiated patients was expressed in granulocytic cells as significant increase myeloperoxidase activity (MPO) (Fig. 5.15) and acid phosphatase (APh) ($p < 0.05$) while reducing the glycogen content compared with the non-irradiated group of patients.

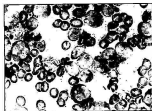


Fig. 5.15. Microphoto bone marrow. MPO in neutrophilic myelocyte (A). Staining by Loeffle $\times 1600$.

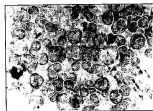


Fig. 5.16. Bone marrow microphoto. Glycogen-positive neutrophils (A). Staining by McManus and Hotchkiss, $\times 1000$.



Fig. 5.17. Microphoto of histological slide of bone marrow patient with osteomyelofibrosis. The development of bone marrow fibrosis, osteoid tissue replacement. Staining with hematoxylin-eosin, $\times 200$.

Glycogen content in the group of sufferers was significantly lower than in the comparison group and was found in all granulocyte cells: in a part of them as a diffuse reaction in the other part — as diffuse-granular (Fig. 5.16).

Peripheral blood lymphocytes of affected patients with CML had elevated levels of acid phosphatase ($p < 0.05$) with no difference in glycogen content in both groups. In bone marrow trepanobiopsy of CML patients against the base of some reduction of cellularity typical fibrotic changes with increased number of megakaryocytes were seen compared with a group of unexposed patients.

In the chronic phase morphological picture of bone marrow tissue in patients with osteomyelofibrosis was characterized by different degrees of the bone marrow architectonics disruption, hypercellularity due to granulocytic and erythroid cells proliferation at different stages of maturation. For patients affected by the Chernobyl accident the enhanced diffuse distribution reticulin fibers and fibrous transformation of varying degrees of manifestation were characteristic. Bone marrow sinuses were narrowed, endothelium — hyperplastic, some vessels contained erythrocytes (Fig. 5.17).

In one third of exposed patients with osteomyelofibrosis (OMF) the bone marrow fibrous tissue contained elongated reticular cells and dramatically deformed, sometimes as many as stratum, altered megakaryocytes.

The most striking change were recorded in megakaryocytic lineage of hematopoiesis in irradiated people with CML, but analysis of the cellular elements distri-

bution by the degree of differentiation showed no difference of the percentage megakarioblasts, promegakaryocytes, basophilic, polychromatophilic, oxyphilic megakaryocytes and free nuclei from the control indices (Fig. 5.18).

The total number of functioning megakaryocytes was significantly higher in comparison with opposite group ($p \leq 0.01$) (Fig. 5.19). Probably higher were percentage of basophilic megakaryocytes that form platelets ($p \leq 0.05$), and lower — polychromatophilic ones ($p \leq 0.05$) in comparison with opposite group. Number of oxyphilic megakaryocytes that form platelets did not depend on exposure.

Moreover, in CML, especially among exposed patients increased one-segmented ($9.33 \pm 1.5\%$) and two-segmented megakaryocytes ($13.70 \pm 1.8\%$) compared to opposite group ($2.8\% \pm 0.6\%$ and $2.5 \pm 0.6\%$, respectively $p \leq 0.01$, $p \leq 0.001$) (Fig. 5.20). These changes are accompanied by reduction in cell number with 4–7 segments of nuclei, which were the largest population of cells in the control group ($p \leq 0.001$). Should also be noted A reduction of hypersegmented megakaryocytes ($p \leq 0.01$; Fig. 5.20) that are the hallmark of osteomyelofibrosis.

Simultaneously, in exposed CML patients the number of micro- ($11.81 \pm 2.2\%$) and dysplastic megakaryocytes ($7.10 \pm 1.5\%$) increased in contrast to the opposite group $0.9 \pm 0.3\%$, $0.6 \pm 0.3\%$, respectively). Aging population of megakaryocytes i.e., cells that have entered the path of apoptosis also increased. This was evident from the increase of megakaryocytes with piknotic nuclei ($9.71 \pm 2.1\%$) and isolated one or more segments of nuclei ($4.29 \pm 0.9\%$) compared with opposite

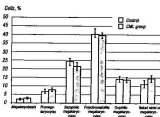


Fig. 5.18. Distribution of the megakaryocytes differentiation degree.

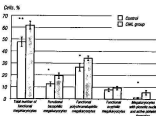


Fig. 5.19. The functional activity of megakaryocytes, depending on the degree of differentiation. **, *** significant difference compared with the control group at $p \leq 0.05$, $p \leq 0.01$, respectively

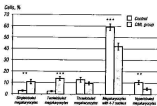


Fig. 5.20. Distribution of the number of megakaryocytes nuclei segments. **, *** likely difference compared with the control group at $p \leq 0.01$, $p \leq 0.001$, respectively).

group ($1.1 \pm 0.4\%$, $p \leq 0.01$; $1.3 \pm 0.8\%$, $p \leq 0.05$). The above data proved for disturbance of bone marrow megakaryocytes differentiation in CML even at the early chronic phase.

Metabolic activity of megakaryocytic lineage cells in patients with OMF showed increased glycogen content in the bone marrow megakaryocytes of patients exposed to IR, compared with the opposite group, $p < 0.05$. Simultaneously, a combination of trends towards reducing the number of cells with low glycogen ($18.27 \pm 2.93\%$ and $26.08 \pm 3.36\%$ respectively, $p < 0.07$) (Fig. 5.21 A) and increase in the number of cells having high glycogen content ($50.04 \pm 3.45\%$ and $40.81 \pm 4.14\%$ respectively, $p < 0.09$) (Fig. 5.21 B).

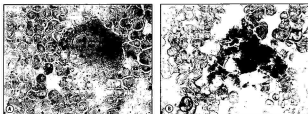


Fig. 5.21. Microphoto of bone marrow of essential thrombocythemia patient. (A) Megakaryocyte with low glycogen activity. Diffusely granular-type reaction. (B) Megakaryocyte piknotic nucleus, cytoplasm which "explosion" is separated into platelets, with a high content of glycogen. PAS-reaction by the method of McManus and Hotchkiss. $\times 1000$.

It should be noted that the majority of cells with a high content of glycogen in affected patients with OMF by morphological characteristics of megakaryocytes belonged to piknotic nuclei, cytoplasm was actively "explosion-like" separated into platelets (Fig. 5.21 B).

The activity of acid phosphatase in bone marrow megakaryocytes of patients affected with OMF was different from the opposite group (2.01 ± 0.10 and 2.07 ± 0.06 , $p = 0.7$) while the same enzyme activity in peripheral blood platelets was reduced compared with control ($p < 0.05$ and $p < 0.005$ respectively). This can be explained by intense thrombocytopoiesis with being in a state of considerable apoptotic fate of bone marrow cells, for which most defined the high activity of acid phosphatase, which resulted in "smoothing" of the negative balance of the average ratio in bone marrow megakaryocytes. Increased percentage of megakaryocytes with a high content of glycogen in patients with OMF irradiated by the Chernobyl accident characterized the energy potential of the cells and shows a much greater percentage of mature and active apoptotic separated platelets megakaryocytes, compared with the opposite group [11].

Cytogenetic and molecular genetic analysis of bone marrow cells in all patients with CML showed the presence of typical translocation $t(9;22)(q34;q11)$ — a so-called Ph^+ -chromosome, which was specific for this disease in the absence of other additional and related clones and clonal karyotype defects were detected only at the stage of disease progression. Patients with CML, who were subjected to ionizing radiation had no other specific violations of the karyotype.

In samples of bone marrow and peripheral blood of patients with CML the expression of chimerical gene *BCR/ABL*, which was formed by the merger of the gene *BCR*, having a gap in the *M-bcr* region (exons b1-b5) with $\alpha 2$ exons of the gene *c-ABL* was noticed. Thus the expression of *M-bcr* mRNA type of b2a2 or b3a2 appeared, leading to protein *bcr/ABL* with a molecular weight of p210 kDa. Sometimes the protein with a molecular weight of p190 (*m-bcr*) appeared, which was an unfavorable prognostic sign of disease.

Three options of transcripts were observed depending on gaps of *BCR* and *ABL* genes localization: b3a2, b2a2, e1a2. Most patients (60%) had transcript b3a2. In 36% cases b2a2 was found, and in 4% co-expression of two transcripts b3a2 and e1a2, or b2a2 and e1a2 was observed. Sometimes this fact is considered as a sign polyclonal process. Cytogenetic studies in these cases revealed the same realignment $t(9; 22)(q34; q11)$ [12].

In 4% of exposed patients with CML additional clonal violations of karyotype were found, such as: 46,XY,der(9)t(9;?)(q34;?)[20]; 47,XX,t(9;22)(q34;q11),+der(22)t(9;22)(q34;q11)[2]/46,XX[18]; 46,XX,t(9;22)(q34;q11),t(16;19)(q13;q34)[20]; 46,XX,der(9)t(9;?;22)(q34;?;q11)[20]; 46,XY,t(9;19;22)(q34;p13.1;q11),+mar[7]/46,XY[13]; 47,XX, t(9;22)(q34;q11),+der(22)t(9;22)(q34;q11)[12]/46,XX,t(9;22)(q34;q11)[17]; 46,XY,t(9;22)(q34;q11)[4]/46,XY,t(2;9;22)(q13;q34;q11)[1]/46,XY[15].

The FISH-analysis, which allows determine the type of translocation for more metaphases is helpful in such cases (Fig. 5.22, 5.23).

During cytogenetic analysis of bone marrow cells of patients with osteomyelofibrosis clonal disorders karyotype was not detected in any case (Fig. 5.24).

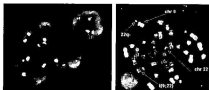


Fig. 5.22. Metaphase and interphase nuclei of bone marrow cells of CML patients with chromosome translocation (9, 22), 10 $\times$ 100.

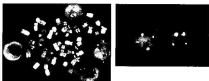


Fig. 5.23. Metaphase and interphase nuclei of bone marrow cells of CML patients with chromosome translocation (9, 22), with additional genetic material, 10 $\times$ 100.



Fig. 5.24. Metaphase and interphase nuclei of bone marrow cells from a patient with osteomyelofibrosis; a normal distribution of hybridization signals *ABL* and *BCR*, 10 $\times$ 100.

Immunophenotype of peripheral blood and bone marrow cells in patients with CML had a certain number of features. So for the chronic phase of CML increase in CD4⁺ cells level by reducing CD8⁺ cells, and CD3⁺, CD5⁺ were inherent to lymphoid cells. For granulocyte populations a decreased CD13⁺ count was characteristic as a possible precedent of blast crisis clinical manifestations. Subpopulation composition of leukocytes was characterized by an increase of eosinophilic subsets.

Discriminating analysis stages of programmed cell death in lymphoid and granulocyte subpopulations of patients with CML in chronic phase of disease (Fig. 5.25) showed that the relative number of cells at early and late stage of apoptosis (Annexin V⁺ and Annexin V⁺ PI⁺ cells) decreased against the background of treatment by tyrosine-kinase inhibitors. First of all, it concerned lymphocytic subpopulation. For myeloid lineage these parameters also decreased with increasing percentage of cells at the late stages of apoptosis. It reflected the delayed response to therapy and the lack of a true exit of cells in the programmed cell death, despite the alertness of cells to Fas-mediated apoptosis (Table 5.8).

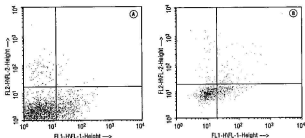


Fig. 5.25. Discriminatory analysis stage of programmed cell death in lymphocytic (A) and granulocytic (B) subpopulations: 1-box — only cells labeled PI (initial stage of necrosis); 2-box — cells, labeled as PI, and annexin-V-FITC (late stage of apoptosis); 3-box — cells that are not labeled or PI, nor annexin-V-FITC (living cells); 4-box — cells labeled only annexin-V-FITC (early stage of apoptosis).

Table 5.8. Comparison of the level of early (annexin-V⁺PI⁻) and late (annexin-V⁺PI⁺) apoptosis in peripheral blood of patients with CML in patients treated by imatinib

Leukocytes subpopulation	Annexin V ⁺		Annexin V ⁺ PI ⁺	
	before treatment	after treatment	before treatment	after treatment
CD45 ⁺ 14 ⁻ lymphocytes M±SD	22.94±8.91	7.90±3.74*	0.59±0.51	0.38±0.18
CD45 ⁺ 14 ⁻ granulocytes M±SD	86.47±8.20	72.25±3.45	3.51±1.65	4.60±2.16

*reliable differences between parameters before and after treatment ($p < 0.05$).

The level of CD38⁺ cells in the bone marrow of patients with CML is a prognostic criterion. Determination of this index showed the presence of correlation between the percentage CD38⁺ cells in the bone marrow of patients (below 30% and above 30%)

and number of metaphase with Philadelphia chromosome in bone marrow cells (1–4) (a method of interval estimation was used, the limits of confidence intervals: 0.72–0.58–0.44; 0.4–0.20–0.09).

A ranking of Cytogenetic responses were ranged by the degree of metaphases with Philadelphia chromosome percentage: 1 — 0%, 2 — 1–35%, 3 — 36–65%, 4 — 66–95% or more. It was found that at a value of up to 30% CD38-positive bone marrow cells in patients with CML was dominated 1 and 2 degree of response, indicating a high efficacy of the treatment by tyrosine-kinase inhibitors. When values of CD38-positive cells were above 30% 3 and 4 degree of cytogenetic response was dominated, which correlated with significantly worse response to therapy.

Thus, determining the level of expression of CD38⁺ cells along with the determination of levels of expression SD95, Bcl-2 and p53 is important for prognosis of chronic myeloid leukemia against a background of treatment by inhibitors of tyrosine-kinase, evaluation of possible responses to treatment, that influences on duration of obtained cytogenetic remissions and life quality of patients with this disease (Table 5.9).

Table 5.9. Predictive criteria for the course CML

Prognostic criteria	Indices
positive	CD95 — level within the normal values Bcl-2 — level within the normal values p53 — level within the normal values CD38 — level to 30%
negative	CD95 — level higher than the normal value Bcl-2 — level higher than the normal value p53 — level higher than the normal value CD38 — level higher 30%

Thus, radiation-associated chronic myeloproliferative disease: CML and OMF are characterized by a number of clinical, morphological, molecular genetic, cytogenetic, immunological features. For the bone marrow of OMF patients is inherent the development of various degrees of fibrosis, which causes clinical and hematological features of course of the disease. CML patients with disturbed differentiation of megakaryocytes results in an increased number of functioning immature megakaryocytes, hyposegmented forms and mikromegakaryocytes in bone marrow. The appearance of additional translocations and koexpression of transcripts b3a2 and eta2 in patients with CML in addition to Philadelphia chromosome suggests the development of polyclonal stage disease progression. Prognostic criteria for evaluating the effectiveness of tyrosine-kinase inhibitors treatment is the level of expression CD38⁺ cells in the bone marrow of patients (30% and above) and the number of Philadelphia chromosome metaphase in bone marrow cells.

Given our own research data optimized the treatment of patients with CML and OMF and criteria for evaluating the effectiveness of therapy.

Until recently the treatment of CML is widely used myelosan (busulfan, mileran), hydroxyurea (hydrea), interferons, cytarabin. However, because of the development of modern technologies in the treatment of malignant haematopoietic system are utilizing various inhibitors that are targeted to tumor cells, affecting the metabolic processes and enhancing the processes of apoptosis, which leads to the disappearance of signs of disease

and normalization of blood-forming system. One of the most famous of this group of drugs is imatinib that led to the revolutionary treatment of CML tactics and because of its high efficiency, a first-line drug for the treatment of Ph-positive cases.

The effectiveness of imatinib treatment estimated by normalization of peripheral blood cell and level of elimination of Philadelphia chromosome and Bcr-Abl, ie depth obtained hematological and/or cytogenetic remission. To determine the therapeutic management must also evaluate the level of expression along with cell CD38, CD95 level, Bcl-2 and p53, which are important for prognosis of chronic myeloid leukemia in patients who receive treatment by tyrosine kinase inhibitors.

Given the presence of unfavorable prognostic criteria of course, getting poor response to imatinib therapy in chronic phase CML, it is desirable to determine the mutational status of the patient, increase the dose of imatinib or switch tyrosine kinase inhibitors 2-nd generation — nilotinib, dasatinib with further cytogenetic and molecular monitoring. Nilotinib is 20–50 times and dasatinib — in 325 times more active as imatinib in those patients in the chronic phase of CML who are refractory to imatinib. All these medications inhibit BCR/ABL mutations except T315I.

The presence of mutation T315I is a direct indication for the allogenic bone marrow transplantation (BMT) (Fig. 5.26).

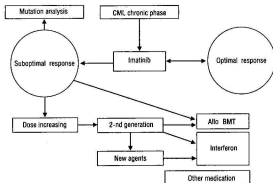


Fig. 5.26. Flow-chart for treatment of CML patients in chronic

Treatment of patients with osteomyelofibrosis depends on the stage, age, presence of complications. At the initial stage of the disease patients do not receive specific therapy, and are under the dynamic monitoring and receiving symptomatic therapy, which is aimed at correction of concomitant diseases. In the case of disease progression treatment is carried out using hydroxyurea, prodymin [N, N'-bis-(b-Brompropionil)]

etylendyamin], α -interferon, small doses of cytosine-arabinosid, corticosteroid drugs. Recently, some success in treating osteomyelofibrosis were achieved using thalidomide in small doses in combination with corticosteroid drugs.

Specific treatment for this disease at this stage of knowledge does not exist.

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5.4 MYELOYDYSPLASTIC SYNDROME

Modern points of view on pathogenesis of myelodysplastic syndrome (MDS) testify to the clonal character of disease, however, hematopoietic cells have a capacity to differentiation, unlike leukemia, when blast cells lose it practically. Clonal abnormalities appear in 40–60% of patients with primary myelodysplasia and increase average to 80% in secondary or "therapy-related" MDS [1–3]. Presence of few cytogenetic abnormalities (complex karyotype) is the reliable criterion to prognose the disease.

There are 3 basic types of the MDS evolution [4, 5]: (1) a stable karyotype with slow progression to acute leukemia (AL); (2) a rapid progression with the development of acute myeloid leukemia (AML) and preliminary appearance of cytogenetic abnormalities; (3) a complex aberrations of karyotype at the stage when MDS is diagnosed and characterized with a rapid transformation to AL.

Variability of detection of chromosomal aberrations in MDS reached 35–70%, sometimes — substantially lower (30–50%) [6]. For refractory anemia with ringed sideroblasts (RARS) patients the percentage of such changes varies from 12.5 to 34%, in refractory anemia (RA) it is considerably higher and reached 32–85%. More often revealed the changes of chromosomes 5, 20, 8 and 7 — del(5q), del(20q), trisomy 8 and monosomy 7, which can combine with other aberrations; translocations (mainly chromosomes 1, 2, 3, 7 and 11) appear rarely. For patients with refractory anemia with the excess of blasts (RAEB) average frequency of difficult changes of karyotype reached 57%. The most unfavorable abnormality is monosomy 7; isolated del(5q) is associated with higher survival of patients. Patients with MDS require the dynamic monitoring because of chromosomal abnormalities are not stable and their amount can grow with the disease progress.

For secondary MDS next changes of karyotype are typical: — 7 (41%), 5q — (28%), — 5 (11%), 21q (9%), 7q, 8 and der12p (8%), t(1;7), — 12 (7%), der17 (6%), der 6p (5%) and der3q, der11q, — 17, — 18, der19 (4%). The average number of cytogenetic abnormalities in secondary MDS is 5.3, while in primary MDS — 2.9. Increased risk of transformation to AL and short life-span is observed for patients with the monosomy of chromosome 7 or with the presence of complex karyotype (three and more aberrations). For secondary AL post MDS are typical aberrations of chromosomes 3, 8, 9, 12, 17 and 21 [7].

The results of cytogenetic investigations today are used as prognostic factors for MDS management. The most known is the International Prognostic Scoring System (IPSS, Greenberg V. et al., 1997) [8, 9]. A risk group according to IPSS is taking into account three criteria — percentage of blast cells in bone marrow (BM), presence of karyotype changes and the degree of cytopenia. A risk group of the MDS patient is determined by scores: low (LR) has 0 scores, intermediate-1 (IR-1) has 0.5–1 score, intermediate-2 (IR-2) has 1.5–2 scores, high (HR) has 2.5–3.5 scores.

Taking into account the adequate estimation of separate karyotype abnormalities for prognosis of disease [10, 11] the frequency of development of AL post-MDS and life-span of patients were estimated. The investigation included three groups: persons with the high risk of MDS acceleration and development AL with multiple abnormalities of karyotype (including — 7/7q, 5q in combination with other chromosomal abnormalities); patients with the intermediate risk of transformation to AL — they had t(17q), isolated cyto-

netic abnormality 12p, trisomy of chromosome 8; patients with the minimum risk of AL (without karyotype abnormalities). There were no dependences between separate subtypes of MDS and type of chromosomal aberrations at the stage of disease diagnosis.

The degree of angiogenesis can be used also as a prognostic factor of MDS management [12]. The secretion of angiogenic cytokines and growth factors correlates with the increase of microvessels density (MVD) in bone marrow tissue in MDS cases. Aguayo et al., 2000 investigated the areas of vessels and their amount in BM of healthy persons and patients with MDS and AL. For all patients with MDS vascularization of BM was considerably higher comparatively with control. The level of vascular endothelium growth factor (VEGF) significantly increased in patients with AML, chronic myelomonocytic leukemia (CMML) and MDS.

Deviations of apoptosis also allow to prognose the MDS course. In most cases of RA, RARS and RAEB the large number of apoptotic cells appeared in BM, including CD34⁺ cells with high expression of Fas-antigen which are absent in healthy persons. It was found out the negative correlation between expression of CD34 antigene and percentage of BM blasts [13, 14]. For MDS patients it was found out the increased secretion of proapoptotic cytokines IL-1b, IL-1a, TNF by stromal cells and correlation of their level with the degree of apoptosis deviations.

The peculiarities of myelodysplastic syndrome were investigated in 89 patients. Peripheral blood (PB) and (BM) counts in patients with myelodysplastic syndrome according to radiation exposure are presented in Tables 5.10, 5.11.

Table 5.10. Peripheral blood counts in patients with myelodysplastic syndrome

PB indexes	Variants of MDS with <5% blasts in BM (RA+RARS+JMDS)			
	Variants of MDS with >5% blasts in BM (RAEB+RAEB-T)			
	Irradiated patients, n=35	Non-irradiated patients, n=19	Irradiated patients, n=15	Non-irradiated patients, n=10
Mean				
Erythrocytes count, T/l	3.23±0.98	2.87±0.28	2.44±0.22	2.52±0.36
Hb level, g/l	93.19±6.69	84.88±8.35	72.43±4.85	67.51±8.96
Platelets count, G/l	143.25±17.04 ¹	128.82±12.80 ¹	72.50±6.53	65.63±5.49
Leukocytes count, G/l	4.06±0.48 ¹	4.45±0.94	9.44±1.21	8.71±1.05
Blasts, %	0	0	2.93±0.52	2.88±0.32
Promyelocytes, %	0	0	1.57±0.42 ^{**}	0.25±0.06
Myelocytes, %	0	0	5.06±0.51 [*]	2.14±0.19
Metamyelocytes, %	0	0	3.71±0.27 [*]	1.63±0.18
Eosinophils, %	3.52±0.58 ¹	2.20±0.36	1.14±0.17 [*]	3.63±0.24
Basophils, %	0.29±0.04	0.42±0.07	0.31±0.06	0.52±0.04
Band neutrophils, %	6.15±0.71 ¹	4.52±0.76 ¹	4.07±0.27 [*]	8.50±0.29
Segmented neutrophils, %	39.07±3.72	45.48±2.82	27.91±4.92	30.91±4.06
Lymphocytes, %	44.91±2.92	37.90±2.11	46.25±4.74	43.23±3.36
Monocytes, %	5.22±0.48	5.73±0.71	10.13±1.91 [*]	4.24±0.45
ESR, mm/h	23.24±3.18 ¹	19.81±3.22 ¹	46.14±7.67	36.71±0.58

¹significant difference in patients with <5% and >5% blasts in BM, $p<0.5$; ^{*}significant difference in irradiated and non-irradiated patients, $p<0.5$ (^{**} $p<0.1$).

Table 5.9. Bone marrow counts in patients with myelodysplastic syndrome

BM indices	Variants of MDS with <5% blasts in BM (RA+RARS+U-MDS)			
	Variants of MDS with >5% blasts in BM (RAEB+RAEB-T)			
	Irradiated patients, n=25	Non-irradiated patients, n=19	Irradiated patients, n=15	Non-irradiated patients, n=10
	Mean			
Myelotaryocytes count, G/l	118.51±13.89	87.67±9.42	121.03±8.15	95.50±4.15
Blasts, %	2.53±0.35 ^{1/2}	3.03±0.52 ^{1/2}	14.71±1.48	12.25±1.69
Promyelocytes, %	1.23±0.15 ^{1/2}	2.73±0.35 ¹	4.18±0.31 ^{1/2}	1.29±0.18
Myelocytes, %	9.95±1.03	10.58±0.89	10.66±0.81	8.96±0.78
Metamyelocytes, %	5.70±0.71	7.96±0.92 ¹	6.67±0.89 [*]	3.18±0.59
Band neutrophils, %	10.19±1.04 ¹	11.52±1.54 ¹	4.24±0.48	6.74±0.72
Segmented neutrophils, %	13.71±1.18	11.24±0.75	7.55±0.21 [*]	15.13±0.98
Eosinophils, %	2.26±0.30	3.08±0.47	0.79±0.03	0.63±0.04
Basophils, %	0.32±0.05	0.24±0.09	0.46±0.05	0.66±0.07
Monocytes, %	3.87±0.47	5.45±0.61	3.05±0.24 [*]	8.19±0.63
Lymphocytes, %	16.53±1.02	14.55±1.69	16.52±2.01	17.89±0.58
Plasma cells, %	1.10±0.21	1.02±0.18	0.68±0.06	1.06±0.09
Erythroblasts, %	1.46±0.15	1.04±0.12	2.84±0.37	1.56±0.17
Normocytes basophilic, %	3.81±0.45	2.48±0.36	2.51±0.35	1.47±0.25
Normocytes polychromatophilic, %	13.51±1.39 ¹	10.22±1.17 ¹	7.79±2.08	5.83±0.42
Normocytes oxyphilic, %	12.53±1.08 ¹	12.74±1.31 ¹	6.72±1.46	5.24±0.27
Megakaryocytes, G/l	58.13±9.78	35.60±7.53	27.37±6.28	22.43±4.27

¹significant difference in patients with <5% and >5% blasts in BM, $p<0.05$ (^{1/2} $p<0.01$); ^{*}significant difference in irradiated and non-irradiated patients, $p<0.05$ (^{**} $p<0.01$).

A dynamic supervision during 14 years did not find out a difference in the survival of irradiated and non-irradiated CMML patients and reached 46.8% and 60.0% accordingly. Hundred percentage lethality of the irradiated RAEB and refractory anemia with the excess of blasts in transformation (RAEB-T) patients was revealed during 2.5 years, in non-irradiated patients — during 3 years.

Histology characteristics of BM biopsies were studied in 22 exposed patients with MDS (RA, RARS, unclassified myelodysplastic syndrome — U-MDS). Histology investigation showed that BM cellularity was different (Table 5.12): diffuse hypercellularity (2 persons), focal hypercellularity (2 persons), diffuse hypocellularity (2 persons), focal hypocellularity (3). Normal cellularity was observed in 13% of patients.

Table 5.12. Histological characteristics of MDS patients

Indices	Number of investigated persons (n=22)
Diffuse hypercellularity	2
Focal hypercellularity	2
Diffuse hypocellularity	2
Focal hypocellularity	3
Normal cellularity	13

There were no significant cytomorphologic peculiarities in the PB and BM samples in the Chornobyl liquidators with MDS (RA, RARS, CMML). All patients had the features of erythroid cells dysplasia. Dysplastic changes in leukocytes and platelets were absent.

Previous literature data revealed that exposed due to the Chornobyl NPP accident MDS patients with incorporated doses of 9.5–30.0 cSv had leukopenia and BM hypocellularity, reduced erythroid hematopoiesis with delayed erythroblasts maturation, dyshemopoiesis features in three lineages.

Qualitative changes of leukocytes lineage in MDS patients exposed to doses from 0.51 cSv to 67.86 cSv, in comparison with nonirradiated patients manifested probability ($p < 0.05$) increasing of frequency of hyposegmentation, hypersegmentation, fragmentation of nuclei, presence of toxic granules, vacuolization, degranulation of the cytoplasm of granulocytes; binuclea, fragmentation of nuclei, vacuolization and basophilia of the lymphocytes cytoplasm; nuclei fragmentation, decreased nuclear/cytoplasmic ratio, frequency of vacuolization and granularity in the cytoplasm of monocytes.

Differences of laboratory indices in MDS patients taking into account the gender were analysed. In the group of the radiation-exposed patients with BM blast cells did not exceeding 5%, women found out the substantial decline of content of PB monocytes and BM erythroblasts and increase of BM monocytes in comparison with the male persons. The non-irradiated MDS patients with the percent of BM blast cells to 5% in accordance with a gender (women were in comparison with men) had an increased amount of PB band neutrophils and reduced count of BM erythroblasts.

The peculiarities of karyotype were investigated in 20 MDS patients suffered after the Chornobyl NPP accident (17 patients with the amount of blast cells in BM to 5%, including RA — 8, RAEB — 4, U- MDS — 5, and every one patient with the diagnosis RAEB, RAEB-T and CMML). Cytogenetic changes were found out in 9 from 20 investigated persons (Table 5.12). The group of investigated persons was composed by 5 residents of 3rd and 4th zones of ChNPP and 5 clean-up workers of 1986 with the incorporated doses of 25.0–67.86 cSv. Additionally 3 MDS patients and 1 ARS grade 1 patient with leukopenia cytogenetic investigation was conducted to determinate radiation markers.

From 20 inspected MDS patients 7 had normal male karyotype — 46, XY, 4 patients had karyotype 46, XX. Karyotype of patients with cytogenetic abnormalities are presented below.

For patient F-ko (RA), resident of contaminated area (3rd zone), karyotype was as follow — 46, XX[29]del(p14)[2], del(q15)[1] (Table 5.13). Karyotype in 29 cells was normal. In 2 cells (6.25%) deletion of long arm of chromosome 4 was revealed, in one cell (3.13%) deletion of short arm of chromosome 12 was revealed, however this aberrations cannot be considered as clonal.

After the investigation of karyotype of patient K-va (RA), clean-up worker of 1986, in 4 (18.18%) from 22 cells a marker chromosome was found out, there was not any other changes in other cells (46,XY+mar[4]/ 46XY[18]) (Table 5.13).

Some chromosome aberrations were revealed in patient K-ko (RA) resident of radionuclide contaminated territory (3rd zone) (Table 5.13). Although the founded abnormalities were not clonal, their quantity required further cytogenetic monitoring. Monosomy of chromosomes X, 16 and 19 observed in one cell (3.45% from a general amount — 29 cells). In one cell a monosomy of chromosomes 12 and 15 took place.

In the third cell, in addition to a monosomy of chromosomes 8 and 9, the translocation $t(5;10)(q35;q24)$ was determined, inversion of chromosome 9 ($inv(9)(p21;q34)$), deletion of chromosomes 10, 11, 13, 18 and 19. Thus, karyotype of this patient was following: 46,XX[25]/43,X,-X,-15,-19[1]/44,XX,-12,-5[1]/44,XX,-8,-9, $t(5;10)(q35;q24)$, $inv(9)(p21;q34)$, $del(10)(q24)$ $del(11)(q23)$, $del(13)(q21)$, $del(18)(p23)$, $del(19)(q21)[1]$.

Clonal aberrations were revealed in patient S-ka (RARS) — in all 12 analysed cells deletions of chromosomes 17–46, XX, $del(7q21)(12)$ were discovered (Table 5.13).

In karyotype of patient A-ka, resident of 4th zone, clone (7 cells — 24,14% of a general amount of investigated 29 cells) was characterized by the loss of chromosome 7 (Table 5.13).

Some cytogenetic abnormalities observed in MDS patient N-ka: 46, XY[36]/46, XY, $del(q-)(31)/46$, XY, $del(q-)(7)/46$, XY, $inv(16)(3)/46$, XY, $t(16;16)(1)$ (Table 5.13). In 3 (6.0%) cells deletion of short arm of chromosome 20 took place. In 7 cells deletion of short arm of chromosome 16 was detected, in 3 cells inversion of chromosome 16 was detected, in one cell a translocation of chromosomes 16 $t(16;16)$ was detected. Taking into account the formal signs (clone must be present not less than 10% from the general amount of the investigated cells) in this patient only deletion of chromosomes 16 $del(16)(q-)$, that was found out in 7 cells (14.0%) can be estimated as clonal abnormality, the presence of multiple chromosomal aberrations testified to severe damage of hemopoiesis. A disease that was diagnosed on the stage of RA later complicated with progressive pancytopenia, and patient died in 57 months.

Transformation RAEB to AML (M2) in 3 months was diagnosed in patient P-ko with karyotype 46, XX, $t(q11.2q22.2)(11)$ (Table 5.13). In BM and PB cells of this patient the expression of chimeric gene *AML1/ETO* was revealed. A patient died because of the progression of AML post MDS.

Karyotype of liquidator of 1986 M-va was following: 46, XY[46], $t(12q;12q)(1)$, $dic(3q;5q)(1)$, $del(q)(1)$, $chbr(7q)(1)$ (Table 5.13). Term of disease was 25.3 months, patient died because of the progressive pancytopenia and intoxication.

The clone of BM cells of CMML patient M-lo was presented by Ph⁺ positive cells (Table 5.13). A monitoring over The patient is under follow-up.

Distribution of investigated patients to IPSS groups was following: LR — 7, IR-1 — 8, IR-2 — 3, HR — 1. In next period 3 patients were lost to follow up. The acceleration of disease caused death of 3 persons: two had pancytopenia (N-ka, variant of RA, and M-v, variant of RAEB) and in 1 patient P-ko (RAEB) was a transformation of disease to AML, M2.

Comparison of the risk groups of MDS patients according to IPSS revealed the significant difference of some laboratory parameters (Table 5.14). Patients from a LR group had a significantly ($p<0.05$) lower indices of PB basophiles (0.40 ± 0.25 against $1.14\pm0.12\%$), PB eosinophiles (1.20 ± 0.45 against $4.75\pm2.50\%$, $p=0.01$), BM erythroblasts (0.53 ± 0.10 against $1.37\pm0.24\%$), BM myelokaryocytes (332.00 ± 37.78 against 672.25 ± 99.41 G/L).

Comparison of indices of patients from LR and IR-2 groups (Table 5.14) testifies to the significant ($p<0.05$) decline in last group the PB platelet count (67.6 ± 16.8 against a 183.60 ± 25.99 G/L), increase of PB eosinophiles percentage (6.16 ± 2.03 against 1.20 ± 0.45) and BM erythroblasts (1.10 ± 0.31 against 0.53 ± 0.10).

Table 5.13. Peripheral blood cytogenetic peculiarities in MDS patients

Patient	Age (years), gender	MDS variant	Karyotype	Term of disease, months	IPSS risk group, (score)	Disease progress
F-ko*	51, f	RA	46, XX(29) del(4)(p14)(2), del(12)(q15)(1)	27	CR-1 (1.0)	absent
P-ko	68, f	RA	46, XX	34	CR-1 (0.5)	absent
K-v**	77, m	RA	46, XY+mar(4)(46XY[18]	38	CR-1 (1)	absent
P-n*	38, f	RA	46, XX	41	NCR (0)	absent
Z-v*	59, f	RA	46, XX	63	CR-1 (0.5)	absent
M-v**	43, m	RA	46, XY	1	CR-1 (0.5)	lost to follow-up
K-ko*	70, f	RA	46, XX(26)(43,X,-X,-16,-19(1)(44,XX,-12,-15(1) 44,XX,-8,-9,5(10)(q35;q24),inv(9)(p21;q34), del(10)(q24), del(11)(q23), del(13)(q21), del(16)(p23), del(19)(q21)(1)	27	CR-2 (1.5)	absent
S-ko	25, f	RARS	46, XX, del(7)(q21)[12]	22	CR-1 (1)	lost to follow-up
B-ly	19, m	RARS	46, XY	7,7	NCR (0)	lost to follow-up
A-k*	19, f	U-MDS	46, XY(22)(46,XY,-12[7]	141,3	CR-1 (1)	absent
L-ko**	36, m	U-MDS	46, XY	98	NCR (0)	absent
N-ko	36, m	U-MDS	46, XY(36) 46, XY, del(16q-)(7)(46,XY,jm(16)(3) 46,XY,del(20)(q-)(3)(46,XY,(16;15)(1)	57 (deceased)	CR-2 (1.5)	pancytopenia
P-k	48, m	U-MDS	46, XY	27	CR-1 (0.5)	absent
P-v	18, m	U-MDS	46, XY	82	NCR (0)	absent
M-v*	24, f	U-MDS	46, XX	89	NCR (0.5)	absent
K-ko	37, f	U-MDS	46, XY	38	NCR (0)	absent
T-v**	33, m	U-MDS	46, XY	76	NCR (0)	absent
P-ko	62, f	RAREB	46, XX,(18,21)(q11.2;q22.2)(11 (chimeric gene AML1/ETO in PB and BM cells presence)	3	CR-2 (2)	AML (M2)
M-v**	47, m	RAREB-T	46, XY(46),(12q)(12q)(1,del(3q;5q)(1), del(20)(q) (1),chr(7)(q)(1)	25,3 (died)	R absent (3.5)	pancytopenia
M-to	34, m	CHMMML	46, XY(16)(46,XY,(9;22)(q34;q11)[4]	17,3	—	absent

*incident of contaminated area (3rd and 4th rows); **chronic worker of 1986

Table 5.14. Laboratory indices of patients with MDS (<5% blasts in BM) according to IPSS

	IPSS group of risk for MDS patients (<5% blasts in BM)	
	low risk, n=5	intermediate risk-1, n=4
	Mean	
PB basophiles, %	0.40±0.25*	1.14±0.12
PB eosinophiles, %	1.20±0.45*	4.75±2.50
Myelokaryocytes, G/L	332.24±37.78*	672.25±99.41
BM erythroblasts, %	0.53±0.10*	1.37±0.24
	Low risk, n=3	Intermediate risk -2, n=5
PB platelet count, G/L	183.60±25.98 [†]	87.60±16.80
PB eosinophiles, %	1.20±0.45 [†]	6.16±2.03
BM erythroblasts, %	0.53±0.10 [†]	1.10±0.31
	Intermediate risk -1, n=4	Intermediate risk -2, n=5
Hb, g/L	112.25±19.53**	66.45±5.24

*reliable difference between the indexes of groups of patients with low and intermediate risk-1 ($p<0.05$); [†]reliable difference between the indexes of groups of patients with low and intermediate risk-2 ($p<0.05$); **reliable difference between the indexes of groups of patients with intermediate risk-1 and intermediate risk-2 ($p<0.05$).

In IR-2 MDS patients in comparison with IR-1 patients the Hb level significantly decreased (Table 5.14).

Positive correlation was revealed between the estimation of the MDS patients status (scores) and status according to ECOG-WHO ($r=0.657$, $p<0.05$), IPSS risk group ($r=0.977$, $p<0.001$), serum VEGF concentration ($r=0.772$, $p<0.01$). A feed-back between the IPSS status (scores) and Hb level ($r=-0.869$, $p<0.01$), PB erythrocytes count ($r=-0.886$, $p<0.01$) was revealed.

The IPSS risk group positively correlated with the IPSS status (scores) ($r=0.679$, $p<0.05$) and negatively correlated with PB erythrocytes level ($r=-0.879$, $p<0.01$), Hb level ($r=-0.824$, $p<0.05$).

Summarizing all above, it should be noted that in the spectrum of karyotype aberrations in irradiated patients (incorporated doses 0.51–67.86 cSv) with MDS (RA, RARS, U-MDS) prevailed deletion of chromosomes 4, 7, 10, 11, 12, 13, 16, 18, 19, 20. The second place was occupied by the losses of chromosomes 8, 9, 16, 19 and X, and occasionally there were translocations, inversions, dicentrics, for one patient was found out a chromosome marker.

Taking into account the changes of karyotype of investigated patients that were found out, we conducted the estimation of patients status in accordance with IPSS. 7 persons were included to LR group, 8 — to IR-1 group, 3 — to IR-2 group (two patients died), 1 — to HR group (patient died). The analysis of results of cytogenetic investigation in MDS patients confirms, that the degree of clonal abnormalities testify to possibility of further MDS acceleration.

According to the previous data [15], 52.1% of the MDS patients sufferers of the ChNPP accident have karyotype abnormalities. Frequency of chromosomal aberrations depended on the variant of MDS and were present in 50–53% of CMML patients and in 75% of RAEB-T patients. Authors divided three risk groups taking into account the transformation of MDS to AL: high (presence of complex abnormalities of karyotype,

anomaly 7q — and monosomy 7), low (normal BM karyotype, single anomalies of 5q-, 7p-, 11p-, 16q-, 20q-) and intermediate (other abnormalities).

The concentration of serum VEGF in MDS patients with the amount of blasts in BM to 5% and in CMML patients was defined (Table 5.15).

Table 5.15. Concentration of serum VEGF in MDS (<5% blasts in BM) and CMML patients

Diagnosis	Concentration of serum VEGF, pg/ml (Mean) in		
	Irradiated patients, n=7	Non-irradiated patients, n=10	Control group, n=20
MDS (<5% blasts in BM)	120.86±36.46 [#] (n=12, p=0.019)	297.12±35.49 [*] (n=5)	15.6–100.0
CMML	356.56±62.41 [*] , [#] (n=6, p=0.018)	111.95±17.40 (n=5)	

^{*}reliable difference between the indices of investigated patients and persons of control group (p<0.05);

[#]reliable difference between the indices of the irradiated and non-irradiated patients (p<0.05).

The concentration of VEGF in non-irradiated patients (297.12±35.49 pg/ml) significantly exceeded normative values (Table 5.15). The concentration of serum VEGF in irradiated MDS patients was significantly lower than in non-irradiated persons — 120.86±36.46 pg/ml against 297.12±35.49 pg/ml (p<0.05).

Comparison of serum VEGF concentration in CMML patients and control group (Table 5.15) revealed that in irradiated patients a concentration was significantly higher (356.56±62.41 pg/mL, p<0.05) than in non-irradiated patients — 111.95±17.40 pg/ml (p<0.05).

Positive correlation was revealed between the serum VEGF concentration in irradiated MDS patients with blast cells count in BM <5% and PB platelets count (Fig. 5.27), BM basophylic normocytes (r=0.77, p<0.01).

For the non-irradiated MDS patients with content of BM blast cells below 5% concentration of serum VEGF positively correlated with BM myelokaryocytes count (r=0.991, p<0.01), percentage of BM myelocytes (r=0.96, p<0.05) and oxyphilic normocytes (r=0.952, p<0.05).

Positive correlation was revealed between the concentration of serum VEGF in irradiated CMML patients and percentage of PB myelocytes (r=0.998, p<0.05), PB basophiles (r=0.998, p<0.05). Negative correlation was revealed between the concentration of serum VEGF and status of CMML patients according to ECOG-WHO scale (r= -0.998, p<0.05).

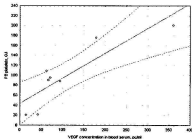


Fig. 5.27. Diagram of dispersion with the regressive line of serum VEGF concentration and PB platelets count in irradiated MDS patients (BM blasts < 5%).

Positive correlation was revealed between the concentration of serum VEGF in non-irradiated CMML patients and their age ($r=0.98$, $p<0.05$), percentage of PB leucocytes ($r=0.999$, $p<0.05$), platelets ($r=0.997$, $p<0.05$) and erythrocytes ($r=0.997$, $p<0.05$), PB lymphocytes (of $r=0.976$, $p<0.05$).

Thus, the investigation of cytogenetic and molecular-genetic peculiarities of hemopoiesis in MDS patients sufferers of the ChNPP accident confirms their role in pathogenesis of disease and gives possibility to prognose myelodysplasia course.

The variance analysis allowed to estimate the influence of IR on variability of basic laboratory indices of the investigated patients and patients of the comparison group with stable leucopenia. A percentage of IR contribution to variability of indices were estimated according to existing views: up to 5% as moderate, 5–20% as middle, over 20% as considerable degree of influence.

Influence of IR on variability of indices of MDS patients (<5% BM blast cells) showed the middle degree of significance for PB erythrocytes count, Hb level, percentage of PB myelocytes, PB metamyelocytes, BM myelocytes, BM band neutrophils and CD45⁺14⁺ PB granulocytes (Table 5.16). More significant contribution was revealed in the percentage of annexin⁺propidium iodide⁺ granulocytes on the late stages of apoptosis and ESR.

Table 5.16. Determination of IR contribution into variability of laboratory indices of MDS patients (<5% blast cells in BM)

Indices	Fisher's criterion (F)	Percentage of IR contribution	p<
PB erythrocytes, T/L	6.97	13.41	0.05
Hb level, g/L	7.01	13.48	0.05
PB myelocytes, %	6.67	14.0	0.05
PB metamyelocytes, %	4.11	9.11	0.05
ESR, mm/h	15.81	26.0	0.001
BM myelocytes, %	6.53	13.45	0.05
BM band neutrophils, %	3.84	8.19	0.05
PB CD45 ⁺ 14 ⁺ granulocytes, %	4.48	18.31	0.05
BM annexin ⁺ PI ⁺ granulocytes, %	8.57	58.83	0.05

For patients with the MDS variants RAEB and RAEB-T IR made a significant contribution into the degree of splenomegalia, percentage of PB granulocytes at the early apoptosis stages, BM oxyphilic normocytes, BM CD10⁺19⁺ lymphocytes, BM CD5⁺20⁺ lymphocytes, CD7⁺33⁺ granulocytes and activated BM T-, B-lymphocytes (Table 5.17).

Table 5.17. Determination of IR contribution into variability of laboratory indices of MDS patients (>5% blast cells in BM)

Indices	Fisher's criterion (F)	Percentage of IR contribution	p<
The size of spleen, mm	4.33	28.51	0.05
PB annexin ⁺ PI ⁺ granulocytes, %	5.0	38.36	0.05
BM oxyphilic normocytes, %	4.86	24.46	0.05
BM CD10 ⁺ 19 ⁺ lymphocytes, %	106.73	98.16	0.01
BM CD5 ⁺ 20 ⁺ lymphocytes, %	15.57	88.62	0.05
BM CD7 ⁺ 33 ⁺ granulocytes, %	151.26	99.94	0.05
BM activated T-, B-lymphocytes, %	30.59	93.85	0.05

For the group of CMML patients the high degree of IR contribution was revealed on duration of disease, serum VEGF level and serum LGH concentration, percentage of PB leukocytes, blast cells, myelocytes, band neutrophils and CD13⁺ HLA-DR⁺ granulocytes (Table 5.18).

Table 5.18. Determination of IR contribution into variability of laboratory indices of CMML patients

Indices	Fisher's Criterion (F)	Percentage of IR contribution	p<
Duration of disease, mths.	11.6	56.31	0.01
Serum VEGF concentration, pg/ml	14.25	78.09	0.05
Serum LGH concentration, U/L	13.78	73.37	0.05
PB leukocytes, %	2.94	24.62	0.05
PB blast cells, %	7.04	43.88	0.05
PB myelocytes, %	5.12	39.0	0.05
PB band neutrophils, %	3.97	30.61	0.05
PB CD13 ⁺ HLA-DR ⁺ granulocytes, %	17.35	85.26	0.05

Variance analysis allowed to work out mathematical models (linear functions) for prognosis of the MDS patients survival taking into account their radiation status.

In the group of the irradiated MDS patients variance analysis allowed to separate the next criteria of survival prognosis: duration of disease (x_1), degree of hepatomegaly (x_2) which was determined in maximal slanting size of right lobe (mm), percentage of PB BCL-2⁺ granulocytes (x_3) and PB T-helpers (x_4). Reliable ($F=18.39$, $p<0.001$) probability of survival prognosis for this patients group is described by next linear functions:

$$y_1 = -77.96 + 0.04 x_1 + 0.97 x_2 - 0.25 x_3 - 0.26 x_4 \quad (1)$$

$$y_2 = -64.39 + 0.08 x_1 + 0.78 x_2 - 0.06 x_3 - 0.03 x_4 \quad (2)$$

According to the conducted variance analysis prognostic criteria were in certain relation to survival of the non-irradiated MDS patients and included next immunological parameters: percentage of BCL-2⁺ granulocytes (x_1), CD34⁺33⁺ cells (x_2) and PB T-helpers (x_3). Linear functions that give possibility to prognose the significant ($F=4.40$, $p<0.05$) probability of survival are following:

$$y_3 = -27.05 + 1.17 x_1 - 0.91 x_2 + 0.89 x_3 \quad (3)$$

$$y_4 = -45.24 + 1.7 x_1 - 1.34 x_2 + 1.08 x_3 \quad (4)$$

It should be noted that the presence of significant differences in laboratory indices of MDS patients were the basis to work out an integral index — Index of prognosis (IPMDS). Criteria of the IPMDS are presented in Table 5.19. The calculation of summarised probability of independent unfavorable prognosis for investigated MDS patients was made by formula (5) of additional probability of independent events.

$$P = 1 - \prod_{i=1}^k (1 - P_i), \quad (5)$$

where P — summarise probability to die when the influence of risk factors occurs,

P_i — probability to die when the meaning of i -th index is critical,

Π — multiplication ($1 - P_i$), i — number of risk factor, k — number of factors.

When patient has all risk factors (Table 5.19), the calculation is as follow:

Table 5.19. Index of prognosis criteria (IPMDS) in MDS patients

Indices	Died MDS patients, n=36		Alive MDS patients, n=17		Student's criterion	p<	Criterion of unfavorable prognosis	Probability of unfavorable prognosis for life on condition that presence of critical index (P), %
	M	m	M	m				
Erythrocytes count, T/L	2.51	0.16	3.25	0.42	-1.86	0.05	<2.5	83.3
ESR, mm/h	38.71	4.17	20.67	5.54	2.24	0.05	>20	81.8
Hepatomegaly (determined in maximal starting size of right lobe), mm	173.94	3.81	192.92	4.01	3.26	0.05	>150	73.0
BM BCL-2 ⁺ granulocytes, %	15.39	1.94	34.35	8.11	-1.87	0.05	<25	44.4
Early apoptotic BM granulocytes, %	7.05	2.92	32.94	7.62	-3.04	0.01	<20	40.0

$$\begin{aligned}
 P &= 1 - (1 - 0.833) \cdot (1 - 0.818) \times \\
 &\times (1 - 0.73) \cdot (1 - 0.444) \cdot (1 - 0.4) = \\
 &= 1 - 0.167 \cdot 0.182 \cdot 0.27 \cdot 0.556 \cdot 0.6 = \\
 &= 1 - 0.0027376 = 0.9972624 = 0.997.
 \end{aligned}$$

When patient has all risk factors the probability of maximally unfavorable prognosis is of 99.7%. Probability of minimum unfavorable prognosis for MDS patients is 40.0%. Relative risks (RR) of opportunity to die during 2.5 years of monitoring in MDS patients were from 2.14 to 26.25.

Summarizing materials of the chapter and taking into account the above-mentioned peculiarities of MDS it is necessary to emphasize that revealed clinical, hematological, immunological, molecular-genetic, cytogenetic characteristics require further study and estimation.

Immunophenotype and programmed cell death

MDS includes the group of syndromes, that are characterized by a clonal cell growth and the ineffective hematopoiesis. Last studies have shown a relationship between the MDS onset and the ionizing radiation exposure. Morphological changes of the haematopoietic cells (HC) of BM and PB are described at MDS in Chernobyl clean-up workers as well as cytogenetic and molecular changes [16, 17]. It is known from the other MDS studies that cell function and death are controlled and associated with a set of membrane receptors and their ligands, which activate both processes of proliferation and apoptosis. However, the number of publications on immunological phenotype of PB and BM cells in a secondary MDS after the radiation exposure is relatively limited.

Myelodysplasia is characterized by a disparity between the high cell proliferation activity and cytopenia syndrome, that allowed to set a theory of an "ineffective hematopoiesis". Most researchers are inclined to consider apoptosis as the basic mechanism of the myelodysplasia phenomenon.

Therefore, the research on the role, specificity, balance of presentation of pro- and anti-apoptotic molecules in the complex cascade of the programmed cell death is a priority direction of the myelodysplasia pathways research. In radiation-exposed the actuality

of apoptosis studies also is proven by the fact that changes in apoptotic pathways have been revealed after radiation exposure including the expression of cellular receptors and markers of apoptosis. Imbalance between the proliferation and apoptosis is an important parameter of the immune system's response to the activating agents, and determines its effectiveness in terms of immune response or immune tolerance.

Pan-leukocyte antigens. The analysis of the white blood cells (WBC) showed no difference between the secondary myelodysplasia in radiation exposed in comparison with idiopathic MDS. Changes correlated with the type of disease. No changes were demonstrated in antigen expression on lymphocytes in a group of patients with RAEB, RAEB-T, as compared with control. Comparative analysis of PB and BM cells showed a significant reduction in expression of CD45 antigen on lymphoid cells. BM granulocytes in a subgroup of patients with RA represented the dominant population with high expression of CD45, which can be regarded as a manifestation of a dysplastic granulocyte maturation. In RAEB and RAEB-T increased BM CD45⁺14⁺ numbers as compared with PC were characteristic.

Lymphocytes. Analysis of the linear antigens expression showed no differences between the exposed and not-exposed in major T-cell (CD5⁺, CD3⁺, CD7⁺) subsets, while high individual variability was typical for antigen expression. Changes were demonstrated mainly in CD4⁺ and CD8⁺ cells at post-thymic stages. A tendency of CD8⁺ subset increase was accompanied by the decline of CD4⁺ cell numbers. In all MDS subtypes an increase of a low density CD56⁺ HLA-DR⁺ cells was shown.

To reveal the influence of a previous ionizing radiation exposure three groups were compared — (1) idiopathic MDS in exposed to natural radiation background, MDS, (2) secondary to low-dose radiation exposure during the Chernobyl accident clean-up, and (3) former clean-up workers without hematological diseases. MDS in group 2 showed the opposite tendencies in CD4⁺8⁺ and CD4⁺8⁺ cell counts as compared with group 1. The negative correlation was demonstrated between the dose of irradiation and CD8⁺ cell numbers ($r = -0.634$; $p < 0.01$) and a positive correlation between of the dose irradiation and CD4⁺ ($r = 0.622$; $p < 0.13$) (Fig. 5.28). B-cells showed signs of activation with an increase of the early B-cell progenitors counts. No difference was demonstrated between groups 1 and 2.

Granulocytes. Existing knowledge supports the MDS onset as a defect of the early myeloid precursors [18]. Blasts cell number is a critical and prognostic factor of MDS transformation to the acute leukemia. The blasts in BM of MDS patients are represented by CD34⁺CD71⁺CD117⁺ cells (Table 5.20).

Table 5.20. Percentage of early hematopoietic progenitors in peripheral blood and bone marrow of MDS patients

Phenotype	RA (n=31) M±SD,%		RAEB/RAEB-T (n=24) M±SD,%	
	PB	BM	PB	BM
CD34 ⁺	0.4±0.30	1.1±0.13*	1.1±0.29	68.0±7.08*
CD71 ⁺	12.2±2.06	24.4±3.06*	5.6±2.44	23.0±3.61*
CD117 ⁺	0.8±0.21	5.2±0.12*	1.8±0.51	11.8±3.01*
CD117 ⁺ 34 ⁺	0.4±0.04	1.3±0.04*	1.0±0.02	65.0±11.20*

*Significant differences between PB and BM, $p < 0.01$.

Among RAEB and RAEB-T patients the percentage of CD34⁺ cells was significantly higher. Inside the subset the decreased counts of CD38⁺ cells level were a characteristic sign of the limited differentiation abilities. CD34⁺117⁺ cell numbers were significantly increased ($p < 0.01$). At all MDS variants considerably higher expression of HLA-DR antigens was demonstrated in BM compared to the PB, as well as an increased CD71⁺ expression. The BM cells-CD38 surface antigen density in RA did not differ from group mean values in PB. In BM of RAEB and RAEB-T patients the density was significantly higher ($p < 0.01$). For other antigens BM and PB changes were parallel.

PB granulocytes study showed changes of CD10, CD33, CD13 antigens expression with a decrease of CD33 expression typical for myelodysplasia. A significant increase was demonstrated in the early progenitor cell subsets expressing CD34, CD33 and CD19 common for myeloid and B-cell lineages. A decrease of the CD33 antigen density was detected on the CD13⁺HLA-DR⁺ cells in group 2 as comparing with group 1. The same tendency was typical for group 3, indicating the common differences in lineage antigens presentation at the late period after the radiation exposure. Differences were also revealed in a CD13⁺ cell numbers with a co-expressed HLA-DR antigen. (Fig. 5.29). Maximal difference with the control figures was shown in patients exposed to the doses up to 1 Sv.

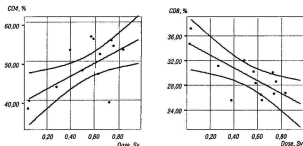


Fig. 5.28. Results of the regression analysis of exposure dose and PB CD4 (A), CD8 (B) lymphocyte counts in Chernobyl clean-up workers with MDS

Phenotype of BM in RAEB, RAEB-T was characterized by the high percentage of CD34⁺ cells — 68.0 ± 7.08 (M $\pm$ SD).

Disparity between the high proliferative activity of hemopoietic cells and cytopenia syndrome at MDS induces researchers to suppose the role of the programmed cellular death. To study first stages of the Fas-mediated apoptosis the number of the CD95⁺ cells and antigen density were studied in lymphocytes and granulocytes. In PB the number of CD95⁺ lymphocytes was significantly increased. For PB granulocytes tendency was the opposite. In RA the positive correlation was demonstrated between Fas-receptor and BCL-2 proteins expression in PB granulocytes ($r = 0.84$; $p < 0.005$). In RAEB and RAEB-T

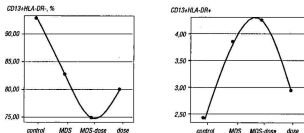


Fig. 5.29. Changes of expression of CD13 (A) and co-expression of CD13/HLA-DR (B) antigens on granulocytes of PB of MDS patients depending from the dose of irradiation.

correlation was negative in lymphocytes ($r = -0.72$; $r < 0.03$) as well as in granulocytes ($r = -0.90$; $p < 0.001$). The obtained data could represent the general mechanisms with a substantial increase of BCL-2⁺ cell counts in combination with the decreased apoptosis that can be related to negative prognosis and primary resistance to the chemotherapy. In BM of RAEB and RAEB-T the increase of CD95⁺ cell counts correlated with CD34⁺ cell counts and BCL2⁺ cell counts decrease.

Annexin-V test data are presented at Fig. 5.30. The number of annexin-V⁺PI⁻ PB granulocytes was increased due to cells on the late stages of apoptosis. Percent of annexin-V⁺PI⁻ and annexin-V⁺PI⁺ among the lymphoid cells of BM was more high than

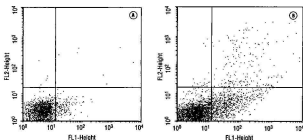


Fig. 5.30. Dot histograms of the cells stained with Annexin-V (FL-1) and propidium iodide (FL-2) showing donor (A) and MDS (B) cells. 1st square — cells are marked only PI (initial stage of necrosis); 2nd square — cells, marked both PI and annexin-V-FITC (late stages of apoptosis); 3rd square — cells marked neither PI nor annexin-V-FITC (living cells); 4th square — cells marked only annexin-V-FITC (early stages of apoptosis).

in the PB at all types of MDS. The number of apoptotic cells among the BM granulocytes was also increased, however in RAEB and RAEB-T these figures were lesser in comparison to RA patients. The decrease of apoptosis can be explained and represent the high risk of MDS transformation into acute leukemia. The highest annexin-V cells counts were revealed in the BM blasts cells and were also confirmed by the mean group values of apoptosis index, which was calculated in obedience to recommendations [19]. A low apoptotic index set for the RAEB, RAEB-T sub-group of patients was probably related to the negative prognosis of these variants of MDS.

Connection between growth of tumour cells and timely start of the programmed cellular death is known. Especially important is a choice of methods of apoptosis induction. Among the results it follows to pay regard to those which showed the individual insensitivity to the inductors of apoptosis in PB granulocytes. It could be the sign of the other pathways that did not lead to the elimination of tumour cells by an apoptosis, and as a result could be related to the high risk of transformation in acute leukemia. The BM myeloid cells usually are not programmed on death by apoptosis, however from the obtained results exactly these cells have shown a substantial response to the inductors that testifies to violation of the corresponding receptor systems and differentiation in BM.

The special interest was drawn to the programmed cellular death among MDS patients in a remote period after irradiation. In a studied group of clean-up workers the increased readiness of lymphocytes to Fas-mediated apoptosis was demonstrated with a high percentage of Fas-positive cells in samples from MDS patients with the previous low-dose radiation exposure in comparison with non-exposed patients and clean-up workers without hematological pathology. Low expression of anti-apoptotic BCL-2 can work to increased apoptosis having possibly a protective significance against cell transformation. An increased number of the CD95⁺ cells was shown with a positive correlation between the level of CD95⁺ cells and dose of irradiation in a group of clean-up workers ($r=0.63$; $p<0.05$). The CD95 antigen density on PB cells was low in both groups of clean-up workers — MDS patients as well as patients without MDS. So, in the late period after radiation exposure an increase of lymphocytes fraction ready for entry to apoptosis is associated with insufficiency of cellular receptors supporting this process that could block the start mechanisms of the programmed cellular death.

Several reports describe the changes in the lipid and protein layers in the WBC membranes of clean-up workers [20]. These structural changes accompany violation of their function and membrane-dependent processes, that could influence transmembrane ion traffic, intercellular co-operation, stimulate apoptosis. One of such examples is a phosphatidilserin molecule that is used in annexin-V assay. Percentage of annexin-V⁺ cells in radiation-exposed MDS patients was significantly increased while both annexin-V⁺PI⁻ and annexin-V⁺PI cell numbers in a group of clean-up workers were not changed. Fluorescence intensity study of annexin-V on annexin-V⁺PI⁻ cells in the groups of clean-up workers and radiation-exposed MDS patients was considerably higher as comparing to control.

Lymphocyte response to verapamil induction of apoptosis *in vitro* was substantially more intensive, both in a group of clean-up workers and among radiation-exposed MDS patients (Fig. 5.31). Such reaction testifies to overactivity of programmed cellular

death pathways in the remote period after irradiation. It is known that an apoptosis next to the proliferation process is the form of lymphocytes response to activating stimuli. Accordingly the analysis was performed of the immune cells activation in MDS patients in the remote period after irradiation.

At the first stage the spontaneous expression of activation markers HLA-DR, CD25, CD71, CD25, CD38 was studied. Among MDS patients without the exposure to radiation a considerable growth of spontaneous expression of HLA-DR and co-expression of HLA-DR and CD38 antigens were revealed. Such tendency is also characteristic for the radiation-exposed patients. HLA-DR antigen FI in MDS patients was decreased. In clean-up workers this index remains within the limits of control values. The similar changes were observed for the IL-2 receptor. Mean values of the activated CD4⁺ cells co-expressing CD25 antigen were decreased only in the group of radiation-exposed MDS patients in all doses range (from 0.04 to 0.92 Sv). The spontaneous expression of receptors to the transferrin (CD71) and the number of antigen-positive cells in the group of MDS patients were enhanced, while among the radiation-exposed persons with MDS the mean group values had a tendency to the decline. In clean-up workers the group values of CD71 fluorescence intensity were also declined.

It is considered that mitogen stimulation of lymphocytes *in vitro* could predict response to specific antigens. Data of concanavalin A (ConA) stimulation in healthy persons showed increased numbers of CD38⁺ and HLA-DR⁺CD38⁺ cells. Similar group changes for CD38 antigen were registered in MDS patients, both those after the radiation exposure and without it. In clean-up workers the mean group values of these receptors expression were not changed in comparison to the pre-incubation indices. The increased intensity of HLA-DR fluorescence in reply to mitogen stimulation was demonstrated in all groups, except of group of radiation-exposed MDS patients. There was an increase of relative cell counts and intensity of CD25 fluorescence on CD4⁺25⁺ and CD4⁺25⁺ cells ($p < 0.01$) after ConA stimulation.

However, the increase of CD25 antigen expression among clean-up workers without hematological pathology was registered on CD4⁺ cells, while in MDS group this was due to CD8⁺ cells. For CD71 antigen while the initial levels of expression were high in MDS patients, an answer to Con A was not so considerable, as among healthy persons. The intensity of this antigen fluorescence after stimulation was also lower than in non-MDS patients, that could be related to the unidirected type of changes of lymphoid and erythroid cells starting from progenitors which took place in BM at MDS. The

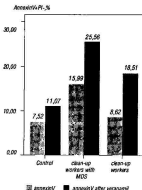


Fig. 5.31. Histogram of the number of annexin-V-PI⁺ P8 lymphocytes after incubation with versapamil in exposed MDS patients in comparison with Chernobyl clean-up workers.

opposite character of changes of fluorescence intensity of CD71 receptor was observed among clean-up workers in a dose range up to 1 Sv. After Con A stimulation intensity of fluorescence both receptor to transferrin and other activation markers (AM) grew considerably, that testifying to a high activation potency of lymphocytes.

The lymphocyte cell cycle studies were performed before and after Con A stimulation by DNA flow cytometry. In RA a balance was shown between the processes of proliferation and apoptosis. Cell cycle analysis has shown an increase of the hypodiploid cell counts in exposed both with MDS and in clean-up workers without MDS. A percent of lymphocytes in G2+M phases was not changed. In non-exposed controls the Con A stimulation *in vitro* led to the enhancement of two alternating processes: apoptosis and proliferation. However, a reliable growth of proliferative activity with the transition of lymphocytes to S/G2 phases of cellular cycle was typical only for lymphocytes from clean-up workers with doses of radiation exposure from 0.25 to 1 Sv. For MDS patients the prevailing response to Con A stimulation was the activation of apoptosis with a lesser effect on cell transition to S/G2 phases (Fig. 5.32).

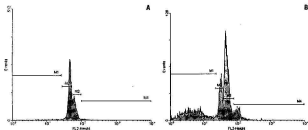


Fig. 5.32. Histogram of lymphocyte distribution by DNA content after Con A stimulation in control group (A) and radiation-exposed MDS patients (B). M1 — hypodiploid cells (apoptosis), M2 — G₀, M3 — G₁, M4 — S+G₂ phases of cellular cycle.

Such phenomenon can be explained by the peculiarity of the MDS pathways with a combination of an enhanced BM proliferation activity and elevated levels of apoptosis in hemopoietic precursors. A competition of two forms of energy-consuming cell reactions to mitogen is realized in a remote period after irradiation. The activation is combined with a shortage of compensatory reactions with a switch to a dominating type of response, i.e. in RA to cytopenic reactions while in RAEB and RAEB-T — to blast cell proliferation.

Peculiarities of myelodysplastic syndrome in acute radiation syndrome persons suffered after the Chernobyl NPP accident

Among the MDS patients 3 persons with ARS were investigated: patient D., variant of RA (convalescent of ARS grade 2), patient S., variant of U-MDS (convalescent of ARS grade 1), patient B., variant of RARS (convalescent of ARS grade 3).

Patient D., born in 1931 (64 years old), professional worker of ChNPP (chief engineer), participant of clean-up works from April, 1986 (worked directly near to the IV reactor at the night 26.04.86, ARS grade 2), was diagnosed RA. Total dose of irradiation was 390 cSv, local dose of irradiation of thyroid gland was 40 cSv. Patient was observed in the clinic of RCRM AMN of Ukraine since 1993.02.10.95 entered clinic with the complains of anemic character, loss of body mass, cough. Peripheral lymphatic nodes and spleen were not increased.

In PB analysis hyperchromic anaemia (erythrocytes count was 2.53 G/L, Hb level was 91 g/L, MCV was 104.3 fL), thrombocytopenia (thrombocytes count was 112 G/L), deviation to the left of blood formula, myelocytes were found out. The qualitative changes of PB included the degranulating of eosinophiles cytoplasm, clived nuclei, plasmatisation and processes of lymphocytes cytoplasm. The macroforms (50%) and giant forms (25%) of thrombocytes prevailed. The nuclei hypersegmentation, vacuolization of nuclei and cytoplasm in monocytes were revealed.

The results of immunophenotyping revealed 10% of CD34⁺ lymphocytes, 13% of CD38⁺ lymphocytes, 1.5% of CD25⁺ lymphocytes, 0.15% of CD34⁺33⁺ lymphocytes, 44% of T-cells, 23% of T-helpers, 21% of T-suppressor-cells, 8% of B-lymphocytes, CALLA-antigen appeared on 0.5% of lymphocytes, antigen HLA-DR appeared on 19% of lymphocytes. Phenotype of granulocytes (CD10⁺33⁺13⁺dr⁺) had not any peculiarities, monocytes slightly expressed HLA-DR-antigen. Humoral immunity indices did not deviate.

Symptomatic therapy was conducted, the amount of erythrocytes after administration of steroids did not change, there was insignificant growth of leukocytes and platelets (Fig. 5.33, 5.34, 5.35).

During the period of observation in the blood formula the percentage of lymphocytes increased to 56.6%, the percentage of monocytes increased to 28% in association with the reducing of segmented neutrophils percentage to 18% (Fig. 5.36).

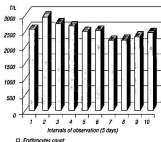


Fig. 5.33. Dynamics of erythrocytes count of patient D., medical record No 1157.

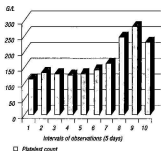


Fig. 5.34. Dynamics of platelets count of patient D., medical record No 1157.

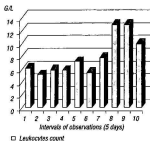


Fig. 5.35. Dynamics of leukocytes count of patient D., medical record No 1157.

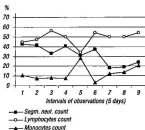


Fig. 5.36. Dynamics of PB segmented neutrophils, lymphocytes and monocytes count of patient D., medical record No 1157.

The patient was consulted in Munich City Clinic 18.01.1996, the diagnosis of MDS was confirmed. According to BM trepanobiopsy myelodysplastic syndrome with activation of megakaryocytopoiesis was revealed. The presence of red BM atrophy in combination with an interstitial oedema testified the toxic damage of BM cells. Patient died in 2 weeks after return home.

Patient S., born in 1951 (45 years old), a younger officer of Ministry of Internal Affairs, had been on duty 26.04.86, total dose of irradiation 170 cSv (ARS grade 2), was diagnosed U-MDS. Patients was being observed in the Clinic of RCRM since 1991. In September, 1996 thrombocytopenia (71–86 G/L thrombocytes) was found out in the PB. Peripheral lymphonodes, spleen were normal.

Hypoplastic BM, amote count of naked nuclears, absence of megakaryocytes (MGKC) appeared in the myelogram. There were the phenomena of mitosis (5:400), presence of macrophages (15:400) and reticular cells (4:400), significant qualitative changes included nuclear and cytoplasm vacuolization in granulocytes, MGKC, normocytes (Fig. 5.37–5.40).

The level of patient PB platelets was reduced moderately during 1996–1997, than their amount reduced to 1–2 G/L causing the hemorrhagic syndrome and severe anaemia (a minimum level of erythrocytes was 0,7 G/L, Hb — 32 g/L) (Fig. 5.41, 5.42). The leukocytes count began to reduce since October, 1998 (minimum level was 0.7 G/l) (Fig. 5.43). Histograms below shows changes of PB indices during the last 2.5 years of patient's life. Deviations of erythrocytes and platelets levels were the results of substitution therapy, infusion of erythrocytes and platelets transfusions.

The changes of leukocytes formula included the decreasing of mature granulocytes percentage (mainly segmented neutrophilias) and to a lesser degree — monocytes count in combination with increasing of lymphocytes count (Fig. 5.44). The periodical deviation of PB formula to the left to single myelocytes and metamyelocytes were being observed during the last 6 months of life (since October, 2001).

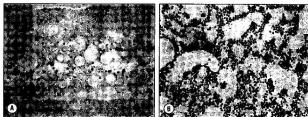


Fig. 5.37. Hypocellular BM of patient S. In dynamic observation (A — myelogram, 16.12.96, medical record No 312; B — myelogram, 08.04.97, medical record No 2814). Microphotographs. Stains are painted after Pappenheim $\times 200$.

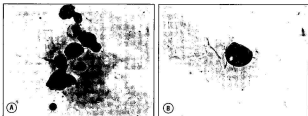


Fig. 5.38. Qualitative changes of BM cells of patient S. (A — dysplastic erythroid MNC with nuclei fragmentation and cytoplasm vacuolization, medical record No 312; B — cytoplasm vacuolization of eosinophilic myelocyte, medical record No 814). Microphotographs. Stains are painted after Pappenheim $\times 200$.

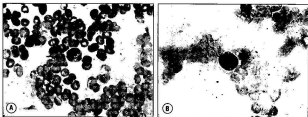


Fig. 5.39. Qualitative changes of BM cells of patient S, medical record No 2814 (A — degranulation and vacuolization of neutrophilic myelocyte cytoplasm; B — vacuolization of basophilic normocyte cytoplasm). Microphotographs. Stains are painted after Pappenheim $\times 200$.

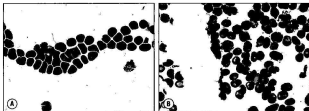


Fig. 5.40. Qualitative changes of patient S. BM cells, medical record No 2814 (A — changes of eosinophilic normocyte nuclei form; B — polychromatophytic normocyte with processes of cytoplasm). Microphotographs. Stains are painted after Papanheim $\times 200$.

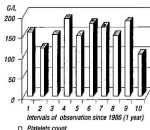


Fig. 5.41. The platelets count changes of patient S. in dynamic observation (1986-1995).

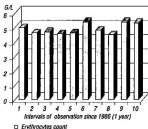


Fig. 5.42. The erythrocytes count changes of patient S. in dynamic observation (1986-1995).

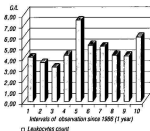


Fig. 5.43. The leukocytes count changes of patient S. in dynamic observation (1986-1995).

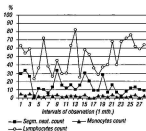


Fig. 5.44. Dynamics of segmented neutrophils, lymphocytes and monocytes count of patient S. in dynamic observation during the last 28 months of life.

In immunological status of the patient the substantial deviation, including the anti-leukocytes (leukoagglutinins, leukolysins) and anti-thrombocytes (thromboagglutinins, thrombolysins) antibodies were adsent.

During the course of treatment the infectious complications (including the bilateral pneumonia in lower lobes of the lungs) and development of acute transmural myocardial infarction caused the death of patient (19.05.2002).

Patient B., born in 1941 (42 years old), professional worker of ChNPP (head of 7 turbine shift of IV energy block, worked at night 26.04.1986), total dose of irradiation 550 cSv (ARS grade 3), was diagnosed RARS. Patient had being observed in the Clinic of RCRM since 1991. The moderate thrombocytopenia (to 60–102 G/L) and leukopenia (to 3,7 G/L) were revealed in PB since 07.12.1992.

Since 02.03.1993 the hemorrhagic manifestations (nasal and gingival bleeding, bloody cough, bloody stool) caused the anaemia. The cytopenia in PB included the reducing of erythrocytes count to 2,66 T/L, Hb — to 68 g/L, platelets — to 46,0 G/L, leukocytes — to 2,95 G/L, PB formula deviation to the left to myelocytes. Taking into account the results of PB and BM morphology (expansion of erythroid lineage with desention of maturation of red cells with nuclei, phenomenon of dyserythropoiesis, dismegakaryocytopoiesis); cytochemical BM investigation — increase of the non-specific esterase activity to mean cytochemical coefficient (MCC) — 0.70–1.65 and increasing content of granular and diffuse glycogen (MCC 0.24–0.52) in nucleated red blood cells; the presence of ring sideroblasts (30–80%) in BM MDS was diagnosed. In spite of administrated of symptomatic therapy (steroids, erythrocytes and platelets transfusions) (Fig. 5.45–5.47).

Threpanobiopstatis (15.03.1997) included the mass of small bone pieces surrounded by fibroreticular fibres, the same fibres located in bone marrow cavities. Forming of new immature collagen fibres was marked. The small areas of hemopoietic cells consisted mainly from red nuclea cells. There were two- and three-nuclear forms, rarely the mature granulocytes cells.

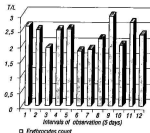


Fig. 5.45. Dynamics of erythrocytes count of patient B., medical record No 447.

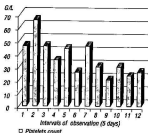


Fig. 5.46. Dynamics of platelets count of patient B., medical record No 447.

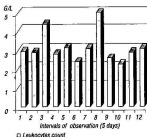


Fig. 5.47. Dynamics of leukocytes count of patient B, medical record No 447.

leukocytes counts (mainly neutrophilic granulocytes) and platelet was observed during this period. Letale progression of disease began after the respiratory virus infection.

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In patient blood serum was found the enhanceable titer of leukoagglutinins — 1:32 (++). The indices of osmotic and mechanical resistance of erythrocytes were substantially reduced (level of hemolyze reached 1%). The amount of reticulocytes reached 1.5%. The level of serum iron was increased to 36 mmol/L.

Decompensation of diabetes, hepatitis and development of acute pneumonia complicated the status of patient, a pulmonary edema developed and on 27.04.1993 patient died. The RARS peculiarities in this patient were the long initial period of disease after the radiation exposure and ARS (7 years), a moderate decrease of

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5.5 CHRONIC LYMPHOCYTIC LEUKEMIA

B-cell chronic lymphocytic leukemia (B-CLL) is characterized by a progressive accumulation of functionally incompetent, long-lived small mature monoclonal B-lymphocytes with a typical phenotype, CD19⁺, CD5⁺, CD23⁺, and low B-cell receptor (BCR) expression resulting from low surface immunoglobulin (Ig) and CD79b expression [1]. The etiology of the disease remains so far elusive. There are no clear genetic predispositions to the development of CLL. Chromosomal abnormalities, that are frequently observed in patients with malignant non-Hodgkin's lymphomas, are heterogeneous and rare among CLL patients with early disease. Therefore, the most authors do not consider them as factors that trigger the development of CLL. The impact of chromosomal abnormalities is more significant after disease progression. The most common cytogenetic alteration is a deletion of 13q14.3. This deletion is evident in about 50 percents of patients with advanced disease. The area of deletion include non-transcribed genes and two microRNA genes, miR-15a, and miR-16-1. MicroRNAs regulate the function of many genes, some of them directly involved in cancerogenesis [2].

Ionizing radiation (IR) is one of the most studied and ubiquitous carcinogens in the environment. It is postulated to be one of the risk factors of chronic myeloid leukemia, acute leukemias and myelodysplastic syndrome, however, CLL was considered to be a non-radiogenic form of leukemia. This was based mainly on the investigation of patients treated with radiotherapy and survivors of Hiroshima and Nagasaki nuclear bombing [3]. However, the significance of these epidemiological data is limited: CLL is a rare disease in Japan and Far East, and most studies of patients treated by radiotherapy include small numbers of cases that were observed almost exclusively within the first 5

years post-treatment, while CLL is the disease of aged people and has long asymptomatic periods. In subsequent decades (in the period of 20–30-year after irradiation) there was an excess of CLL mortality reported among 14,000 ankylosing spondylitis patients who had been treated with X irradiation [4] and among 12,955 female patients who had been submitted to radiotherapy for benign gynecologic disorders [5].

The Chernobyl NPP accident, the most serious accident in the history of the nuclear power industry, led to release of enormous quantities of radionuclides and damaged the health of many thousands people, namely, accident recovery workers (clean-up workers), the workers at the nuclear power plant, inhabitants of radionuclide contaminated areas, evacuees from the most suffered settlements of Chernobyl region (Prypiyat and the 30-km zone around Chernobyl). Since 1986 many efforts have been made to investigate the IR-induced effects and minimize their impact on health of sufferers. Preliminary results of the joint USA-Ukrainian epidemiological project for Study of Leukemia and Other Hematological Diseases Among Clean-up Workers at the Chernobyl Nuclear Power Plant (National Cancer Institute, USA and ICRF, Kyiv, Ukraine), based on the estimation of a cohort of 110,000 clean-up workers of the Chernobyl NPP accident from 6 regions of Ukraine, have demonstrated that CLL is the most frequent form of leukemia among observed persons and revealed increased frequency of CLL among clean-up workers whose estimated dose was <100 mSv [6]. In addition, some differences in the course of CLL in sufferers were noted: younger age, more advanced symptoms at the onset of disease, more aggressive course with rapid progression, poor response to standard therapy with alkylating agents and purine analogues [7]. These results were the background for more advanced studies of CLL in clean-up workers and other groups of IR-exposed persons.

We have studied the basic molecular-genetic characteristics of CLL leukemic cells, namely peculiarities of immunoglobulin heavy chain variable region (IGHV) and kappa (IGK) or lambda (IGL) light chain genes rearrangements. It is known that immunoglobulin's molecules are the main component of B-cell receptor.

It was established that CLL leukemic cells express a restricted number of functional IGHV genes for the construct of BCRs and their repertoire differs significantly from those in non-neoplastic B-cells. In normal CD5⁺ B-cells only 6 of the 50 functional IGHV genes were used to a significant extent such as IGHV3-23 (13.9%), IGHV4-59 (6.3%), IGHV3-30.3 (8.3%), IGHV3-30 (6.3%), and IGHV3-07 (5.6%). IGHV1-69-expressing B-cell clones were absent in approximately 11% of healthy individuals while were found in the rest of persons with the mean frequency of 4.7% in the young, and 4.3% in the elderly [8]. The distribution of IGHV genes in CLL patients is different. The most frequent genes in CLL are IGHV1-69* (10.5–17.4%), IGHV4-34* (10.7–14.5%), IGHV3-23* (6.4–9.2%), and IGHV3-07* (5.4–8.1%), and prevalence of IGHV1-69 or IGHV4-34 genes is statistically significant comparing with healthy persons. On the contrary, CLL cases with IGHV3-30.3 gene usage occur rarely.

Another peculiarity of CLL leukemia cells is the expression of stereotyped BCRs with homologous amino acid and nucleotide sequences, including heavy chain complementarity-determining region-3 (HCDR3), in significant part of patients from different geographical areas [9, 10]. More than 150 subsets of stereotyped BCR are known, including new subsets UA/ref1-18, and UA1-9 identified in joint Ukrainian-Italian study [11].

The main hypothesis of the recent study was as follows: since assumed influence of IR on CLL development should be realized by a restricted number of mechanisms, it can be expected that CLL in IR-exposed patients may be a more homogeneous disease with regard to IGHV gene usage, somatic hypermutation status, and expression of stereotyped BCRs.

Materials and methods

Three hundred nine patients with CLL observed in the Department of Hematology and Bone Marrow Transplantation of RCRM and in the Department of Hematology of Poltava Regional Hospital, were studied. Total RNA extraction, polymerase chain reaction amplification, and nucleotide sequence analysis were performed as described previously [12]. Most of the reported sequences were deposited into the GeneBank database under the access numbers EF091900-EF091931, EF175384-EF175440, EF407822-EF407849, EF441744-EF441761, EF583659-EF583683, EU350410-EU350430, EU433859-EU433883, EU667594-EU667611, FJ694872-FJ694889, and HM173321-HM173333.

Observed patients were divided into two groups according to IR exposure. The main group included 71 IR-exposed CLL patients: 53 clean-up workers of Chernobyl NPP accident, 5 evacuees, 12 inhabitants of radionuclide contaminated areas, and one convalescent of acute radiation syndrome (ARS) due to the Semipalatinsk nuclear explosion test.

Information about estimated doses was available for 34 patients. Among 53 clean-up workers 28 patients were clean-up workers of "iodine period", April–May 1986, (estimated doses were available for 8 persons — 5.7; 21.0; 26.0; 27.8; 32.2, 75.0, 100.0, and 120.0 cSv), 15 patients were clean-up workers of June–December 1986 (estimated doses were available for 8 persons — 0.14; 2.2; 2.7; 5.0; 8.4; 8.9, 9.02, and 10.77 cSv), 5 patients were clean-up workers of 1987 (estimated doses were available for 3 patients — 9.85; 9.75, and 20.15 cSv), and 5 were clean-up workers of 1989 (estimated doses were available for 3 patients 2.8; 4.8, and 4.9 cSv). Accumulated doses (since 1986 to the diagnosis of CLL) in 8 residents of contaminated areas (0.23; 0.39; 0.54; 0.73; 0.73; 1.24; 1.62, and 2.12 cSv) were calculated on the basis of the data on ^{137}Cs soil contamination density (mean age dose typical for a given level of local contamination) and information on measurements of radioactive cesium in the body at the CLL diagnosis (if available). Absorbed doses for 4 evacuees from Prypiyat (3.74; 4.9; 5.2, and 5.23 cSv) were reconstructed taking into account date and route of evacuation, date of removal from contaminated territory, and iodine precaution. Calculations were made by S. Nechaev (RCRM).

The control group included 238 CLL patients without influence of IR in the anamnesis.

The diagnosis of CLL was based on clinical history, lymphocyte morphology, and confirmed by immunophenotypic analysis which was performed in the Department of Clinical Immunology of RCRM (head of the Department is Prof. D.A. Bazyka). Stage of disease was established according to classifications of Rai, and Binet [13, 14]. Baseline clinical characteristics of observed CLL patients are presented in Table 5.21.

Table 5.21. Baseline clinical characteristics of observed CLL patients

Characteristics	The main group, n=71	The control group, n=238	P
Median age, y (range)	58 (39–88)	57 (29–78)	0.449
Gender, n (%)			0.001
Male	62 (87)	161 (67)	
Female	9 (13)	77 (33)	
Rai stage at diagnosis, n (%)			0.625
0	8 (11)	15 (7)	
I	22 (31)	90 (38)	
II	31 (44)	95 (40)	
III	6 (8)	25 (10)	
IV	4 (6)	12 (5)	
Binet stage at diagnosis, n (%)			0.435
A	32 (45)	110 (46)	
B	32 (45)	92 (39)	
C	7 (10)	36 (15)	
Initial WBC counts, $\cdot 10^9/L$ (range)	25 (6.0–270.0)	34 (6.0–485.0)	0.188

As seen from the table, the most of clinical characteristics of patients at diagnosis were similar in the main and in the control groups. However, there was overrepresentation of male patients in the main group.

Results

Characteristics of IGHV, IGHD, and IGHJ gene usage in CLL leukemic cells

In the main group the distribution of IGHV gene families was as follows: IGHV1 — 18 cases (25.4%), IGHV3 — 37 cases (52.1%), IGHV4 — 14 cases (19.7%), and IGHV2 and IGHV5 — each one case (each 1.4%). The most frequently used genes were IGVH1–69 (13 cases), followed by IGHV3–30 (8 cases), and IGVH3–21 (5 cases). Using criteria of ERIC group, 50 (70.4) nucleotide sequences with >98% homology to the closest germline gene were considered to be unmutated (UM), and 21 (29.6%) with <98% homology were classified as mutated (M) with a mean mutation frequency of 7.3% (range, 3.6% — 11.6%). The most represented IGHV genes within M and UM sequences were IGHV3–07 and IGHV1–69, correspondingly.

In the control group IGHV genes used were derived from all 7 known IGHV gene families: IGHV1 — 86 cases (36.1%), IGHV3 — 95 cases (39.9%), IGHV4 — 44 cases (18.5%), IGHV5 — 8 cases (3.4%), IGHV6 — 1 case (0.4%), and IGHV2 and IGHV7 — each two cases (each 0.8%). The most frequently employing genes were IGHV1–69 (56 cases), followed by IGHV4–34 (19 cases), IGHV1–02 (17 cases), and IGHV3–33 (14 cases). The overwhelming majority of nucleotide sequences (162 cases, 68.1%) were UM, and the remaining 76 (31.9%) were M (mean mutation frequency of 6.0%; range, 2.2–12.4%). IGHV4–34 (15 cases) and IGHV1–19 (55 cases) were predominant in M and UM sequences, correspondingly.

The differences in frequency of IGHV gene usage and mutation status of IGHV genes between the main and the control group were non-significant (Table 5.22).

Table 5.22. The most frequent IGHV genes used in observed CLL patients, number of cases (%)

	IGHV genes used in observed CLL patients, number of cases (%) in					
	the main group			the control group		
	total, n=71	mutated, n=21	unmutated, n=50	total, n=238	mutated, n=76	unmutated, n=162
IGHV1-69	13 (18.4)	0	13 (26.0)	56 (23.5)	1 (1.3)	55 (34.0)
IGHV1-02	1 (1.4)	0	1 (2.0)	17 (7.1)	3 (4.0)	14 (8.6)
IGHV1-58	0	0	0	5 (2.1)	0	5 (3.1)
IGHV1 others genes	4 (5.6)	1 (4.8)	3 (6.0)	8 (3.4)	1 (1.3)	7 (4.3)
All IGHV1 genes	18 (25.4)	1 (4.8)	17 (34.0)	86 (36.1)	5 (6.6)	81 (50.0)
IGHV3-21	5 (7.1)	2 (9.5)	3 (6.0)	9 (3.8)	7 (9.2)	2 (1.2)
IGHV3-07	4 (5.6)	4 (19.0)	0	6 (2.5)	4 (5.3)	2 (1.2)
IGHV3-33	3 (4.2)	0	3 (6.0)	14 (5.9)	5 (6.6)	9 (5.6)
IGHV3-11	0	0	0	9 (3.8)	0	9 (5.6)
IGHV3-48	1 (1.4)	0	1 (2.0)	10 (4.2)	5 (6.6)	5 (3.1)
IGHV3-09	4 (5.6)	2 (9.5)	2 (4.0)	5 (2.1)	2 (2.6)	3 (1.8)
IGHV3-30	8 (11.3)	3 (14.3)	5 (10.0)	11 (4.6)	4 (5.3)	7 (4.3)
IGHV3-53	4 (5.6)	3 (14.3)	1 (2.0)	2 (0.8)	1 (1.3)	1 (0.6)
IGHV3-23	1 (1.4)	1 (4.8)	0	6 (2.5)	5 (6.6)	1 (0.6)
Others IGHV3 genes	7 (9.8)	1 (4.8)	6 (12.0)	23 (9.7)	11 (14.5)	12 (7.4)
All IGHV3 genes	37 (52.1)	16 (76.2)	21 (42.0)	85 (39.9)	44 (57.9)	51 (31.4)
IGHV4-34	3 (4.2)	1 (4.8)	2 (4.0)	19 (8.0)	15 (19.7)	4 (2.5)
IGHV4-69	3 (4.2)	1 (4.8)	2 (4.0)	10 (4.2)	2 (2.6)	8 (4.9)
IGHV4-39	2 (2.8)	0	2 (4.0)	6 (2.5)	2 (2.6)	4 (2.5)
Others IGHV4 genes	6 (8.5)	1 (4.8)	5 (10.0)	9 (3.8)	3 (4.0)	6 (3.7)
All IGHV4 genes	14 (19.7)	3 (14.3)	11 (22.0)	44 (18.5)	22 (28.9)	22 (13.6)
Total	71 (100)	21 (100)	50 (100)	238 (100)	76 (100)	162 (100)

In the main group the IGHD genes were identified in 64 sequences. Their distribution was following: IGHD3 family — 30 cases (46.8%), IGHD6 family — 17 cases (26.5%), IGHD2 family — 8 cases (12.5%), IGHD4 family — 6 cases (9.4%), IGHD1 family — 2 cases (3.1%), and IGHD7 — one case (1.6%). The most frequently used IGHD gene was IGHD3-3, which was found in 13 (20.3%) of revealed IGHD genes and used mainly in UM cases (12 UM, 1 M; $p < 0.001$). The rest of IGHD genes showed similar distribution in M and UM subsets.

In the control group IGHD genes were identified in 220 cases. The most frequently used genes were IGHD3 (113 cases, 51.4%), then IGHD2 (50 cases, 22.7%), and IGHD6 (24 cases, 10.9%) ones. The frequency of IGHD6 gene usage was lower than in the main group ($p = 0.001$). IGHD3-3 gene was found in 49 cases (20.2%), mainly in combination with UM IGHV genes ($p = 0.001$) as well as in the main group.

The distribution of IGHJ genes was similar in both observed groups of CLL patient (Fig. 5.48).

All productive IGHV sequences in our CLL groups were compared with sequences from public databases and with sequences published previously for search of CLL cases

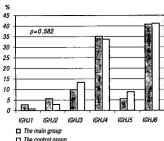


Figure 5.48. IGHJ gene usage in the main and in the control group

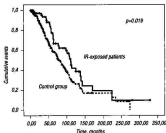


Figure 5.49. Overall survival of observed CLL patients. Kaplan-Meier method.

treatment period was similar in both groups (median 12 months, $p=0.226$).

Medians of progression-free-survival (PFS) did not differ between IR-exposed patients and the control group and were 64.0 and 45.0 months respectively ($p=0.124$). At the same time IR-exposed patients had better overall survival (median 111.0 months) than patients of the control group (median 78.0 months), $p=0.019$ (Fig. 5.49).

The frequency of autoimmune disorders did not differ between two observed groups of patients. Autoimmune haemolytic anemia (AIHA) developed in six and autoimmune thrombocytopenia (AIT) developed in one IR-exposed patients (27 AIHA cases and one AIT case in the control group; $p=0.842$). The criteria for AIHA were as follows: anaemia ($Hb < 12$ g/dL), laboratory signs of haemolysis (increased bilirubin, reticulocytosis), and the presence of antierythrocyte autoantibodies detected by the direct antiglobulin test. The criteria for AIT were as follows: rapid (< 2 weeks) and severe

with expression of stereotyped BCRs. For the confirmation of relevance of some CLL cases to clusters with stereotyped BCRs, the amplification of IGH or IGL light chain genes was also performed. The frequency of stereotyped CLL cases was similar in the main and the control groups (36.6% and 39.1%, correspondingly; $p=0.709$) as well as the distribution of clusters. In the main group we found homologous sequences corresponded to CLL clusters 1, 3, 6, 7, 9, 11, 26, 41, 71, 89, 93, N7, and UA/ref2, UA/ref5, UA/ref11, UA/ref18, UA1, UA4, UA5, UA8, and UA9. In the control group clusters 1–7, 9, 10, 12, 16, 19, 22, 28, 33, 34, 44, 59, 69, 79, 88, 95, N7, UA/ref1–4, UA/ref6–10, UA/ref12–17, and UA1–9 were presented.

Thus, the analysis of IGHV gene rearrangement did not reveal any significant differences between IR-exposed patients and the control group.

Some clinical data of observed CLL patients

During the observation, 60 CLL patients of the main group (84.5%) and 183 patients of the control group (76.5%) needed the treatment because symptomatic or progressive disease developed. Duration of time-to-treatment

decrease (half of the initial level and below $100 \cdot 10^9/L$) of platelets counts, normal or augmented megakariocytes in bone marrow, absence of or limited splenomegaly (not palpable), absence of cytotoxic treatment in the preceding month, and improvement in platelet counts in response to steroids [15].

In the control group patients with AIHA had a higher frequency of expression of UM IGHV3-11 (18.52% vs. 1.89% in patients without AIHA; $p=0.001$), and UM IGHV4-59 (18.52% vs. 2.37%; $p=0.001$) genes. The frequency of expression of UM IGHV1-69 gene did not differ in CLL patients with (22.75%) or without AIHA (29.63%); however, a 1/3 of all AIHA cases were observed among CLL patients that expressed IGHV1-69. As a result, 18 out of 27 AIHA cases were found in patients with expression of UM IGHV1-69, IGHV3-11, or IGHV4-59 genes (odds ratio = 5.404; 95% CI 2.296–12.717; $p=0.0001$). Furthermore, the period of AIHA development was significantly shorter for patients expressing UM IGHV3-11, IGHV1-69 or IGHV4-59 compared to CLL patients expressing other UM IGHV genes (Fig. 5.50).

In the main group we did not find association between AIHA and IGHV gene usage. Autoimmune disorders developed in IR-exposed patients with expression of IGHV1-69 (one case), IGHV3-21 (2 cases), IGHV3-49 (one case), IGHV4-59 (one case), and IGHV5-a (one case) genes. However, in both groups patients with AIHA more frequently expressed stereotypic BCRs than patients without AIHA ($p=0.022$). Stereotypic BCRs were revealed in 3 out of 6 CLL patients with AIHA in the main group, and in 16 out of 27 CLL patients with AIHA in the control group.

The difference in frequency of Richter transformation (development of diffuse large B-cell lymphoma) was not significant between IR-exposed and IR non-exposed patients: 9 (12.7%) and 18 (7.6%) cases respectively, $p=0.181$. In both group the probability of Richter transformation was similar in patients with homologous or heterologous BCRs ($p=0.502$) as well as for the patients with M or UM IGHV genes ($p=0.283$) and did not appear to be correlated with IGHV gene usage or any subset.

Notably, there were significant differences in frequency of solid tumors between the main and the control groups. Secondary non-lymphoid tumors were found in 16 IR-patients (22.5%) comparing with 24 (10.1%) patients of the control group; $p=0.006$.

For the confirmation of these data, we included in our study representative data from the National Cancer Registry of Ukraine concerning all 740 CLL cases developed in male residents of Kyiv between 1991 and 2008 (code C91.1, chronic lymphocytic leukemia, according to ICD-10). Among them 56 patients were clean-up workers of Chernobyl NPP accident (list of personalities was checked by the comparison with the

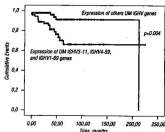


Figure 5.50. Median time of development of autoimmune haemolytic anaemia (AIHA) in the control group of observed CLL patients depends on the expression of separate UM IGHV genes.

State Ukrainian Register of persons who suffered due to Chernobyl NPP catastrophe), and 684 males without influence of IR in the anamnesis (the group of comparison).

Secondary tumors were found in 74 (10.0%) CLL patients. In three CLL patients a double carcinoma was diagnosed, and a total number of revealed tumors in this group was 77 (Table 5.23).

Table 5.23. The frequency of solid tumour in CLL patients (male residents of Kyiv) depending on IR exposure, according the data of National Cancer Registry of Ukraine

Characteristics	Clean-up workers, n=58		The group of comparison, n=684	
	n	%	n	%
CLL patients with solid tumours:	13	23.2	61	8.9
All solid tumours	14	25	63	9.2
before CLL diagnosis	5	8.9	22	3.2
simultaneously or after CLL diagnosis	9	16.1	41	6.0
Origin of tumours:				
1. Skin and soft tissues	7	12.5	27	3.9
basal cell carcinoma	5	8.9	22	3.2
squamous cell carcinoma	1	1.8	3	0.4
melanoma	1	1.8	2	0.3
2. Digestic system	2	3.5	12	1.7
gastric cancer	0	0	1	0.1
colorectal cancer	2	3.5	10	1.5
pancreas cancer	0	0	1	0.1
3. Urogenital system	3	5.3	14	2.1
prostate cancer	3	5.3	6	0.9
testis cancer	0	0	2	0.3
renal cancer	0	0	5	0.7
bladder cancer	0	0	1	0.1
4. Lung cancer	0	0	7	0.9
5. Mesothelioma	0	0	1	0.1
7. Thyroid cancer	1	1.8	1	0.1
10. Meningioma	0	0	0	0
11. Cancer of oral cavity	1	1.8	2	0.3

The most frequent tumors were basal cell carcinoma (27 cases), followed by colorectal cancer (12 cases), and prostate cancer (9 cases). The frequency of solid tumor was higher in clean-up workers (25%) comparing with IR non-exposed CLL patients (10.1%; $p=0.001$). Differences were significant due to a high frequency of basal cell carcinoma and prostate cancer among clean-up workers.

So, compared with IR non-exposed patients, CLL patients with IR exposure demonstrated a significantly increased risk of solid tumors development (OR of 3.29, 95% CI 1.61–6.62; RR of 2.71, 95% CI 1.63–4.52).

The excess of malignant diseases after IR exposure is considered to be a one of the most serious medical consequences of Chernobyl NPP catastrophe. It is known that irradiation with therapeutical aims increased the risk of development of secondary cancers in oncological patients [16, 17]. The increasing risk of malignant tumors of different origin has been found in survivors of atomic bombing in Hiroshima, including dose-dependent effect for non-melanoma skin cancer (the most representative secondary tumor in clean-up workers with CLL in our group) [18]. It is the reason why a direct tumor-inducing action of IR might be responsible for increasing of secondary cancer development in IR-exposed CLL patients. According to the epidemiological data, the risk of certain cancers, namely thyroid cancer and some leukemias, was more pronounced in clean-up workers of Chernobyl NPP accident [6, 19, 20]. However, only leukemia risk has been established in analytic epidemiological study [6]. Furthermore, while dose-dependent effect was found for all leukemias as a whole, elevated CLL risk did not reach statistical significance. Thus, in our study we have possibly observed modifying influence of two factors.

In addition, there are some publications in the literature in which the impairment of T-lymphocyte-mediated immunity in CLL patients as promoting factor for development of second tumors is discussed [21]. Our previous studies revealed also considerable disturbances in cellular and humoral immunity among different groups of sufferers of Chernobyl NPP catastrophe [22]. It is possible to suggest joint influence of IR and presence of CLL for deterioration of T-cell immunity and following development of secondary cancer. It cannot be excluded also that genetic susceptibility for development of CLL and some others tumors is believed to be influenced by IR.

Association between peculiarities of IGHV rearrangement, clinical data, and IR anamnesis of CLL patients

The group of CLL patients, suffered due to Chernobyl NPP accident, was heterogeneous by absorbed doses of IR, duration and intensity of IR exposure, etc.

First of all, we compared clean-up workers with all other IR-exposed CLL patients (evacuees and inhabitants of contaminated areas). Such parameters as the frequency of M cases (29.6% vs 29.4%, $p=0.926$), the distribution of IGHV gene families (IGHV1 family — 24.5% vs 29.4%; IGHV3 family — 50.4% vs 52.9%; IGHV4 family — 20.8% vs 17.6%; $p=0.993$), and the frequency of stereotyped BCRs were similar in both groups. The frequency of solid tumors in clean-up workers (26.4%) was much higher than in the control group ($p=0.001$), while the frequency of solid tumors in other IR-exposed CLL patients (11.1%) was similar to the control group ($p>0.05$).

The clean-up workers of April–May 1986 received the highest doses of irradiation compared with all other groups of IR-exposed CLL patients ($p=0.013$) (Table 5.24). The majority of solid tumors (9 cases) and Richter transformation (5 cases) developed just in this group — either solid tumor or Richter transformation were revealed in 57.1% clean-up workers of “iodine period” compared with 25.6% among the other IR-exposed patients ($p=0.007$) and 17.6% in the control group ($p=0.001$). Clean-up workers of April–May 1986 also were the most homogeneous group with regard to mutation status of IGHV genes. Only three patients (10.7%) from this group had M IGHV genes in comparison with 41.8% of the other IR-exposed patients ($p=0.004$) and with 31.9% in the control group ($p=0.02$).

Table 5.24. Mutation status of IGHV genes and some clinical data of CLL patients depending on IR anamnesis

Patients' subgroups	Average absorbed dose, cSv, mean $\pm$ SD	Solid tumours	Richter transformation	MUM IGHV genes
Clean-up workers April–May 1986, n=28	50,1 $\pm$ 19,4	9	5	3/25
Clean-up workers of June–December 1986, n=15	16,4 $\pm$ 7,2	3	2	8/7
Clean-up workers of 1987–1989, n=10	8,7 $\pm$ 2,6	2	1	4/6
Inhabitants of contaminated area, n=12	1,0 $\pm$ 0,2	1	0	3/9
Evacuees, n=5	4,6 $\pm$ 0,4	0	1	2/3
Convalescent of ARS, n=1		1	0	0/1
The control group, n=238		24	18	78/162

In conclusion, we have found some distinctive features of leukemic cells and clinical data of IR-exposed CLL patients, especially clean-up workers of April–May 1986: a high frequency of solid tumors and Richter transformation development, and mainly unmutated status of IGHV genes. These preliminary findings do not contradict to an assumption about possible influence of IR on CLL development and are in need of confirmation in more extensive study.

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5.6 STEM CELL: RESULTS OF CLINICAL RESEARCH

Multistage process of differentiation of immunocompetent cells throughout the human life is supported by bone marrow stem cells, in turn immunocompetent cells constantly monitor the size of a pool of stem cells, their maturation and differentiation. High radiosensitivity of cell components combines these systems by the mechanism of formation of remote consequences of radiation exposure

The number of CD34-positive cells is very small in the peripheral blood. This minor population contains stem cells and hematopoietic progenitors. Radiation increases the number of CD34-positive cells in the peripheral blood and modifies their surface phenotype [1, 2].

The objects of research were two groups of patients: clean-workers of the Chernobyl accident (average cumulative dose 1.77 ± 0.41 Sv) and individuals exposed to prolonged radiation in the range of low doses (approximately 15 mSv) in the process of their occupation activity. Mononuclear cells from heparinized peripheral blood were separated and phenotyped by flow cytometry analysis of surface staining for negative and positive markers (CD34, CD90, CD10, CD123, CD38, PCNA, CD117, and CD45).

It was determined that the number of circulating CD34-positive cells increased in clean-workers of the Chernobyl accident (liquidators) and individuals exposed to prolonged radiation in the range of low doses. Liquidators had also increased number of cells with an undifferentiated surface phenotype $CD34^{+high}CD90^{-}CD10^{-}CD123^{-}CD38^{-}PCNA^{-}CD117^{-}CD45^{-low}$ in the total population of CD34-positive cells in peripheral blood. Individuals exposed to prolonged radiation at low doses, had a level of cells with an «undifferentiated surface phenotype» $CD34^{+high}CD90^{-}CD10^{-}CD123^{-}CD38^{-}PCNA^{-}CD117^{-}CD45^{-low}$ in the circulating CD34-population, which did not differ from control values (Fig. 5.51).

A similar study in a group of non-irradiated patients with depression in the immune system, that was formed against a background of chronic somatic diseases

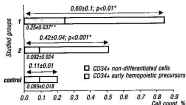


Fig. 5.51. Content of undifferentiated cells with the immunophenotype $CD34^{+}CD90^{-}CD10^{-}CD123^{-}CD38^{-}PCNA^{-}CD117^{-}$ in total $CD34^{+}$ population of peripheral blood and the size of the circulating pool of $CD34^{+}$ -positive cells in groups: 1) clean-up workers in remote periods after acute radiation exposure; 2) individuals exposed to prolonged radiation in the range of low doses (approximately 15 mSv) in the process of their occupation activity and control *the difference with control; 2) **the difference with control and group 2

also revealed significantly ($p < 0.01$) increased $CD34^{+}$ circulating pool, while the number of cells expressing only stem-cell marker $CD34$ («undifferentiated surface phenotype») also did not differ from control values. These findings confirm that this effect of increased number of circulating $CD34$ positive cells is non-specific.

Circulating $CD34$ positive cells were morphologically homogeneous but heterogeneous in the surface phenotype. They contained mainly early hematopoietic progenitors with suf-

ficient expression of CD90, CD117, PCNA antigens in individuals exposed to prolonged radiation in the range of low doses. The composition of the circulating pool of hematopoietic precursors in the clean-up workers of the Chernobyl accident was characterized by high expression of stem-cell marker CD34 and low expression of CD90, CD117 antigens.

The proportions of cells with phenotypes of CD34⁺ PCNA⁺ and CD34-PCNA⁺ exceeded the control level by 7–10 times in liquidators and individuals exposed to prolonged radiation in the range of low doses, that was the evidence of functionally active state of the circulating pool of hematopoietic precursors and may indicate on radiation-associated functional characteristics of cytoplasmic protein PCNA.

Dynamic study of the quantitative characteristics of circulating pool of CD34-positive cells in liquidators-ARS convalescents (average cumulative dose 2.91 ± 0.41 Sv) showed significant ($p < 0.01$) decrease the total amount of circulating CD34⁺ pool and no change in cells count with expression only stem-cell marker CD34 through 2–3 years after the first study, that had been held through 16 years after acute exposure. In the individuals exposed to prolonged radiation at low doses (average effective dose in the first study 7.56 ± 0.88 mSv; in the second study 10.04 ± 0.98 mSv) through 1–1.5 years after the first study of probable difference of these indicators could not were established (Fig. 5.52).

These data are evidence of saving the modified phenotype of circulating CD34⁺ pool for a long time. The presence of increased numbers of cells with expression only stem-cell marker CD34 may indicate a possible significant role of this indicator in assessing long-term effects, when such a high value components irreversible failure may contribute to the realization of the consequences of radiation on human body. We know little about how radiation affects sensitivity of hematopoietic compartment of stem cells. However, these findings are consistent with a delayed and long-lived tissue reaction to radiation injury characteristic of an inflammatory response with the potential for persisting bystander-mediated damage [1, 3–5].

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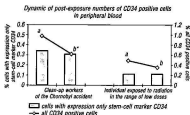


Fig. 5.52. Comparison of the size of the circulating pool of CD34 positive cells and numbers of cells with expression only stem-cell marker CD34 in groups of irradiated patients (a — first study; b — second study) *statistically significant differences, $p < 0.01$

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5.7 STEM CELLS — FUNCTIONAL ACTIVITY

Post-irradiation repair of the hematopoiesis depends on the state of the stem cell pool of the hematopoietic system, which is very sensitive to the effects of the ionizing radiation [1, 2]. Today the complete picture of the post-irradiation repair of the hematopoiesis is not fully understood [3, 4]. However, it is determined that regeneration of the maturing cells pool and recovery of their numbers in peripheral blood is determined primarily by the speed and completeness of the repair of the hematopoietic progenitor cell clone [5] and stromal cells of the bone marrow [6].

For determination of the peculiarities of function of human hemopoiesis in the conditions associated with Chernobyl accident, we investigated the state of the committed progenitors in 206 patients from 32 to 70 years old that were exposed to ionizing radiation in a broad dose range during acute and prolonged periods after the accident.

Comparative analysis was performed in the clean-up workers of Chernobyl accident exposed during 1986–1987 to doses lower than 1.0 Gy; the acute radiation syndrome (ARS) convalescents irradiated in the doses from 1.0 to 3.0 Gy; inhabitants of the contaminated areas of the Zhytomyr and Kyiv regions of Ukraine and evacuated from the 30 km zone with absorbed doses from 0.015 Gy to 0.120 Gy, and a group of patients with malignant diseases of the hematopoietic system (as comparative control).

Enrichment of the hematopoietic cells with CD34⁺ cells was achieved by implementation of the immunoselection of the CD34⁺ cells using microselective multiwell plates of the Allied Immune Sciences Inc. (UK).



Fig. 5.53. Eosinophilic colony from the culture of the bone marrow of patient with ARS grade 2. Giemsa-Romanovsky staining, $\times 500$

In the early period after the exposure to ionizing radiation, reduction of colony-forming units (CFU) in culture was observed in ARS patients, which was followed by proliferation of immature forms of granulocytes, destruction of cells and eosinophilic differentiation of the cells (Fig. 5.53). CFU efficiency with ARS grade 1 was 12.6 ± 0.6 , and in patients with ARS grade 2 it was 9.4 ± 2.6 per 10^5 explanted cells (Table 5.25).

Table 5.25. Comparative analysis of the cultivation results of the hematopoietic progenitor cells in patients who survived ARS depending on the times of the investigation

Investigated patient groups	1988		1990	
	Quantity of patients	CFUdc	Quantity of patients	CFUdc
ARS grade 1	9	12.6±0.6*	12	38.5±3.6*
ARS grade 2	6	9.4±2.8*	5	40.0±3.4*
Control	11	36.5±1.8	25	38.7±2.3

*statistically significant differences between observed values ($p < 0.001$).

Hemopoietic potential of the progenitor cells could be recovered if the post-exposure recovery was favorable after the exposure doses of more than 1.0 Gy during post-accident periods of 6 month to 10 years (Fig. 5.54), in contrary to leukemias, where even during stable remission stages none of the qualitative or quantitative characteristics of the progenitor cells could be recovered. However CFU activity of the granulomonocytic progenitor cells in the peripheral blood was increased.

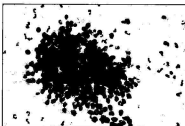


Fig. 5.54. Granulocytic-monocytic colony from the bone marrow culture of the reconvalescent of the ARS grade 1. Giemsa-Romanovsky staining, $\times 400$.

During depression of the CFU activity in the prolonged periods after the Chernobyl accident, during prolongation of the cultivation duration of bone marrow mononuclear cells of the reconvalescents of the ARS grade 1 and 2 in the gel diffusion capsules, an interesting phenomenon was determined, that was not common to the donors of bone marrow, where the CFU activity was pronounced for 2 week period with subsequent maturation of the cultured cells and their diffusion into the media. In the control samples after two week cultivation time, CFU efficiency was optimal and summed up to 38.7 ± 2.3 per 10^5 explanted cells. Bone marrow of the irradiated patients during the process of prolonged cultivation with apparent reduced levels of the CFU activity for the first two weeks, would give rise to colonies after 3–4 weeks of culture, which can be explained by the reparation of the of the stem cell hemopoietic elements. Results of the cultivation were similar to control ones and were 35.5 ± 2.5 per 10^5 explanted cells (Table 5.26).

Such effect would probably be valid only in the cases when transplanted bone marrow along with late progenitors some pluripotent stem cells were present or at least their closest progenitors, which under the influence of the growth factors went through maturation and induced the formation of the new colonies. Hence, during culture of the bone marrow cells of the patients exposed to the doses higher than 1.0 Gy in the gel diffusion capsules in vivo, at least two generations of the granulocytic-macrophage colonies could be determined, early (14th day) and late (28th day).

Table 5.26. CFU efficiency of the granulocytic-macrophage progenitors in the patients with ARS grade 1 and grade 2 depending on the duration of culture

Studied persons	n	CFU level	n	CFU/10 ⁵ at day of culture	
				14 th	28 th
ARS grade 1 and 2	34	low	7	21.3±3.2*	35.5±2.5*
		high	27	36.8±2.9	38.2±3.2
Control	25			38.7±2.3	37.5±4.3

n — quantity of investigated persons; *statistically significant differences between observed values ($p < 0.001$).

Quantity of the circulating progenitor cells in the patients with ARS grade 1 and 2, was 20.5 ± 2.7 , which is statistically significantly higher than in control (12.5 ± 2.1). Prevailing growth of the eosinophilic progenitors in the cultures was observed in 17% of the studied subjects, which was most probably connected with activation of the T-helper cells of the second rang that are responsible for the humoral reactions and synthesize growth factors for eosinophilic differentiation.

During radiation exposure doses from 0.25 to 1.0 Gy along with ordinary CFU activity, a significant increase of the CFU efficiency of the granulocytic-macrophage lineage is observed if compared to control ($p < 0.05$; Table 5.27). As well as no differentiation disturbances was observed in granulocyte-macrophage progenitors of the bone marrow of patients affected with such radiation exposures, however hypersegmented forms of the neutrophilic leucocytes were observed and prevailing proliferation of the maturing eosinophilic elements could be noted (Fig. 5.55).

Table 5.27. CFU efficiency of the hematopoietic progenitor cells of the bone marrow of clean-up workers of Chernobyl accident

Groups	Exposure doses, Gy	Quantity of patients	CFU per 10 ⁵ cells
1	>0.25-0.5	17	45.6±2.2*
2	0.5-0.75	10	46.3±2.6*
3	>0.75<1	9	48.0±3.5*
Control		25	38.7±2.3

*statistically significant differences between observed values ($p < 0.05$)

Activation of the erythroid lineage in the clean-up workers of the Chernobyl accident was developed by increase of the CFU efficiency of the early erythroid progenitors (BFU-E), which formed bursts mainly from young proliferating cells in comparison to maturing and mature cells as observed in control (Fig. 5.56).

CFU efficiency of the BFU-E equalled to 32.1 ± 2.8 , while the same parameter in control was at 23.0 ± 3.9 clones per 10⁵ explanted cells. As it was shown that the nature of the erythroid progenitors development was closely linked to biochemical processes at the level of morphologically identifiable erythroid cells, especially with the prevailing synthesis of the appropriate hemoglobin type [7], investigation of the most informative biochemical parameters was performed according to the set tasks (level of the fetal hemoglobin, its quantitative distribution in the separate erythroid progenitors, and presence of the ligand forms of hemoglobin in peripheral blood).

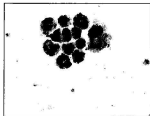


Fig. 5.55. Granulocytic cluster in *in vitro* culture of the ARS convalescent bone marrow. Giemsa-Romanovsky staining, $\times 900$

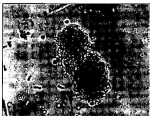


Fig. 5.56. Erythroid colonies of irregular shape from the culture derived from bone marrow of the ARS patient (inverted microscopy), $\times 200$

Glycolysis intensity and direction were studied based on the investigation of the enzymatic activities and based on the level of 2,3-bisphosphoglycerate as a main allosteric regulator of the hemoglobin. Performed investigations had shown that level of fetal hemoglobin (HbF) in the peripheral blood erythrocytes of the Chernobyl accident clean-up workers equaled to $5.9 \pm 0.3\%$, while level of HbF containing erythrocytes was $6.1 \pm 0.26\%$, at the same time in control these parameters were $3.9 \pm 0.2\%$ and $3.2 \pm 0.18\%$ respectively. Erythrocytes containing HbF, which were circulating in peripheral blood, can be a result of the elevated proliferation of the BFU and elevated levels of differentiation of erythroid progenitors.

Elevated levels of meth- and carboxyl forms of the hemoglobin in the clean-up workers to $9.9 \pm 0.9\%$ and $11.0 \pm 1.6\%$ (in control $1.1 \pm 0.3\%$ and $0.64 \pm 0.26\%$ respectively) showed development of hypoxia, in response to which activation of the major glycolysis enzymes was revealed (such as hexokinase, phosphofructokinase, and piruvate kinase), with activities of 17.76 ± 0.9 ; 79.9 ± 1.8 and 200.8 ± 6.2 $\mu\text{mol}/\text{hour}/\text{ml}$, in ARS convalescents (grade 1 and 2) 17.78 ± 0.8 ; 80.3 ± 2.1 and 195.8 ± 4.5 , and in control — 12.56 ± 1.5 ; 67.03 ± 1.5 , and 174.4 ± 4.9 $\mu\text{mol}/\text{hour}/\text{ml}$, respectively. Elevated activity of the major enzymes was probably linked with disturbance in the lipoprotein complexes, which leads to reduced anchorage of the membrane bound enzymes. Elevation of their catalytic activity leads to increase in the glucose consumption, which leads to accumulation of the 1,3-bisphosphoglycerate that is used up in 2,3-bisphosphoglycerate conversion with the 2,3-diphosphoglycerate formation. Content of 2,3-bisphosphoglycerate in erythrocytes of the clean-up workers was 0.90 ± 0.02 nmol/nm , in ARS convalescents — 0.95 ± 0.03 , that was significantly higher ($p < 0.05$) than in control (0.69 ± 0.1 nmol/nm).

Obtained results of the biochemical investigations are pointing out the observation that in patients affected by the Chernobyl accident, erythroid cells possess higher levels of the HbF, increased levels of the cells with this type of hemoglobin and activated glycolysis with intensive elevation of the 2,3-bisphosphoglycerate conversion that allosterically controls hemoglobin-mediated oxygen supply to the tissues. Comparative analysis of morphological, functional and biochemical parameters of the erythroid elements in the tested patients shows that in the case of increase in the levels of CFU efficiency of

the early progenitors, at the level of maturing and mature cells HbF accumulation is prevalent with increased numbers of cells filled with this type of hemoglobin. Direction of the mentioned changes in the biochemical cell processes can have an adaptive character. Significance of the latter is confirmed by changes of the qualitative hemoglobin content as well as adaptations in the glycolysis, directed to the oxygen supply to the tissues due to the increased level of allosteric regulator of hemoglobin function.

As a result of prolonged observation of the patients exposed to ionizing radiation due to the Chernobyl accident, a group of patients with stable inconsistencies of the hematological parameters was identified. The bone marrow of 18 patients was investigated in cell cultures. Four of them had moderate normochromic anemia, 5 patients had moderate leucopenia, 7 patients had neutrophilic leukocytosis, in two cases leucopenia was linked to thrombocytopenia. During cytological analysis of the peripheral blood smears, qualitative disturbances of the hemopoietic system were determined. Analysis of the myelograms indicated the presence of dishemopoiesis, which may develop into myelodysplasia. In 5 patients CFU efficiency of bone marrow in cell culture *in vivo* was higher than in control culminating up to 51.6 ± 2.5 per 10^5 explanted cells, in 3 patients it was on the lower limit of normal level — up to 36.9 ± 2.1 per 10^5 explanted cells. In 10 patients depression of colony-forming in culture was observed. CFU efficiency of their cells was 21.3 ± 3.8 per 10^5 explanted cells.

Diagnosis of the hematological pathology using culture-based method was proposed in 14 patients with various disturbances in hematopoiesis. In 4 patients myelodysplasia was diagnosed, which course passed as refractory anemia with ringed sideroblasts, in 3 patients it was a variant of refractory anemia, in 1 patient it was chronic myelomonocytic leukemia, in 1 patient it was hypoplastic anemia, and in 5 patient it was acute myeloblastic leukemia. Rough quantitative and qualitative disturbances of bone marrow cells clones of the patients were observed in tissue culture *in vivo*. CFU efficiency was significantly depressed and was up to 7.3 ± 1.5 in case of myelodysplasia, 3.0 colonies in case of hypoplastic anemia, and 4.3 ± 1.9 colonies per 10^5 explanted cells in case of acute myeloblastic leukemia. Morphological analysis of the cultured cells indicated alteration of the ratio of early and late granulocytes and presence of qualitative changes, which were revealed in fragmentation and pyknosis of cell nuclei, hypersegmentation, and appearance of large band and mature neutrophilic leukocytes, toxic granules and whimsical forms of cells.

To reveal alterations in the level of progenitor cells in irradiated patients concerning the risk of oncohematological pathology comparative analysis the growth character was performed in gel diffusion chambers with cultures of malignant cells in case of different leukemia forms, together with comparison of the data with clinical and laboratory data of the investigated patients. For convenient comparison we offered the index of differentiation (ID), which is the ratio between maturing and mature granulocytes, obtained in culture after two weeks of cultivation. Thus, if ID is higher than 1, that means maturing cells are prevailing.

In leukemia patient no connection was determined between the number of blast cells in myelograms and the CFU efficiency. No connection was found between CFU and clinical and laboratory data, obtained as a result of bone marrow and peripheral blood assessment. At the same time combination of such parameters as increase of maturing

granulocytes on the colonies ($ID > 1$), elevated levels of circulating progenitor cells in peripheral blood (CFUpb) and reduction of the bone marrow CFU activity (CFUbm) are considered unfavorable prognosis for the leukemic process. In case of disturbance of cell differentiation in clones with prevailing growth of low-differentiated cells, when ID is higher than 1, prognosis for therapy efficiency was unfavorable even in the cases when CFU efficiency was normal. And finally all cases with observed release of the progenitor cells into circulating blood stream, in the absence of two other preconditions, was a negative prognosis factor.

Complex functional assessment of the state of hemopoietic potential of committed progenitor cells of the patients exposed to ionizing radiation and of the persons of comparison group, including donors and patients with malignant diseases of hemopoietic system, is the basis for selection of most informative criteria for hemopoiesis assessment in culture and forming risk groups as for malignant hematological diseases. Disturbances in the ability of bone marrow to form colonies in culture were shown, as well as activation or depression of proliferative activity of the cells in clones, differentiation, eosinophilic direction of differentiation of the cells in clones, increased CFU of circulating progenitor cells in peripheral blood, development of destructive alterations in the cells, stimulation of metabolic and glycolytic processes.

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CHAPTER 6 SOLID CANCERS

6.1 THYROID CANCER. EPIDEMIOLOGY

Thyroid cancer is the most common malignancy of the endocrine system. However, it accounts only for a small fraction of all human cancers. In eighties, the annual incidence of thyroid cancer varied considerably in different European registries, ranging from 0.6 to 6.2 in males and from 1.0 to 8.3 in females per 100,000 individuals [1].

Before the accident at the Chernobyl Nuclear Power Plant in 1986, the thyroid cancer incidence rate in Ukraine increased slowly but steadily. After the accident this increase became steeper [2]: from 1989 to 2007, the thyroid cancer incidence rate increased from 0.9 to 1.6 in males and from 2.7 to 6.2 in females, per 100,000 individuals. The overall fraction of thyroid cancer among all human cancers increased about twofold for both genders: from 0.33% to 0.62% in males and from 1.59% to 3.28% in females [3, 4].

The vulnerability of the thyroid to ionizing radiation has already been known before the accident; therefore it gained particular attention after the Chernobyl accident. For this reason, since 1989, thyroid cancer has been singled out as a separate entry in the official statistics on cancer incidence by the Ministry of Health of Ukraine, while before it was included into the category "others". Furthermore, thyroid cancers diagnosed up to an age of 29 years were specified by 5-year age classes, allowing to obtain more detailed information about cancer incidence in children and adolescents.

Due to radioiodine released to the environment after the Chernobyl accident, a large number of inhabitants of Ukraine, Russia and Belarus received radiation doses to the thyroid that were much higher than doses to other organs and tissues [5]. It is also remarkable that the Chernobyl accident became an issue not only in adjacent but also in remote countries and promoted research of its possible consequences in respective populations [6].

The increase in thyroid cancer is one of the most recognized health consequences of the Chernobyl accident in those territories of Ukraine, Belarus and Russia surrounding the Chernobyl nuclear power plant. In fact, the first radiation-induced thyroid cancer cases were registered four years after the Chernobyl accident [7, 8].

A number of studies estimated thyroid cancer risk of people exposed to Chernobyl radiation in childhood and adolescence. Although significantly increased radiation risk has been demonstrated in all of them, estimates vary quite widely.

As to adult population, three different affected groups have been commonly explored: recovery operation workers, evacuees, and residents of the most heavily contaminated territories. In all these groups an excess of thyroid cancer incidence was registered (compared with national levels), but it is disputable to which extent this is due to ionizing radiation, because of the potential influence of screening and introduction of modern ultrasound diagnostic equipment [9, 10, 11].

Materials and methods

Information on thyroid cancer cases in Ukraine from 1989 through 2008 was collected from the National Cancer Registry of Ukraine (NCRU); gender- and 5-year-age-specific annual data for the population of each region in the country were obtained from official publications of the State Committee of Statistics.

All regions of Ukraine were classified into two groups based on ^{131}I thyroid doses that were reconstructed for the entire Ukrainian population aged 1–18 years at the time of the accident [12]. Assuming that the ranking of regions by the distribution of accumulated dose in those individuals aged 1–18 years was similar to that in older age groups in the same regions, we designated the regions “high-exposure” if thyroid dose exceeded 35 mGy (Fig. 6.1).

Although the average individual thyroid dose was 32 mGy in Kyiv city, we included it into the high-exposure regions, because a significant proportion of all recovery operation workers (over 23%) and evacuees from the city of Prypyat and from the 30-km zone (over 15%) reside in Kyiv city. These high numbers are characteristic for the Kyiv region only, while the fraction of recovery operation workers and evacuees in all other high-exposure regions is substantially lower. For instance, these two risk groups account for 4.6% and 2.6%, respectively, in Chernihiv region [13]. It should also be noted that according to our calculations that were done on the basis of the available data [5], the average thyroid dose of the evacuees is 328 mGy.

Thus, Cherkassy, Chernihiv, Kyiv, Rivne, Zhytomyr regions and Kyiv city were designated as high-exposure regions while all other locations were designated as low-exposure regions.



Fig. 6.1 Average accumulated thyroid doses (bold numbers, given in mGy) in all regions of Ukraine, for individuals aged 1–18 years at the time of the Chernobyl accident [12]

We calculated both the age-specific and age-adjusted thyroid cancer incidence rates for each year and for aggregated time periods, where age adjustment was done using the World Standard Population. For aggregated age groups (such as 0–19, 20–39, 40–59, 60+ years), truncated age-standardized incidence rates (TASR) were calculated. The reference period was limited to 1989 only because the first radiation-induced thy-

roid cancer cases were registered in 1990 [7, 8] and because we considered 1989 the last year of the latency period. The data for earlier years were not available, as already pointed out above.

The rate ratio (RR) was used to compare thyroid cancer incidence rates between high-exposure and low-exposure regions, and calculated 95% confidence intervals (95% CI) assuming Poisson distribution for the annual incidence of thyroid cancer. The lower and upper limits of the confidence interval for the ratios of two age-standardized rates (ASR1 and ASR2) are:

$$(ASR_1 / ASR_2)^{1 \pm (z_{\alpha/2} / X)}, \quad (1)$$

where $z_{\alpha/2}$ denotes the 100 $(1-\alpha/2)$ percentile of the standard normal distribution, and $z_{\alpha/2} = 1.96$ for $\alpha = 0.05$. For X , we used an approximation proposed by Smith (1987):

$$X = \frac{(ASR_1 - ASR_2)}{\sqrt{\{SE(ASR_1)\}^2 + \{SE(ASR_2)\}^2}}, \quad (2)$$

where SE denotes the standard error.

The study period for Chernobyl accident recovery operations workers (CRW) was 1994–2007 and that for evacuees from the 30-km restriction zone around the Chernobyl NPP and residents of contaminated territories was 1990–2007. The local cancer registry was used as the main data source for cancer cases among residents of the contaminated territories. It had been established to perform a retrospective (since 1980) and current collection of information on all cancer cases in the Luginy, Narodychs, and Ovruch districts of the Zhytomyr region and the Borodyanka, Ivankiv, Polisske, and Chernobyl (1981–1985) districts of the Kyiv region. These six districts are referred to in the text below as the territories most heavily contaminated with radionuclides. Since 1999 when the NCRU was established, these two institutions have shared information on new cancer cases registered in contaminated territories.

From 1999 through 2004, this study was supported within the frame work of the French-German Initiative for Chernobyl, Project No 3 “Health effects of the Chernobyl accident”, Subprojects 3.1.1 “Solid cancer incidence in the most highly contaminated region of the Ukraine”, and 3.1.1S “Thyroid cancer in adolescents and adults in the most affected territories of the Ukraine after the Chernobyl accident”.

At the time of the accident, the total population of the most contaminated districts was 360.7 thousand including 74.4 thousand children aged 0–14 years [8]. In 2006, the population of six districts excluding the now evacuated Chernobyl district was 193.3 thousand including 29.9 thousand children [14]. For the data collection, all relevant medical documents (including emergency notifications of new cancer cases as well as death certificates) were obtained from all medical institutions where these patients were diagnosed and treated. These documents were cross-checked to eliminate duplicates and were then entered into the final data base. 12,458 new cases of cancer were registered in 1990–2006.

The data of the State Registry of Ukraine on Chernobyl victims were used to investigate cancer incidence among CRW (1986–1987 were the years of participation in clean-up activities) and among evacuees. The 1986–1987 data for CRW relate to a group of 105.4 thousand persons in 2006, namely those who resided in the Dniprop-

etrovsk, Donetsk, Kharkiv, Kyiv, and Lugansk regions and in Kyiv city. In addition the evacuees from Prypiat and the 30 km zone, resettled in the territory of Ukraine, a group that included 53.4 thousand persons in 2006 were used. The data were compared with the data base of the NCRU. After this procedure, all duplicates and cases without validated diagnosis were eliminated. During 1994–2006, there were 6451 new cancer cases registered among CRW and, in 1990–2006, 2500 among evacuees [15]. The analysis was carried out with the standard methods of descriptive epidemiology: calculation of crude, age-specific, and age-adjusted incidence rates with standard errors and confidence intervals. The world population structure was used as the standard. For indirect standardization (calculation of standardized incidence ratio – SIR) the age-specific cancer incidence rates of the Ukrainian population in 1998 were used. To reveal possible tendencies, linear regression coefficients were calculated.

Results

In the 20 years following the Chernobyl accident in Ukraine, as a whole, thyroid cancer incidence exceeded spontaneous rates by a factor of two in males and three in females. (Fig. 6.2)

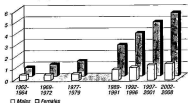


Fig. 6.2. Age-standardized average annual thyroid cancer incidence rates in Ukraine in separate time periods (males and females)

As it was mentioned above, the first cases of thyroid cancer in children residing in the most contaminated territories were observed in 1990. Before that time, no case of the disease had been registered for the children of these territories.

Starting from that period, an increasing incidence was marked not only in children, but also in adolescents and adults [16]. Investigation conducted in

the frame of the French-German Initiative for the period 1990–1999 in 3 regions with substantial ^{131}I fall-outs showed for the first time a relationship between the level of radiiodine fall-outs and the thyroid cancer incidence rate (Table 6.1).

Table 6.1. Truncated age-adjusted incidence rate (TASIR) in 1991–1999 in adolescents and adults inhabiting the Zhytomyr, Kyiv, and Chernihiv regions in territories with different levels of ^{131}I deposition

Gender	TASIR per 100 000 population on territories of ^{131}I deposition (kBq/m ²)		
	< 100	100–200	≥ 200
Male	1.53±0.26	2.20±0.20	2.56±0.25
Female	3.94±0.26	10.36±0.41	10.21±0.46

The truncated age-standardized incidence rate in territories with a level of contamination <100 kBq m⁻² did not exceed 2 cases per year per 100,000 males and 5 cases per year per 100,000 females. However, in territories with medium and high levels of contamination (100–200 kBq m⁻² and >200 kBq m⁻², respectively), a significant increase in the thyroid cancer incidence rate was registered. The excess amounted to 4 cases per year per 100,000

males and 16 cases per year per 100,000 females in 1998–1999. The effect of the exposure to radiiodine, i.e. the excess of thyroid cancer, kept increasing during the study period.

The thyroid cancer incidence rate and its dynamics in the two groups of regions were quite different. The level and average annual increase of thyroid cancer incidence were higher in the high-exposure regions than in the low-exposure regions, both for males and females (Fig. 6.3).

Throughout the period of the present study (1989–2008), age-adjusted thyroid cancer incidence rate in females of the high-exposure regions increased from 3.34 to 10.99 per 100,000, i.e. 3.3-fold, while in females of the low-exposure regions it increased from 2.51 to 5.69 per 100,000, i.e. 2.3-fold. In contrast, in males the thyroid cancer incidence rate was 0.87 per 100,000 at the beginning of the observation (1989) for both the high-exposure and low-exposure regions. In 2008 it increased to 2.64 in the high-exposure and 1.37 in the low-exposure regions, corresponding to factors 3.0 and 1.6, respectively. The slopes of the regression lines through the four trends in Fig. 6.2 are as follows: 0.079 (standard error (SE) = 0.011) for males of the high-exposure regions and 0.019 (SE = 0.004) for those of the low-exposure regions; 0.405 (SE = 0.029) for females of the high-exposure regions and 0.133 (SE = 0.011) for those of the low-exposure regions. This means that in the high-exposure regions, the thyroid cancer incidence rate increased faster compared to that in the low-exposure regions both for males and females, over the period of observation. The difference in the regression coefficients for deduced for the high- and low-exposure regions is statistically significant for both genders ($p < 0.01$).

A comparative analysis of the thyroid cancer incidence rate in different groups of the affected population (Table 6.2) suggests that the most significant excess over the national level during the study period occurred in CRW. This amounted to a factor of 5.3, while it was 5.2 for the evacuees. Among the residents of the territories that were most heavily contaminated with radionuclides, a statistically significant excess of thyroid cancer incidence (by a factor of 1.4) was registered for the time period 1990–2008.

Table 6.2. Standardized incidence rates (SIR) values for thyroid cancer (Code ICD-10 C73) in different groups of the Ukrainian population affected by the Chernobyl accident

Groups of observation (period of observation)	Numbers of cases		SIR (%)	95% CI
	observed	expected		
Residents of contaminated territories (1990–2008)	317	232.8	136.2	121.2–151.1
Recovery operation workers 1986–1987 (1994–2007)	299	53.0	564.2	500.2–628.1
Evacuees from 30 km zone (1990–2007)	231	52.3	441.7	384.7–498.6

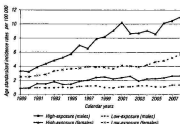


Fig. 6.3. Trends in thyroid cancer incidence from 1989 through 2008 in Ukraine by gender and dose category

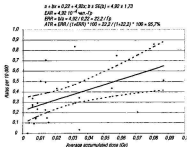


Fig. 6.4. Parameters of a linear regression equation between average regional thyroid doses of children and adolescents (1–18 years old) in 1986 and thyroid cancer incidence rates in this cohort in 2006 in regions of Ukraine

There is a correspondence between factorial (doses) and observed results (thyroid cancer incidence rate in 2006 in irradiated cohort 1–18 years old at the moment of accident) ($r=0.5023$, $p<0.01$). This is the reason we searched for the linear regression equation between dose and incidence rate (Fig. 6.4).

The abscissa axis represents the average accumulated dose (Gy), and the ordinate axis represents the incidence rate per 10,000 population. Therefore, the regression coefficient b actually reflects a rate change per 10,000 of 1 Gy. That is, the excess absolute risk is $4.92 \cdot 10^{-4}$ per Gy. The ratio of b to a (the incidence rate for an average accumulated dose equal to 0) indicates an excess relative risk of 22.2/Gy.

Note that in this cohort attributive risk = $\frac{ERR}{(1 + ERR)} \cdot 100 = 95.7\%$. (3)

Therefore, most thyroid cancer cases in this cohort have a radiogenic origin.

Fig. 6.5 presents thyroid cancer incidence at the age of diagnosis of 5–9, 10–14, 15–19 and 20–24 years for those individuals of the high- and low-exposure regions born in 1982–1986 and 1987–1991. In the high-exposure regions, thyroid cancer incidence rates at the age of diagnosis of 5–9, 10–14 and 15–19 years were significantly higher in those born in 1982–1986 compared to those born in 1987–1991, while in the low-exposure regions no such difference was observed.

Since the level of soil ^{131}I contamination is known to have been essentially zero in 1987 [17], it is possible to use the maximal values (to avoid an overestimation of the screening effect) of the thyroid cancer incidence rate in the rest three groups of individuals presented in this figure (conditionally, low-exposed): residents of the high-exposure regions born in 1987–1991 and in those of the low-exposure regions at each age of diagnosis for estimating the rate in individuals not affected by radioactive iodine. At ages of 5–9, 10–14, 15–19 and 20–24 years, these were 0.10 (low-exposure, born in 1987–1991), 0.49 (low-exposure, born in 1982–1986), 1.40 (high-exposure, born in

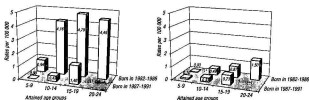


Fig. 6.5 Thyroid cancer incidence rate per 100,000 by birth year and age at diagnosis in the high-exposure regions (left panel) and low-exposure regions (right panel)

1987–1991) and 1.50 (low-exposure, born in 1982–1986) per 100,000, respectively. Subtraction of these values from the corresponding rates in the high-exposure regions for individuals born in 1982–1986 yields the estimates of radiation-induced thyroid cancer incidence per 100,000: 0.84 (0.94–0.10) for those diagnosed at 5–9 years, 3.67 (4.16–3.49) for those at 10–14 years, 3.39 (4.79–1.40) for those at 15–19 years and 2.95 (4.45–1.50) for those at 20–24 years. Thus, the proportion of radiation-induced thyroid cancer cases can be estimated as 89.2% in those diagnosed at 5–9 years; 88.3% at 10–14 years; 70.8% at 15–19 years and 66.4% at 20–24 years.

Comparative analysis regarding for age at the moment of the Chernobyl accident (Table 6.3) shows that in 1991 age-specific thyroid cancer incidence rate in “high exposure” territories significantly exceeded those in “control” regions in persons aged 0–4, 5–9, 25–29, 30–34 at the time of accident. However, in 2001 this excess was registered in all 5-years age subgroups in age range 0–54 years old at the time of Chernobyl accident (i.e. born in 1932–1986). Noteworthy, in 2006 the mean of incidence ratio in “high exposure” and “control” regions decreased in all age groups.

Table 6.3 Rate ratios (95% confidence intervals) for age-specific thyroid cancer incidence rates in population (both males and females) of the high- and low-exposure regions in Ukraine by age at the time of the Chernobyl accident and year of diagnosis

Age at the time of the Chernobyl accident (years)	Rate ratios (95% confidence intervals) at the year of diagnosis			
	1991	1996	2001	2006
0–4	28.80 (2.16, 383.75)	8.54 (3.59, 20.33)	5.53 (2.75, 11.11)	2.98 (1.69, 5.25)
5–9	22.52 (3.25, 156.20)	2.54 (1.21, 5.35)	3.22 (1.76, 5.88)	2.59 (1.60, 4.19)
10–14	2.02 (0.73, 5.63)	2.29 (1.02, 5.13)	3.69 (2.26, 6.03)	2.35 (1.53, 3.62)
15–19	1.73 (0.66, 4.52)	1.17 (0.66, 2.10)	2.19 (1.34, 3.58)	2.17 (1.45, 3.24)
20–24	1.85 (0.79, 3.43)	1.56 (0.98, 2.50)	2.70 (1.83, 4.00)	2.35 (1.66, 3.32)
25–29	2.09 (1.21, 3.59)	1.78 (1.19, 2.68)	2.69 (1.83, 4.00)	2.27 (1.69, 3.05)
30–34	1.68 (1.04, 2.70)	2.23 (1.54, 3.22)	2.31 (1.70, 3.13)	2.30 (1.73, 3.04)
35–39	0.85 (0.54, 1.33)	1.89 (1.35, 2.64)	2.10 (1.58, 2.79)	1.81 (1.39, 2.36)
40–44	1.57 (0.95, 2.60)	1.59 (1.09, 2.30)	2.36 (1.60, 3.49)	1.86 (1.17, 2.97)
45–49	1.37 (0.95, 1.99)	1.79 (1.30, 2.48)	1.99 (1.47, 2.68)	1.91 (1.39, 2.61)
50–54	1.38 (0.89, 2.15)	1.99 (1.32, 3.01)	2.03 (1.32, 3.10)	1.47 (0.91, 2.38)
55–59	1.06 (0.70, 1.59)	0.91 (0.63, 1.33)	1.50 (0.99, 2.27)	1.13 (0.74, 1.72)

Discussion and conclusions

The present analysis revealed significant differences in trends of the thyroid cancer incidence rate in the two groups of regions investigated: the increase in this malignancy was higher in high- than in low-exposure regions; the incidence rate varied among the attained age groups. The steepest increase was registered in the young age groups (up to 19 years) of the high-exposure regions suggesting that the thyroid is particularly vulnerable to radiation in childhood and adolescence. The incidence rate in these groups substantially decreased in 2005–2009 probably because the majority of the members of these groups (and all since 2006) are individuals born in 1987 or later who were not exposed to ^{131}I from the Chernobyl accident.

In the groups of other ages at diagnosis, the age-specific incidence rate was characterized by a steady increase which was significantly higher in the high- than in the low-exposure regions. The least clear tendency was observed in the oldest age group of 60+ years in the low-exposure regions. This could be explained by the lower vulnerability of the thyroid to radiation in adults and by a possible increase in the period of latency with age.

The significant increase in thyroid cancer incidence observed in the low-exposure regions for all age groups at diagnosis was not as steep as in the high-exposure regions. This increase could be due to both an increase in background and screening effects.

In all population groups affected by the Chernobyl accident, a significant increase of thyroid cancer incidence was registered. The increase was found not only in children but also in adolescents and adults. It appears to be associated, at least partly, with the fall-out of radiiodine. At the same time, we cannot disclaim the significant role that external irradiation played in the total dose received by clean-up workers.

The results of the present study evidently confirm the high vulnerability of young age groups to radiation carcinogenesis. Especially sensitive is the youngest age group born in 1982–1986 aged 0–4 years at the time of the Chernobyl accident. The observed dramatic increase in the thyroid cancer incidence rate in this age group is predominantly attributed to ionizing radiation. This conclusion is strongly suggested by the results obtained when the incidence in this age group was compared between the high- and low-exposure regions, where other confounding and modifying non-radiation factors (e.g. quality of registration, screening) were similar.

It should be stressed that for the first time, a comparative analysis of thyroid cancer incidence in Ukraine after the Chernobyl accident was performed in a cohort that is almost as large as the general population. The finding of a probable excess of radiation-induced thyroid cancer cases in adults, especially in females, may be due just to the adequately high study power. From the international literature it is well known that the excess thyroid cancer rates due to exposure to radioactive iodine tend to increase over time for more than 20 years, mainly for those exposed as young children. In the present ecological study it was difficult, however, to evaluate the potential contribution of an increased screening of the thyroid glands, which may partly have contributed to the observed increase of thyroid cancer cases, for example because of a more frequent use of ultrasound devices in medical diagnostics. We performed such evaluation of the screening effect in young age groups.

However, it should be acknowledged, that our investigation was not free from ecological biases and limitations. Precise estimation of radiation risks requires thoroughly designed cohort or case-control studies including detailed information about doses and confounding factors (both endogenous and exogenous) that might also affect the investigated thyroid cancer incidence, in addition to ionizing radiation.

The present study also suggests that the latency period of radiation-induced thyroid cancer development may depend on age at exposure: the peak of rate ratio in youngest age groups at the time of the Chernobyl accident was in 1991, but in elder ages it shifted to later years (Table 6.3). Previous investigations of this problem are very limited, but [18] pointed out the possibility of such dependence in patients subjected to radiation therapy. Further studies are necessary to confirm these observations.

The decrease in incidence rate ratios observed in 2006 in all groups of age at the time of accident may imply that the peak of excess of radiation-induced thyroid cancer is already passed. This statement, however, should be taken cautiously because similar annual data presented by [19] for persons aged 0–14 and 15–18 years at the time of the Chernobyl accident do not comply with our observations.

It was difficult to evaluate completely in the present descriptive and ecological study the contribution of increased screening of the thyroid glands to the observed increase of thyroid cancer. Based on the experience of previous studies on health effects of irradiation, the excess of solid cancer including that of the thyroid was observed decades after exposure. Therefore, we should continue to monitor thyroid cancer in groups of affected populations. The data suggest the necessity for epidemiological monitoring of thyroid cancer and a concentration of efforts to perform analytical epidemiological studies that will evaluate radiation risks at low doses of irradiation.

The documentation of dosimetric information will be essential for future attempts to examine – and possibly improve – current estimates of the risk of radiation associated thyroid cancers.

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6.2 THYROID CANCER IN EXPOSED AS CHILDREN AND ADOLESCENTS IN UKRAINE

Today a significant increase in the incidence of thyroid cancer (TC) after the Chernobyl catastrophe among high risk groups (0–18 years old at the time of the accident) is finally proved; it is recognized as the main health consequences of the Chernobyl accident by leading medical and scientific institutions in the world.

In 25 years after the Chernobyl accident children and adolescents who have experienced the most significant effect of radioactive iodine, moved to the category of adults, so now special attention should be paid to information on the TC among young adults, finding out the dynamics of changes in macro-microscopic characteristics and invasive properties of tumors.

The development of malignant tumors is clearly stochastic effects of radiation, but people who were exposed to the Chernobyl accident in childhood, especially up to 4 years of age, there was a clear dependence of the additional degree of TC incidence on thyroid irradiation. More higher prevalence of thyroid cancer in higher doses in people aged under 18 at the time of the Chernobyl disaster was observed during the screening and research for the Ukrainian-American thyroid project. Moreover, morbidity in children born before the accident, is 15 or more times higher than the rates in children born after the accident, which further confirms the nature of radiation “children post-Chernobyl” thyroid cancer.

Analysis of clinical and morphological data register of Institute of Endocrinology and Metabolism named by V.P. Komissarenko, Academy of Medical Sciences of Ukraine found that in Ukraine during post-Chernobyl period (1986–2008) 6049 persons were operated born in 1968–1986 (0–18 years old at the time of the accident) with morphologically confirmed diagnosis of «thyroid cancer» among them 4480 (74.1%) were in childhood (0–14 years; Fig. 6.6) and 1569 (25.9%) — in adolescence (15–18 years old at the time of the accident; Fig. 6.7).

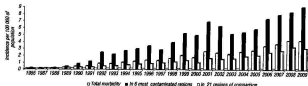


Fig. 6.6. Incidence of thyroid cancer (per 100 thousand of child population aged 0–14 at the time of the Chernobyl disaster; data of SI “Institute of Endocrinology and Metabolism named by V.P. Komissarenko, Academy of Medical Sciences of Ukraine”)

Ratio of women: men for these age groups increased by age at the time of accident: 3560:920 (3.9:1) for children and 1312:257 (5.1:1) for adolescents. The incidence gradually increased from 1990 to 2008. The number of new cases of thyroid cancer in children at the time of the Chernobyl accident in 2009 was 463 (as in the previous

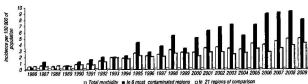


Fig. 6.7. Incidence of thyroid cancer (per 100 thousand of child population aged 15–18 at the time of the Chernobyl disaster; data of SI "Institute of Endocrinology and Metabolism named by V.P. Komissarenko, Academy of Medical Sciences of Ukraine")

2008). The number of cases in adolescents at the time of the accident was 129 and also did not differ from the previous year.

More likely relative index, i.e. incidence per 100 thousand children and teenagers at the time of the accident also rose steadily from 1990 to 2008. In 2009, the incidence was 4.13 in children and 4.87 in adolescents, not exceeding the figures for 2008 (Fig. 6.6, 6.7). Thus, it is possible that in 2008–2009 the peak incidence of thyroid cancer in radiation-risk group was determined, and in 2010 a gradual reduction of such diseases will be expected. Increase in the incidence of TC to some extent could be explained by a gradual increase in the age of designated cohort during 1986–2008. But the difference between the incidence of TC in the six most affected northern regions compared with the rest of the country not only conserved, but increased in 2006–2008 compared with the previous period of research in children and adolescents at the time of the accident (Fig. 6.6, 6.7), indicating an association between increased TC incidence and radiation factor, not with increasing age over time that has elapsed since the accident.

By the age at the time of surgery (Fig. 6.8) in recent years TC in children and adolescents was found only among born after the Chernobyl disaster. In adults born after the accident first cases of TC were reported in 2006. During 2006–2009 their number amounted to 91 compared with 2223 cases identified among people who were children and adolescents at the time of the accident (Fig. 6.8).

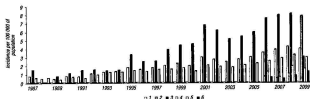


Fig. 6.8. Incidence of thyroid cancer (per 100 thousand population, 19–40 years old at the time of surgery; born before 1987 and in 1987 and later; data of SI "Institute of Endocrinology and Metabolism named by V.P. Komissarenko, Academy of Medical Sciences of Ukraine") 1 — Ukraine, born before 1987; 2 — Ukraine, born in 1987 and later; 3 — 6 most polluted regions, born before 1987; 4 — 6 most polluted regions, born in 1987 and later; 5 — 21 region of comparison, born before 1987; 6 — 21 region of comparison, born in 1987 and later.

Total number of cases of TC, registered over the years 1986–2009, was 6448, among them 6049 were born before the accident and 399 — after the accident. In young adults born before the accident the incidence of TC and morbidity in Ukraine in general and in the most contaminated regions in 2009 remained at the 2008 level (Fig 6.8).

Incidence among children, adolescents and young adults of 19–22 years old, born after the Chernobyl accident (in 1987 and subsequent years) in all years of observation was significantly lower than in the corresponding age groups born before 1987 (Fig. 6.8).

According to morphological characteristics of TC operated in 1990–2008 in patients whose age at the time of the accident did not exceed 18 years, 92.2% of cases were papillary carcinoma.

With time that has elapsed since the accident, raised the age of exposed, but also significantly changed the morphological characteristics of papillary carcinoma towards a gradual reduction of the percentage of carcinomas of a solid structure from 24.4% in 1990–1995 to 5.7% in 2006–2009 ($p < 0.01$ per test χ^2) and increase the percentage of papillary carcinomas and mixed typical structure: from 12.0% in 1990–1995 to 34.0% in 2006–2009 and 25.5% in 1990–1995 to 43.8% in 2006–2009 respectively ($p < 0.05$). With the increase in latent period varied the combination of structural components of the mixed variant of papillary carcinoma as well: the percentage of tumor of solid-follicular structure significantly decreased (from 72.7% in 1990–1995 to 25.4% in 2006–2009, $p < 0.01$), while the percentage of tumor of papillary-follicular structure increased (from 10.9% in 1990–1995 to 43.8% in 2006–2009, $p < 0.01$).

Analysis of the invasive properties of papillary carcinomas has established two major dependencies: age and time.

Under the age dependency extra-thyroid signs of invasion, presence of regional and distant metastases consistently significantly declined from children of 4–14 years old to adults of 19–40 years old at the time of surgery.

Over time dependence by combining all cases of patients of 4–40 years a reduction of the mentioned above parameters was determined with the increase of latent period (time elapsed from the Chernobyl accident to the surgery). Of special interest are the data on reduction the percentage of patients with the occurrence of distant metastases from 23.0% in 1990–1995 to 1.8% in 2006–2009 ($p < 0.001$).

Moreover, the percentage of encapsulated tumors increased from 7.4% in 1990–1995 to 29.4% in 2006–2008 ($p < 0.001$) as well as “small” carcinomas up to 10 mm: from 4.1% in 1990–1995 to 29.4% in 2006–2009 ($p < 0.001$).

Thus it can be summarized that the morbidity of thyroid cancer per 100 thousand children and adolescents (age at the time of the accident) were highest in 2006–2008 and stabilized in 2009.

Meanwhile, saving significant difference in morbidity levels between the most and least affected regions of the country (2.5 times in children and 1.9 times in adolescents), evidence for the fact that in Ukraine 24 years after the Chernobyl disaster was kept effect of the radiation factor on the incidence of thyroid cancer among persons at high risk.

The data also show that with time elapsing after the Chernobyl disaster and age of patients at the time of surgery, prevailing among thyroid cancers papillary carcinomas experienced significant morphological changes, which reflected the reduction of aggres-

sive disease, as evidenced by a significant decrease in the percentage of cases with signs of extra thyroid spread, appearance of regional and distant metastases.

The gradual accumulation of cases among persons born after the Chornobyl accident, compared with adequate relevant age group each year adds evidence to support the radiation nature of TC in children and adolescents exposed to radiation from the Chornobyl disaster.

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6.3 BREAST CANCER

Breast cancer is the most frequent tumor in female population in Ukraine and majority countries of Europe and Northern America. This pathology has tendency to increase.

Several factors have been identified as potentially responsible for increasing of the breast cancer risk, but their mechanism of action is unclear [1]. Early menarche, late first pregnancy, low parity and late menopause have all been associated with an increased risk of breast cancer and pointed out that reproductive history plays significant role as a determinant of the disease risk. Increasing of the breast cancer risk was noted in women exposed to estrogen treatment. Oral contraceptive use has been suspected as the trigger factor of increasing the breast cancer risk, especially after long-term use before age 25 or before the first pregnancy, but the evidences are still limited.

The role of dietary factors has been carefully investigated and it was suggested that diet, by influencing the pattern of estrogen exposure, may also be an important determinant of the risk.

Also the risk of the breast cancer is positively associated with socio-economic status. It should be pointed out that it is difficult to assess the specific role of each of the potential risk factors, given their strong inter-correlation and the difficulty in adjusting for confounding. The well identified role of genetic predisposition and ionizing radiation should also be noted.

During recent decades mammary cancer has been studied intensively in irradiated women. Strong evidence was revealed that breast cancer might be associated with exposure to ionizing radiation. This evidence includes studies of nuclear workers and other persons potentially exposed to ionizing radiation.

A possible increase in breast cancer incidence was observed among females who where employed for at least one year between 1969 and 1978 in Los Alamos National Laboratory [2]. Also possible increase in breast cancer death was observed in a study of 1,073 women first employed between 1947 and 1974, and followed up through 1990 in a nuclear materials production plant in Oak Ridge, Tennessee [3].

Possible increase of breast cancer death rates was observed with increasing doses of external irradiation in a study of 65,984 women employed at 10 Department of Energy facilities between start-up and 1980 [4].

After the Chornobyl accident interest to breast cancer was growing because of its high susceptibility to radiation. But studies of this pathology were performed mostly in a frame of descriptive models.

Analysis of breast cancer rates among subjects affected by Chernobyl accident indicated a significantly increased incidence compared to the general population especially in women who participated in recovery works in 1986–1987 [5–11]. Increase of the breast cancer incidence over time was also reported in the Mogilev region of Belarus [12].

A more detailed ecological study was therefore conducted to describe the spatial and temporal trends in breast cancer incidence in Belarus and Ukraine [13]. A significant increase in risk was observed during the period of 1997–2001 in districts with highest average dose levels vs least exposed districts. The magnitude of this increase is greater than would be expected based on current risk estimates [14]. Due to the public health importance of breast cancer, these findings warrant further investigation.

Thus quoting sources suggest that the women breast has relatively high sensitivity to radiation carcinogenesis, especially in those irradiated in young age.

Susceptibility of the breast to induction of radiogenic tumors justifies an extensive epidemiological study of breast cancer in affected groups of population after the Chernobyl accident.

The study was performed in a frame of scientific activities of RCRM for continuous registration of the radiation doses and cancer data among the major groups of the affected population in Ukraine.

The objective of the study was to examine hypothesis of possible influence of Chernobyl accident factors on breast cancer incidence rate in female population of Ukraine as a whole and main affected groups (participants of recovery works in 1986–1987, evacuees from Prypiat town and 30 km zone around the Chernobyl NPP and residents of the most contaminated by radionuclides areas).

Materials and methods

The current study has been started to determine and document the frequency of malignancies including breast cancer after the exposure of a significant part of the Ukrainian population due to the Chernobyl accident [5–11].

Objects of the study

- Female population of Ukraine according to administrative-territorial distribution, period of observation 1976–2008.
- Female residents of the most radionuclides contaminated districts of Ukraine: Lugyny, Narodychi, Ovruch districts of Zhytomyr region, Borodyanka, Ivankiv, Polisske districts of Kyiv region. Period of observation 1980–2008 (100,0 thousand in 2008, total number of person-years of observation 3948.1 thousand). An average accumulated individual effective dose of residents of the most contaminated territories was 22.4mSv. In territories with level of fall-out of ^{137}Cs more than 555 kBq $^{-2}$ the dose could reach 50 mSv [15, 16, 17].
- Representative sample of female-participants of recovery works in 1986–1987 (11.7 thousand females in 2007, period of observation 1994–2007, total number of the person-years of observation 144.8 thousand) inhabitants of Kyiv city, Dnipropetrovsk, Donetsk, Kyiv, Lugansk, Kharkiv regions. Average whole body dose of the external irradiation of the male recovery workers in 1986–1987 was estimated to be 50–200 mSv [16]. Females did not take part in emergency works, but worked in communal and health services therefore accumulated doses were significantly lower and could be comparable with evacuees doses.

- Female-evacuees from Prypiat town and 30 km zone (30.9 thousand in 2007, period of observation 1990–2007, total number of the person-years of observation 547.6 thousand) resettled in all regions of Ukraine. Average effective dose of the external irradiation of evacuees estimated to be 10–30 mSv. The estimated average external dose of the evacuees should be doubled to account also for the contribution of incorporated radioactivity [15, 16].

To study cancer incidence rate (including breast cancer) the local cancer registry was established in 1987 for residents of territories that had been most contaminated with radionuclides, in order to perform a retrospective (since 1980) and current collection of information on all cancer cases in the abovementioned districts. These six districts are referred to in the text below as territories most heavily contaminated with radionuclides. In addition, the information on all cancer cases including breast cancer in the former Chernobyl district during 1981–1985 was collected and was included in the database of the local cancer registry. Since 1989 when NCRU was established the linkage of information with this institution was performed to identify new cancer cases registered in the residents of contaminated territories.

From 1999 to 2004 the study was supported within the framework of the French-German Initiative for Chernobyl, Project No 3 "Health effects of the Chernobyl accident", Subprojects 3.1.1 "Solid cancer incidence in the most highly contaminated region of the Ukraine"[10].

For the data collection all relevant medical documents (including emergency notifications of new cancer cases as well as death certificates) were obtained from all medical institutions where these patients were diagnosed and treated. These documents were cross-checked to eliminate duplicates, and were then entered into the final data base. 1,533 new cases of breast cancer were registered in 1980–2008 (Table 6.4).

The data of the SRU were used to investigate the cancer incidence among female participants of recovery works in 1986–1987 and among female evacuees. The first counted 11.7 thousand persons in 2007. In addition, there were the evacuees from the of Prypiat town and the 30 km zone, who resettled in the territory of the Ukraine, a group that included 30,9 thousand persons in 2007. The data were compared with the data base of the NCRU [18]. After this procedure, all duplicates and cases without validated diagnosis were eliminated. During 1994–2007, there were 226 new breast cancer cases registered among female participants of recovery works and in 1990–2007 and 245 among evacuees. The analysis was carried out with the standard methods of descriptive epidemiology: calculation of crude, age-specific and age-adjusted incidence rates with standard errors and confidence intervals. The structure of world population was used as a standard. For indirect standardization (calculation of standardized incidence ratio – SIR) the age-specific cancer incidence rates of Ukrainian population in 1998 were used. To reveal possible tendencies, linear regression coefficients were calculated.

The increase of the total number of female participants of recovery works in 1986–1987 and evacuees during the observed period needs explanation. The source of these data is the SRU established in 1986. During the reported time the activity of its local agencies improved with regard to a more comprehensive registration of all victims, which may have resulted in an extension of registered groups.

Table 6.4. General characteristics of female populations affected by the Chernobyl accident

Groups of population	Follow up years	Studied territory	Population size (thousand) at the		Total number of the breast cancer cases registered in the period of follow up.
			start of follow up	end of follow up	
Residents of the territories most heavily contaminated with radionuclides	1980–2007	Zhytomyr region: Lugyny, Narodychi, Ovrach districts; Kyiv region: Borodyanka, Chornobyl, Ivankiv, Polisske districts.	197.1	99.0	1,494
Female participants of recovery works (PRW) in 1986–1987	1994–2007	Kharkiv, Dnipropetrovsk, Donetsk, Kyiv, Lugansk regions and Kyiv city	6.6	11.7	226
Evacuees from Prypiat town and 30 km zone	1990–2007	Whole territory of Ukraine	28.8	30.9	245

Results

First of all the general demographical and epidemiological situation in Ukraine in comparison with European and other countries should be estimated. In contrast to the Western Europe where the average life expectancy continues to increase (4.5%), a decrease of this index was found in Ukraine from 1990 to 2006 (–3.3%) (Table 6.5).

Table 6.5. Average life expectancy (years) of females population of Ukraine and Western Europe

Year	Ukraine*	Western Europe**
1990	74.9	79.4
2001	73.8	81.4
2006	72.5	83.0

*Data of Institute for Demography and Social Study, National Academy of Science of Ukraine; **Insermet "Health for All" (HIA) Database 2007

Therefore in accordance with decrease average life expectancy in female population of Ukraine it could be expected decrease of cancer cases number due to diminution of probability to reach the age with highest cancer risk. So the analysis of dynamic models of breast cancer incidence rates during 30 years period (Fig. 6.9) represents great interest.

There is a stable increase tendency during all period of observation. But increment in 1976–1990 was significantly higher (regression coefficient of 0.81 ± 0.05) in contrast with the next period — 1991–2008 (regression coefficient of 0.39 ± 0.04). These peculiarities of the trend models could be partly related to decrease of the average life expectancy since 1990 and as a consequence decrease of risk of this pathology.

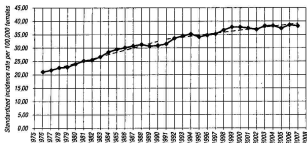


Fig. 6.9 Age-standardized breast cancer incidence rates (10^{-5} years $^{-1}$) in female population of Ukraine in 1975–2007. Regression coefficients 0.81 ± 0.05 (1975–1990), 0.39 ± 0.04 (1991–2007).

Table 6.6 Age-standardized breast cancer incidence rate in Ukraine and other countries 1983–1997 [18].

Country/territory	Incidence rate per 100,000 women
Ukraine	34.8
Kyiv city	47.1
Russia, Saint Petersburg	46.1
Croatia	47.3
Czech Republic	52.9
Spain, Granada	45.8
Poland, Warsaw	53.7
Cracow	50.4
Slovakia	42.2
Sweden	78.5
Switzerland, Geneva	97.0
Great Britain, England	74.4
Japan, Nagasaki	29.8
Hiroshima	35.6
USA, California, Los Angeles (whites)	103.9
Canada, Newfoundland	68.3

Because of the great difference in breast cancer incidence rates in different world populations comparison of these data for Ukraine and for some countries of the world is of no disputable interest (Table 6.6).

Breast cancer incidence rate in several Eastern European countries (Slovakia, Czech Republic, Croatia) is 20–35% higher than in Ukraine. In large cities such as Kyiv, Warsaw, Krakow, St. Petersburg the figures are very close. As to other countries the highest risk of the breast cancer is registered in West European and North America countries, the lowest – in Asia. Even within Western Europe there is almost a two-fold difference between the highest incidence rate, seen in Geneva (Switzerland) and the lowest in Granada (Spain). A similar range is seen in North America between white females in the San Francisco (USA) and those in Newfoundland (Canada). Noteworthy that the

breast cancer incidence rates in countries are in close relationship with average life expectancy except Japan.

Long-term monitoring of the breast cancer incidence in Ukraine and its regions give the base for conclusion about some territorial peculiarities of this pathology. This

is evidenced by data on medical-geographical analysis during more than 25 years, presented in Fig. 6.10. Medical-geographical analysis in two periods: 1977–1979 and 2004–2006 suggests the stable agglomeration of region with high, medium and low indices of morbidity. Highest breast cancer incidence rate was revealed in south and south-east parts of Ukraine, lowest — in the west and north (except Kyiv city and Kyiv region) parts of Ukraine.

Analysis of territorial variability of the breast cancer incidence rate in Ukraine (1977–1979, 2004–2006) (Table 6.7) testify the diminution of difference between regions. It is demonstrated by decreasing of maximum/minimum incidence rate ratio in regions (from 2.5 to 1.9), as well as variation coefficients (from 31.2% to 16.0%). Coefficients of correlation for two periods are 0.84 ($p < 0.01$).

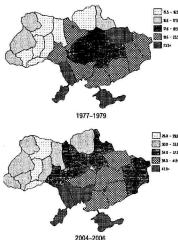


Fig. 6.10. Territorial characteristics of the breast cancer incidence in Ukraine (standardized indices by 100,000 female population) in 1977–1979 and 2004–2006.

Table 6.7. Territorial variability of the breast cancer incidence rate in regions of Ukraine in 1977–1979 and 2004–2006

Period of observation	Standardized incidence rate per 10 ⁵ females		max/min ratio	Coefficient of variation (%)	Correlation coefficient for two periods
	maximal	minimal			
1977–1979	40.4	15.9	2.5	31.2	0.84 ($p < 0.01$)
2004–2006	52.8	28.5	1.9	16.0	

Analysis of temporal and territorial peculiarities of the breast cancer incidence proves that factors responsible for forming the level of morbidity in Ukraine are unchangeable for a long period of time. This suggests an absence of the influence of Chernobyl accident negative factors to the breast cancer incidence rate in Ukraine as a whole during the period of observation.

Nevertheless the actual question is if there is such influence to the breast cancer incidence rate in main groups of population affected by the Chernobyl accident. In women-inhabitants of the most contaminated areas (Fig. 6.11) breast cancer incidence was characterized by moderate increase in 1987–1991 in comparison with 1980–1986. The level and its increment were lower than in Ukraine as a whole, Kyiv and Zhytomyr

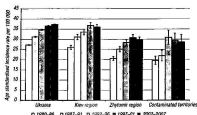


Fig. 6.11 Age-standardized breast cancer incidence in Ukraine as a whole, Kyiv, Zhytomyr regions and in the most contaminated territories in 1980–1986, 1987–1991, 1992–1996, 1997–2001 and 2002–2007.

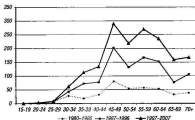


Fig. 6.12 Age specific breast cancer incidence rates in female residents of the most radionuclide contaminated territories in 1980–1986, 1987–1996 and 1997–2007.

Exceptions are age groups of 30–34 and 45–49. But statistically significant difference was registered only in the age groups 55–59 and 65–69. It should be noted that more appropriate method of comparison consists in the corresponding birth cohorts of those affected by Chernobyl accident in young age; for example 10–19 (1967–1976 years of birth) and 20–29 (1957–1966 years of birth). But small size of these cohorts does not give possibilities to provide sufficient confidence.

As to comparative evaluation of the breast cancer incidence rate in three groups of affected population (Table 6.8) the significant excess was revealed only in female participants of recovery works in 1986–1987 in comparison with national data. In 1994–2007 observed numbers of cases was 1.5 times higher then the expected one.

Analysis of these figures in inhabitants of contaminated territories and evacuees proves that in spite of significant increase of the breast cancer incidence rate its values are lower than national level. It should be stressed that both groups of affected population inhabited territories with one of the lowest breast cancer incidence rates before

regions, which include contaminated districts. The highest increase (40.9%) was observed in 1992–1996. Due to that morbidity was leveled with indices for Zhytomyr region. In the next two periods of time (1997–2001, 2002–2007) some decrease of the breast cancer incidence rate was observed either in the most radionuclide contaminated areas or in Kyiv and Zhytomyr regions. It could be resulted from the shortening of the average life expectancy especially in contaminated areas. In Ukraine as a whole the moderate increase was registered but not such intensive as in previous years.

Comparison of the age-specific breast cancer incidence rate for 3 periods: 1980–1986, 1987–1996 and 1997–2007 proves the increase of indices almost in all age groups within two last periods in comparison with preaccidental one (Fig. 6.12).

the Chernobyl accident. In contrast to that female, participants of recovery works in 1986–1987 lived in regions with high level of morbidity.

Table 6.8. Standardized breast cancer incidence ratios (SIR) in different groups of affected by Chernobyl accident female population of Ukraine

Group of observation	Period of observation	Number of cases		SIR (%)	95% CI
		observed	expected		
Residents of the most contaminated territories	1980–1991	625	1,248	50.2	46.2–54.1
	1992–2007	888	1,304	66.6	62.1–71.0
PRW*, 1986–1987	1994–1999	54	41	155.1	117.9–194.3
	2000–2007	162	108	150.0	126.9–173.1
Evacuees	1990–1997	72	122	59.0	45.4–72.6
	1998–2007	166	222	74.8	63.4–86.1

*Participants of recovery works

Conclusions

The presented long-term observation of epidemiological situation of the breast cancer in Ukraine has revealed spatial and temporal peculiarities of this pathology. Stable agglomeration of regions with high, intermediate and low incidence level was noticed. Dynamics of the breast cancer incidence was characterized by smooth increase. Recent years (1991–2008) this increase was less then in 1976–1990. The epidemiological situation of breast cancer in Ukraine as a whole and in its regions over 20 years after Chernobyl accident is stable.

Results of the study suggest that factors responsible for the forming of the breast cancer incidence before Chernobyl accident are still predominant in the post accidental period.

Among separate groups of affected population (female, participants of recovery works, evacuees and inhabitants of the most radionuclides contaminated areas) significant increase of the breast cancer incidence rate exceeding national level was registered only in women who participated in activities related to Chernobyl accident in 1986–1987.

Statistically significant increase of this pathology incidence was noted in inhabitants of contaminated areas and evacuees. Nevertheless morbidity in these groups was lower then in Ukraine as a whole.

Presented data evidence an urgent need to continue the epidemiological monitoring of the breast cancer taking into account the long period of radiation carcinogenesis in breast. Implementation of the more informative analytical methods of the epidemiological study (cohort, case-control) could give the possibility to evaluate the radiation risks of the breast cancer after irradiation with small doses due to the Chernobyl accident.

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6.4 OTHER SOLID CANCERS

Malignant tumors including leukemia are the most significant late effects after radiation exposure. The main sources for evaluating the risk of malignant neoplasms occurrence are studies on A-bomb survivors in Japan [1], on populations of radiation exposed due to nuclear tests [2] and on patients exposed to radiation in medical diagnostics and therapeutic procedures. Based on these studies the risk of fatal cancer has been evaluated as 10–12% per 1 Sv [2].

The radiation exposures due to the Chernobyl accident are not fully comparable to the types of exposure in the studies mentioned above, because the dose rates are much lower and the exposure times much longer. In line with this difference the risk of fatal cancer from exposures due to the Chernobyl accident has been estimated to be half as large, namely about 5% per 1 Sv [3]. This estimate being of considerable consequence, both with regard to the assessment of the health impact of the reactor accident and to radiation protection practice in general.

The RCRM considers it as one of its major tasks to register in a long term follow-up the radiation doses and cancer data among the major groups of the affected population in the Ukraine. These groups are: the Chernobyl accident recovery operation workers (CRW) who participated in recovery operation works in 1986–1987, the evacuees from Prypiat town and the 30 km zone, and the population still living in the territories that were most heavily contaminated with radionuclides.

The present report summarizes within the regular documentation of the follow-up the observations in the Ukraine up to and including the year 2006. These observations can be seen in the context of current interpretations and risk estimates, but no systematic evaluation is here intended, since the data are merely a part – although an important part – of the total body of observations that will emerge from all populations affected by the Chernobyl reactor accident and that is going to be the ultimate basis for risk assessment by National and International expert groups.

Materials and methods

The current study has been started to determine and document the frequency of malignancies after the exposure of a significant part of the Ukrainian population due to the Chernobyl accident [4–8].

Goals of the study:

- to collect information about all cases of cancer in the studied groups of the population;
- to create an electronic database containing all cancer cases registered in these groups;
- to collect and analyze demographic data by gender and age structure in the groups affected by the Chernobyl accident;
- to collect and analyze official data and scientific publications on the collective doses received by the different groups;
- to perform descriptive epidemiological analyses of cancer incidence rate in the groups under study;
- to provide some additional information that will facilitate future risk estimates by national and multinational expert groups.

The local cancer registry was established in 1987 for residents of the territories, that were most heavily contaminated with radionuclides, in order to perform a retrospective (since 1980) and current collection of information on all cancer cases in Luginy, Narodychi, Ovruch districts of the Zhytomyr region and the Borodyanka, Ivankiv, Polisske districts of the Kyiv region. These six districts are referred to in the text below as territories most heavily contaminated with radionuclides. In addition, the information on all cancer cases in the former Chernobyl district during 1981–1985 was collected and was included in the database of the local cancer registry. Since 1989 when NCRU was established a linkage of information with this institution about new cancer cases registered on contaminated territories was being performed.

Since 1999 to 2004 this study was supported within the frame work of the French-German Initiative for Chernobyl, Project No 3 "Health effects of the Chernobyl accident", Subprojects 3.1.1 "Solid cancer incidence in the most highly contaminated region of the Ukraine", Subproject 3.2.2 "Leukemia incidence in the Zhytomyr and Kyiv regions of the Ukraine" and 3.1.1S "Thyroid cancer in adolescents and adults in the most affected territories of the Ukraine after the Chernobyl accident".

At the time of the accident the total population was 360.7 thousand including 74.4 thousand children aged 0–14 years [6]. In 2007, the population of six districts excluding the now evacuated Chernobyl district was 186.8 thousand including 29.0 thousand children [9]. For the data collection all relevant medical documents (including emergency notifications of new cancer cases as well as death certificates) were obtained from all medical institutions where these patients were diagnosed and treated. These documents were cross-checked to eliminate duplicates and were then entered into the final data base. Total 21671 new cases of cancer were registered in 1980–2007 (Table 6.9).

Table 6.9. General characteristics of populations affected by the Chernobyl accident

Population	Years of follow up	Population size at the start of follow up (thousand)	Total number of cancer cases registered in period of follow up.
Residents of the territories most heavily contaminated with radionuclides (Zhytomyr region: Luginy, Narodychi, Ovruch districts; Kyiv region: Borodyanka, Chernobyl, Ivankiv, Polisske districts)	1980–2007	1986 — 360.7, including 74.4 children 2007 — 186.8, including 29.0 children	1980–2007 — 21671
Evacuees from Prypiat town and 30 km zone	1990–2007	1990 — 50.2 2007 — 53.7	1990–2007 — 2718
Chernobyl accident recovery operation workers 1986–1987 years of participation (Kharkiv, Dnipropetrovsk, Donetsk, Kyiv, Lugansk regions and Kyiv city)	1994–2007	1994 — 84.6 2007 — 103.4	1994–2007 — 7190

The data of the State Registry of Ukraine on Chernobyl victims were used to investigate the cancer incidence among CRW in 1986–1987 and among evacuees. The 1986–1987 data for CRW relate to a group that counted 103.4 thousand persons in 2007, namely those that reside in the Dnipropetrovsk, Donetsk, Kharkiv, Kyiv, Lugansk regions and in Kyiv city. The evacuees from Prypiat and the 30 km zone, who resettled in the territory of Ukraine, a group that included 53.7 thousand persons in 2007 were in addition. The data were compared with the data base of the NCRU. After this procedure all duplicates and cases without validated diagnosis were eliminated. During 1994–2007 7190 new cases were registered among CRW and 2718 cases among evacuees within 1990–2007 [7, 8]. The analysis was carried out with the standard methods of descriptive epidemiology: calculation of crude, age-specific and age-adjusted incidence rates with standard errors and confidence intervals. The structure of world population was used as standard. For indirect standardization (calculation of standardized incidence ratio – SIR) the age-specific cancer incidence rates of Ukrainian population in 1990 were used. To reveal possible tendencies, linear regression coefficients were calculated.

Collective and average individual accumulated doses were evaluated on the basis of three National reports (1996, 2001 and 2006) [10–12] and of general dosimetry-passport data of settlements contaminated with radionuclides (2001). Scientific publications were analyzed as well. These matters are summarized in Table 6.10.

The increase of the total number of CRW and evacuees during the observed period needs explanation. The source of these data is the SRU established in 1986. During the reported time the activity of its local agencies improved with regard to a more comprehensive registration of all victims, which may have resulted in an extension of registered groups.

Table 6.10. Estimated collective and average per capita dose equivalent for various groups in the Ukrainian population

Population group	Period	Population	Estimated collective dose (man Sv)	Mean effective dose (mSv)
Recovery operation workers*	1986–1987	141 000	22 946	162.7
	1986	98 000	18 130	185
	1987	43 000	4 816	112
Evacuees of Prypiat, Chernobyl and 30-km zone*		91 406	2800	30.6
Inhabitants of the most highly contaminated areas**	1986–2005	164 625	3584.2	22.4

* UNSCEAR Report (2000) [13]; ** Data of National Report (2006) [12]

According to the National Report (2001) [11] the average per capita effective dose varied between 100 and 200 mSv for CRW in 1986 and between 50–100 mSv for CRW in 1987. For the evacuated former residents of Prypiat town the average external dose was evaluated as 10–12 mSv, in evacuees from Chernobyl it was 20 mSv, in evacuees from settlements of the 30-km zone it was 20–30 mSv. The estimates of the evacuees' average external dose should be doubled to account also for the contribution of incorporated radioactivity. According to the National Report (2006) [12] residents of the most contaminated territories obtained an average individual effective dose of 22.4 mSv.

Only a minority of the CRW was monitored for incorporation of radiiodine in their thyroid, and, accordingly, no relevant information on thyroid doses (due to radioactive iodine) has been provided for CRW.

In children and adolescents among the residents of the contaminated territories the mean thyroid dose was 524 mGy with estimates ranging from a minimum of 68 mGy to a maximum of 2,671 mGy in the various settlements of the study districts [12].

The total thyroid collective dose in evacuees was 2,996 person-Gy and the average individual dose was 328 mGy. In this group the strong dependence of the mean thyroid dose on the age at the time of the accident needs to be noted. In the age group 0–15 years the average thyroid dose was 852 mGy, in the age group 16+ it was 184 mGy [13].

Resulting data

The subsequent information needs to be seen against the background of the general demographic and epidemiological situation in the Ukraine (Table 6.11, 6.12).

Table 6.11. Average life expectancy (in years) of males and females of Ukrainian and Western European population

	Males		Females	
	Ukraine	Western Europe	Ukraine	Western Europe
1990	65.6	72.8	74.9	79.4
2001	63.0	75.2	73.8	81.4
2006	61.5	78.0	73.4	83.0

(V.S.Stetsenko, 2001, 2004; I.O.Kurilo, 2007)

In contrast to the West European countries where the average life expectancy continues to increase, there is a decrease of this index in the Ukraine during 1990–2006, which amounts to 6.3% in males and 2.0% in females. Therefore in Ukrainian population (especially in male) due to shortening average life expectancy could be expected decrease of number of cancer cases because probability to attain most vulnerable age to cancer is diminished. In this connection special attention needs to be paid to the time trend of the rate of cancers of all sites and to the rates of specific cancer types in the Ukraine and its contaminated regions.

Time trends for the Ukraine are indicated in Table 6.12. For male until 1992 an increase of age-standardized cancer incidence rate was noted with regression coefficient being 4.48 ± 0.31 , but in 1993–2006 the rates decreased with regression coefficient (-1.79 ± 0.21) . For female in 1976–1992 regression coefficient was 1.88 ± 0.19 . In the next period of time (1993–2006) this increase was not so significant (only 0.88 ± 0.08). This may seem to be related to the decline in economic — industrial and agricultural activities and the resulting reduction of environmental pollution with toxic and carcinogenic substances, but in actuality the time scale appears to be too short for such an association become visible.

Particularly notable among the specific forms of cancer is the continued increase of incidence rates of cancer of oropharyngeal cavity, rectum, female breast, prostate, urinary bladder, kidney, and thyroid. For cancer of oesophagus, larynx, trachea, bronchus, and lung an increase was observed until 1992, subsequently there was a decrease. Also there has been a continued decrease of lip and stomach cancer incidence rates dur-

ing the entire period of observation. The skin cancer incidence rate remained constant throughout the period of observation.

Table 6.12. Time dependence of cancer incidence in the male and female population of the Ukraine in 1978–2007 (regression coefficients (b_{tm}) of the age standardized incidence rates (in 10⁻³/year))

Site of tumor	ICD-10 code	Males		Females	
		Period of observation	Regression coefficient, b _{tm}	Period of observation	Regression coefficient, b _{tm}
All cancers	C00–C97	1976–1992	4.48±0.31	1976–1992	1.88±0.18
		1993–2007	-1.68±0.15	1993–2007	0.94±0.08
1 Lip	C00	1976–2007	-0.31±0.01	1976–2007	-0.03±0.001
2 Oropharyngeal cavity	C01–C14	1976–2007	0.31±0.02	1976–2007	0.046±0.008
3 Esophagus	C15	1976–1992	0.21±0.01	1976–1992	-0.005±0.002
		1993–2007	-0.11±0.01	1993–2007	-0.02±0.004
4 Stomach	C16	1976–2007	-0.67±0.02	1976–2007	-0.29±0.01
5 Colon	C18	1998–2007	0.16±0.04	1998–2007	0.17±0.02
6 Recto sigmoid junction	C19–C21	1976–2007	0.22±0.01	1976–2007	0.065±0.01
7 Larynx	C32	1976–1992	0.28±0.03	1976–1992	0.002±0.0016
		1993–2007	-0.15±0.01	1993–2007	-0.003±0.0019
8 Trachea, bronchus, lung	C33, C34	1976–1992	1.49±0.12	1976–1992	0.11±0.01
		1993–2007	-1.60±0.08	1993–2007	-0.15±0.01
9 Skin	C43–C44	1982–2007	0.05±0.03	1982–2007	0.05±0.03
10 Breast	C50	1976–2007	—	1976–2007	0.60±0.02
11 Cervix uteri	C53	—	—	1976–1991	-0.40±0.03
		—	—	1992–2007	0.15±0.02
12 Corpus uteri	C54	—	—	1998–2007	0.22±0.04
13 Prostate	C61	1989–2007	0.48±0.01	—	—
14 Kidney	C64	1989–2007	0.28±0.02	1989–2007	0.15±0.01
15 Urinary bladder	C67	1989–2007	0.11±0.02	1989–2007	0.004±0.01
16 Brain	C70–C72	1998–2007	0.01±0.01	1998–2007	0.02±0.01
17 Thyroid	C73	1989–2007	0.04±0.01	1989–2007	0.19±0.01
18 Leukemia and lymphoma	C81–C96	1976–1990	0.31±0.02	1976–1990	0.19±0.01
		1991–2007	0.04±0.01	1991–2007	0.04±0.01

The rate of cervical cancer behaved quite differently. Until 1991 this pathology decreased significantly, but since 1991 there was an increased incidence due to changes in the young age groups.

The cancer incidence rates in the population still resident in the most heavily contaminated territories are presented in Fig. 18.1. For comparison this includes also the data for Kyiv, the Zhytomyr regions and the entire Ukraine. During 1980–2006 the cancer incidence rate in the contaminated territories was somewhat lower than in the comparison regions. In the same period, the time trends of cancer incidence rate were similar in all compared territories: the annual standardized incidence rates showed a gradually increasing trend. However, since 1993 some decrease of the rates is observed in all com-

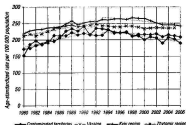


Fig. 6.13. Cancer incidence rates ($10^{-5}/\text{yr}$) in Ukraine, Kyiv, Zhytomyr regions and the territories most heavily contaminated with radionuclides in 1980–2006. Regression coefficients: Ukraine — 1980–1992 — 3.17 ± 0.35 ; 1993–2006 — $(-0.67) \pm 0.15$ Kyiv region. — 1980–1992 — 2.45 ± 0.18 ; 1993–2006 — $(-1.78) \pm 0.37$ Zhytomyr region. — 1980–1992 — 3.58 ± 0.55 ; 1993–2006 — $(-0.90) \pm 0.30$; contaminated territories — 1980–1992 — 5.69 ± 0.94 ; 1993–2006 — $(-3.04) \pm 0.41$

are, especially, leukemia, thyroid cancer, breast cancer, lung cancer, stomach cancer, colon cancer and ovary cancer.

Table 6.13. Standardized incidence ratios (SIR) for all forms of cancer (Code ICD-10 C00–C97) in different groups of Ukrainian population affected by the Chernobyl accident

Group of observation and time period	Number of cases		SIR (%)	95% confidence interval
	actual	expected		
Residents of contaminated with radionuclides territories (males + females)				
1990–2007	3678	4753	77.4	74.9–79.9
Chernobyl accident recovery operation workers, 1986–1987 years of participation (males)				
1994–2007	7190	6649	108.1	105.6–110.6
Evacuees from Prypiat and 30 km zone (males + females)				
1990–2007	2718	3318	81.9	78.9–85.0

Very demonstrative are studies of leukemia incidence rate which could be indicator of oncogenetic influence of radiation in population still living on most contaminated with radionuclides territories. The study was performed in frame of French-German Chernobyl Initiative during 1999–2004 in population of Kyiv and Zhytomyr regions which includes most contaminated with radionuclides territories. Observed period 1980–1999 evidences lack of radiation induced excess of leukemia incidence in studied territories.

There is a slow increase of leukemia incidence rate over time with small fluctuations in separate years in the regions under study. Differences with national level of incidence rate and time trends are insignificant. This tendency is characteristic for

comparison territories. There is no significant difference between the regression coefficients.

The comparison of standardized incidence ratios (SIR) in different groups of the affected population (Table 6.13) suggests no statistically significant excess against the national level, both for the evacuees and the residents of the contaminated territories. However, in CRW a statistically significant excess is seen. Today, 20 years after the Chernobyl accident, certain types of malignant tumors are of particular interest, because earlier studies have established a clear linkage to radiation. These

both genders, males and females separately. Analogical results were obtained by in contaminated territories of Chernihiv and Sumy regions. This does not contradict the current radiobiological knowledge that cancer can be caused by ionizing radiation but reflects the fact such a possible increase on the population level is so small that it is not statistically detectable.

Conclusions

The study of cancer incidence in the major part of the population affected by the Chernobyl accident (CRW of 1986–1987; evacuees from Prypiat town and the 30 km zone, and the population still living in the territories most heavily contaminated with radionuclides) has provided characteristic trends. The total cancer incidence rates were higher than the national rates only in the population of CRW, many of whom participated in recovery operation works in 1986–1987.

The comparatively small number of cases of specific types of cancer and the difficulty to assess the influence of migration make it advisable to extend the monitoring beyond the contaminated areas. Special attention needs to be given to regions with a large and stable fraction of persons who were relocated from the contaminated zone.

These problems need to be solved with assistance of the SRU and with linkage to the NCRU and regional registers. The documentation of dosimetric information will be essential for future attempts to examine – and possibly improve – current estimates of the risk of radiation induced malignancies in the light of the observations after the reactor accident.

Because the latency of radiation induced cancers varies and can extend to very long periods, special attention should be given not only to thyroid, breast cancers and leukemia, but also to cancers of lung, stomach, colon, ovary, bladder and liver, and to multiple myeloma.

Presented data suggest the necessity of epidemiological monitoring of cancer and concentration of efforts to perform analytical epidemiological studies as case control or cohort. That will give possibility to evaluate radiation risks at low doses of irradiation.

Finally, the needs of particular interest consist in the follow-up of the age groups of those who were young at the time of the reactor catastrophe, i.e. the persons who were exposed to radiation *in utero*, in childhood or as adolescents.

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6.5 MOLECULAR MECHANISMS OF THE MALIGNANT TRANSFORMATION IN THE URINARY TRACT EPITHELIUM, INFLUENCED BY CHRONIC LONG TERM LOW DOSE IONIZING RADIATION EXPOSURE

SI "Institute of Urology, National Academy of Medical Sciences of Ukraine"(IU), had accumulated the experience in the application of the modern clinical and histological methods for studies at the area of diagnostics, classifications, prophylactic measures and treatment of bladder cancer, renal-cell carcinoma, prostate cancer and testicular tumours over the several years before the Chernobyl accident in Ukraine. The pathogenesis as well as morphogenesis of precancerous lesions and the importance of some background states for bladder carcinogenesis were also investigated. This experience was represented in many articles, 7 monographs and some manuals that became a good background as well as they became the control groups for the investigations at the area of the new molecular peculiarities of the carcinogenesis after the Chernobyl accident.

Effects of chronic long term low dose ionizing radiation on the renal-cell carcinoma and the peritumoral tissue

Over the last 20 years we had investigated patterns of the renal-cell carcinomas (RCCs), renal oncocytomas and peritumoral renal tissue collected from Ukrainian patients with different exposure to ionizing radiation. The epidemiological, histological, immunohistochemical and molecular methods were applied. The immunohistochemical study and molecular genetic analysis were performed in the Department of Pathology, Medical School of Valencia University, Spain (Professor and Chairmen Dr. Antonio Llombart Bosch). We thank Luisa Morell-Quadreny, M.D. Ph.D., Jose Antonio Lopez-

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Epidemiology of renal cancer in Ukraine after the Chernobyl accident.

The epidemiological study showed the increasing number of patients with the primary find out renal cancer from 3771 in 1998 to 4877 patients in 2009.

Morbidity of malignant renal tumors in adults during the period of 1986–2009 increased from 4.7 to 10.6 per 100,000 of total population in Ukraine (from 6.0 to 13.6 per 100,000 of the males and from 3.6 to 8.1 per 100,000 of the female population) [1]. Seventeen years of internal exposure to ^{137}Cs at low doses, which were constantly concentrated and eliminated by kidneys of about $10\cdot 10^6$ dwellers of the radiation contaminated areas of Ukraine as well as Kyiv (over $31\cdot 10^6$ people), could possibly influence the incidence of kidney tumors in Ukraine. The internal exposure is the most dangerous, ^{137}Cs constitutes 80–90% of it. This radionuclide is known to be concentrated and eliminated through kidneys and 90% of the more labile pool of ^{137}Cs is excreted via urine [2].

Radiometric measurement of ^{137}Cs was conducted using a Standard V-Radiometer RUB-01 (Ukraine) with urine collected over 24 hours from all patients of groups I–III before surgery was performed. Significant relation of ^{137}Cs urine levels with level of radiocontamination residence territory was showed and possibility of group dividing was confirmed. Furthermore, apart from radionuclides, the synergetic effect influenced by the different chemical factors including potentially carcinogenic substances took place at the contaminated territories of Ukraine. The levels of the chemicals contamination in Ukraine is 6,5 times higher than in USA, and 3,2 times higher than in Europe.

Pathology and proliferative activity of RCCs in Ukrainian patients with different radiation exposure.

An investigation of the histological and prevalence patterns of RCCs as well as tubular nuclear atypia and carcinoma *in situ* (CIS) of peritumoral kidney tissue in association with dose radiation exposure was performed. We selected five groups of patients with RCCs in Ukraine, which represented varying degrees of internal exposure to radiation and who were operated in 2 different periods of time (1993–1996 and 1997–1999) following the Chernobyl accident. The control group of analog patients with RCCs was selected in Valencia (Spain) for the present study. Three hundred forty-eight patients from Ukraine and Spain underwent radical nephrectomy due to RCC without previous treatment; 236 patients from Ukraine underwent surgery in the IU and were divided as groups I to V, according to the level of radiation contamination and duration of exposure. The Spanish group included 112 patients who underwent surgery in the University Clinic, Hospital of Valencia, thus representing control group VI. Characteristics of all patients are summarized in Table 6.14.

In all cases surgical specimens were revised with tumoral size as a variable for study. RCCs were classified in accordance with the clinical and histological criteria defined by the Heidelberg UICC classification.

TNM stage and pathological features of RCCs and peritumoral kidney tissues in the different groups are summarized in Table 6.15. Mean and standard deviation of tumoral size was 7.7 and 4.9 cm, respectively, for the Ukrainian groups, and 8.6 and 4.4

cm, respectively, for the Spanish group, without significant differences. Comparative analysis between all Ukrainian and Spanish groups showed more frequent incidences of high grade RCCs in Ukrainian patients with the significant differences that depended predominantly on groups II and III. Analysis of statistical relations between different groups showed that the distributions by grade in group I and the Spanish group were similar, and tumors of both these groups were significantly more differentiated than those of groups II, III, IV and V.

Table 6.14. Patient characteristics

Group	I		II		III		IV		V		VI	
	male	female	male	female	male	female	male	female	male	female	male	female
Quantity of patients	30	27	30	25	43	29	11	3	22	16	74	38
Median age (range)	55 (27-79)		56 (34-75)		55 (18-77)		51 (27-75)		53 (41-75)		57 (26-77)	
Years of surgery	1993-1999		1993-1996		1997-1999		1993-1996		1997-1999		1975-1989	
Levels of soil contamination (Ci/km ²)a	not contaminated		0.5-5		0.5-5		5-30		5-30		not contaminated	
	0.5-0.49											

Table 6.15. Incidences of clinical and pathological features of renal-cell carcinomas and peritumoral kidney tissues in different groups of patients

Patients		TNM stage				Histological type**						Grade			Sarcomatoid changes	Peritumoral changes ³			
groups	number of cases	I	II	III	IV	1	2	3	4	5	6	1	2	3		1	2	3	4
I	57	28	17	7	5	39	13	—	—	4	1	8	37	12	12	32	3	33	27
II	55	31	16	6	2	37	7	3	3	2	3	8	20	27	11	33	4	45	35
III	72	36	27	6	3	40	11	5	2	9	5	12	23	37	25	55	9	49	63
IV	14	7	5	1	1	10	1	1	—	—	2	3	8	3	4	10	1	9	10
V	38	21	11	6	—	18	6	1	1	8	4	10	16	12	10	30	6	22	37
VI	112	43	38	14	17	67	16	3	3	12	11	21	59	32	11	8	1	97	—
Statistical significance		$\chi^2 = 7.22$, pns				$\chi^2 = 27.2$, pns						$\chi^2 = 29.6$, p<0.005			$\chi^2 = 12.4$, p<0.001	$\chi^2 = 133.66$, p<10 ⁻⁶			

* I —, II —; **1 — conventional; 2 — papillary; 3 — chromophobe; 4 — collecting ducts; 5 — oncocytoma; 6 — others. *** 1 — nuclear atypia; 2 — carcinoma in situ; 3 — inflammation; 4 — radiation nephropathy.

It is interesting that RCCs from Ukraine presented more frequently sarcomatoid changes with significant differences, compared with the Spanish group. The high extent of sarcomatoid changes was seen in groups III, IV and V, with significant differences in groups I and II. Nevertheless, groups I and II also showed differences in sarcomatoid changes with the Spanish group ($\chi^2 = 4.99$; $p < 0.05$). Peritumoral inflammatory response of Ukrainian RCCs was significantly less frequent than the Spanish group, which showed differences with all Ukrainian groups except group II.

Peritumoral medullar and cortical tubular nuclear atypia and CIS demonstrated significant differences between the Ukrainian and Spanish groups. Moreover, predominantly medullar nuclear atypia of collecting ducts occurred more frequently in groups III, IV and V, being less frequent and of low grade in groups I and II. Similarly, areas

of CIS were detected more frequently in groups III and V, with significant differences against the other groups.

Irradiation-like lesions, predominantly of medullar and less cortical peritumoral tissue, were detected only and in all Ukrainian groups (Table 19.2). Microscopically they appeared in cortex patterns of radiation sclerosing, proliferative atypical nephropathy, with pyknotic epithelial cells of proximal and distal tubules. Furthermore, focally tubular degeneration and necrosis with glomerular changes, simulating membranous nephropathy, were detected. Large areas of pyknotic epithelial cells in collecting ducts with following epithelial detachment, degeneration, regeneration and desquamation with development of widened enlarged loops that were already naked and empty, resembling "cheese holes", were seen in medulla. It is important to note that the above mentioned irradiation nephropathy was more frequent in groups III and V than in other Ukrainian groups, with significant differences.

Molecular pathogenesis of renal-cell carcinomas in Ukraine after the Chernobyl accident.

Our study shows the tumour suppression genes (and coded proteins) alteration associated with the high-level oxidative stress markers in tumor and peritumoral tissue which was caused by the influence of chronic low doses radiation. As it is known, the expression of $p16^{INK4A}$ gene is activated by oncogenic stress, whereas the expression of $p15^{INK4B}$ gene is related to the negative growth factor, transforming growth factor- β [3]. The tumor suppressor activity of $p16^{INK4A}$ and $p15^{INK4B}$ is attributed to their biochemical ability to bind both cyclin-dependent kinase (CDK) 4 and 6, thus inhibiting the catalytic activity of the CDK4-6/cyclin D enzyme complex required for retinoblastoma protein (pRb) phosphorylation and cell-cycle progression. The result is that both $p16^{INK4A}$ and $p15^{INK4B}$ prevent E2F-dependent transcription, blocking cell-cycle progression pathway and the G1/S restriction point.

Recently, accumulated evidence indicated a close link between 5'CpG island methylation in the promoter region and transcriptional block of $p16^{INK4A}$ gene in various human tumors [4]. Extensive analysis of $p16^{INK4A}$ promoter methylation in human head and neck squamous cell carcinoma has also demonstrated a high level of inactivation that correlates exactly with the absence of $p16^{INK4A}$ protein expression that was immunohistochemically indicated [5]. Numerous additional studies have now established the importance of $p16^{INK4A}$ methylation as a major mechanism of carcinogenesis in many different tumors in human [6].

We try to determine whether there is any manifestation of oxidative stress associated with $p15^{INK4B}$ and $p16^{INK4A}$ genes alteration in renal cell carcinomas from patients continuously exposed to low doses of ionizing radiation in correlation with levels of ionizing radiation exposure, TNM stage and histological features of these tumors. Three groups of the Ukrainian RCCs with varying degrees of exposure to IR were selected for comparison.

Data from molecular analysis revealed no homozygous deletions of the $p16^{INK4A}$ and $p15^{INK4B}$ genes in any sample. However, aberrant hypermethylation of the 5'CpG islands in the promoter region of these genes was observed in seven (32%) and five (23%) cases for the $p16^{INK4A}$ and $p15^{INK4B}$ genes, respectively. A higher incidence of

cases with hypermethylation of $p16^{INK4A}$ gene was observed in high-grade tumors. Three tumors of high stage and grade (one with sarcomatoid metaplasia and two with conventional renal cell carcinomas) showed a coincident hypermethylation of both genes. A high proportion of cases with hypermethylation of the promoter region of the $p16^{INK4A}$ gene were observed in tumors with CIS and cellular atypia in distal tubules and medullar collecting ducts of peritumoral kidney, particularly in undifferentiated tumors with sizes of more than 7 cm, all corresponding to patients from the radiation contaminated areas. No association between any of these parameters and hypermethylation of the $p15^{INK4B}$ gene has been found.

Thus, we have documented for the first time that RCCs from patients living in the radiation contaminated areas of the Ukraine showed aberrant hypermethylation of $p16^{INK4A}$, and fewer $p15^{INK4B}$ genes with immunohistochemically decreased expression of proteins, associated with dramatically increased expression of cyclooxygenase 2 (COX2), accompanied by the high incidence of radiation nephropathy in peritumoral tissues. This data confirmed that $p16^{INK4A}/pRb$ pathway was damaged by oxidative stress during chronic long term low doses radiation exposure that leads to activation of the RCC progression and to the development of the more aggressive tumors.

The complex study of the molecular alterations of those tumor suppressor genes located on chromosome 9p21 ($INK4A/ARF$ locus and $p15^{INK4B}$), MAPK signal transduction pathway's marker p38MAPK protein, alterations of p53 tumor suppressor gene and genetic amplification of the *cyclin D1*, *mdm2* in association with the immunohistochemical protein expression of the same genes in the same samples of 26 primary renal-cell epithelial tumors and peritumoral kidney tissues from patients with different radiation exposure after the Chernobyl accident in Ukraine resulted to unique date.

Formalin fixed paraffin embedded 112 tissue blocks (approximately, 4 blocks per each case) and frozen material from 26 patients with RCETs (including the peritumoral kidney tissue), who underwent surgery at the Institute of Urology in Kyiv (Ukraine) were investigated. The control group I of patients from so called "clean" area (without indicated radiocontamination), group II — from the less contaminated city of Kyiv; and group III patients were from heavily contaminated areas. All patients resided in the same areas during pre — and post — Chernobyl accident periods. Radiometric measurement of ^{137}Cs was conducted with urine collected over 24 hours from all patients before surgery.

P53 protein nuclear overexpression occurred in 3 (13%) conventional RCCs with >10% and <50% of stained nuclei and in 4 (18%) from 26 cases (one papillary-, one-Bellini and two conventional RCCs) with less than 10% of stained nuclei predominantly from groups II and III. RCCs from radiation contaminated areas expressed higher quantities of $p14^{ARF}$ protein, in relation to tumors from "clean" regions. There was direct relation between $p14^{ARF}$ protein and *mdm2* expression in RCCs from groups I–III with the increased overexpression of $p14^{ARF}$ and *mdm2* in radiation contaminated areas, associated with wild type p53 protein (negative staining).

Proliferative activity showed differences between groups with Ki67 stained nuclei and was the highest in groups II and III. Relationship between Ki67 immunostaining and histological variables demonstrated the increase of mean values of positive cells in high grade RCCs, with significant differences.

Regular homogenous or granular p38 MAPK cytoplasmic immunostaining in RCCs was detected in 100% and 78% of cases from groups II and III respectively. RCCs from radiation contaminated areas showed the highest frequency of $p14^{INK4A}$ gene hypermethylation and strongest intensity of p38MAPK overexpression ($p=0.003$) to compare with RCCs from group I. Furthermore, $p16^{INK4A}$ protein immunostaining showed irregular tendency in relation to groups. Direct relation was presented between increased nuclear overexpression of cyclin D1 and cytoplasmic expression of p38MAPK in tumors from groups II and III. Moreover, relation between low or negative $p16^{INK4A}$ protein expression in many cases of RCCs and cyclin D1 nuclear overexpression in the same tumors from groups II and III, accompanied by hypermethylation of the 5'CpG islands in the promoter region of $p16^{INK4A}$ gene in 10% of these tumors could be detected. Interestingly, hypermethylation of $p16^{INK4A}$ and $p14^{ARF}$ genes was never observed in the same samples. Hypermethylation of the promoter region of the $p14^{ARF}$ and $p16^{INK4A}$ genes occurred eventually in peritumoral kidney tissues, predominantly in cortex with cellular atypia.

This study represents the first finding of a specific lesion in *INK4a/ARF* locus of chromosome 9p21 in primary human RCETs and peritumoral kidney tissues from patients chronically exposed to low doses of IR, as they lived in the radiation contaminated areas of Ukraine. The present radiometric study confirmed significant increase of ^{137}Cs in one-day urine of these patients, so the long-term radiation exposure of the kidney tissue could be easily supposed. There was no dose-response relationship in the range 2–12 Gy, suggesting that an instability phenotype may be induced by quite low radiation doses.

Our study has documented that RCCs from patients living in the radiation contaminated areas of Ukraine showed aberrant hypermethylation of $p14^{ARF}$ and $p16^{INK4A}$ genes associated with increased mdm2 and cyclin D1 proteins levels. Moreover, $p14^{ARF}$ promoter hypermethylation occurs independently of the $p16^{INK4A}$ methylation status, but in relation to the p53 stable status, following by increased levels of nuclear mdm2 and $p14^{ARF}$ proteins in the same tumors. In the group of RCCs $p16^{INK4A}$ promoter hypermethylation was never combined with $p14^{ARF}$ promoter hypermethylation. The most common situation was the simultaneous absence of methylation in both promoters, but after this, the two possible scenarios ($p16^{INK4A}$ methylated alone, $p14^{ARF}$ methylated alone) were similarly represented. These data suggest the existence of multiple levels of "cross-talk" between the $p16^{INK4A}$ /pRb and $p14^{ARF}$ /p53 pathways in RCCs.

We found significant relation between strong immunostaining against p38MAPK and RCCs of radiation contaminated areas. Constantly elevated p38MAPK activation leads to constitutive elevation of nuclear factor — kappa B (transcription factor) response element transactivation in the malignant phenotype and furthermore, malignant progression [7, 8].

Our recent study, which use tissue microarray technique, include 56 Ukrainian patients with RCCs including 18 patients living in "clean" Ukrainian regions in control group and 41 patient living in contaminated regions of Ukraine. The similar group of 18 patients operated in University of Valencia's clinic (Valencia, Spain) was used as control. Significant increase of expression of the apoptosis promoter BAX and death receptor DR-5 in cytoplasm and cell membrane of tumour cells were detected. The same cells

show reduced expression of apoptosis inhibitors Bcl-2 and Bcl-x combining with high expression of p65 and p50 subunits of nuclear transcription factor NF- κ B. Our findings indicate that long-term low dose radiation exposure initiates the apoptosis regulation system alteration, thereby resulting to tumour progression and apoptosis resistance of tumour cells.

Conclusions:

Significant increase of RCCs incidence in "contaminated" by radionuclides regions (CRR) of Ukraine combined with changing of tumors biological features gives the evidence for presence of ecological pathomorphosys in Ukraine after the Chernobyl accident. Increase of proliferative activity and aggressivity accompanied by hyperexpression of K-ras and PCNA in RCCs, especially in patients from CRR associated with chronic radiation nephropathy in peritumoral tissue are correlated with the duration of radiation exposure and confirms the influence of chronic and persistent radiation exposure on renal carcinogenesis.

The new histological form of nephropathy — radiation sclerosing proliferative atypical nephropathy, which was occurred in association with the areas of epithelial nuclear atypia and CIS in patient living in CRR, was defined. Persistent oxidative stress induced by long-term influence of low dose ionizing radiation exposure on renal cell carcinomas (RCCs) in patients living in CRR induces destruction of intracellular signal system, accompanied by inactivation of $p16^{\text{INK4A}}/p53$ pathway, plays a significant role in tumor progression

$p14^{\text{ARF}}$ promoter hypermethylation in peritumoral renal tissue could be explained by G1-S cell cycle regulation disorders and promotes the development of precancerous lesions in kidney as well as the progression of RCCs. Molecular promoters and inhibitors of apoptosis are the target for the long-term low-dose ionizing radiation exposure in renal-cell carcinogenesis.

Urinary Bladder Lesions Induced by Persistent Chronic Low-dose Ionizing Radiation (IR)

Urinary bladder lesions in male-patients with benign prostate hyperplasia (BPH) who underwent open transbladder prostatectomy were studied in the IU from 1993 until 2007. Multiple mapping biopsies of the bladder urothelium (including areas of bladder neck and both ureter orifices) were obtained from every patient. Furthermore, the histological and immunohistochemical studies were performed in collaboration with the Department of Pathology in the Uppsala Medical University, Sweden (Professor & Chairman Dr. Krister Busch) in 1993-1994, in collaboration with the Department of Pathology of the Innsbruck Medical University, Austria (Professor & Chairman Dr. Gregor Mikuz) in 1995-1996. Histological and immunohistochemical study accompanied by molecular biology were performed from 1996 until 2007 in collaboration with the Department of Pathology (Professor & Chairman Dr. Shoji Fukushima), Osaka City University Medical School in Japan.

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Chornobyl cystitis

We have documented for the first time that chronic long-term, low-dose IR in parts of the Ukraine leads to development of a previously unknown urinary bladder disease, radiation-induced chronic proliferative atypical cystitis or so-called Chornobyl cystitis [9]. This is characterized by multiple areas of dysplasia and CIS of the urinary bladder urothelium in strong association with sclerosis of connective tissue and strongly increased angiogenesis without a marked inflammatory reaction in the propria mucosa.

The incidences of urinary bladder dysplasias and carcinomas in 164 patients living in 5–30 Ci/km² (group 1), 0.5–5 Ci/km² (group 2) soil contamination areas or non-contaminated areas (group 3) are summarized in Table 6.16. Urinary bladder epithelium biopsied from 131 male patients with BPH from groups 1 and 2 demonstrated multiple areas of dysplasia with strong epithelial abnormalities such as extensive cellular pleomorphism and nuclear hyperchromism, frequently combined with increased thickness of urothelial epithelium. Foci of dysplasia were observed in 97% and 83% of cases in groups 1 and 2, respectively, and multiple areas of CIS with neoplastic changes of urothelium that frequently involved von Brunn's nests or cystitis cystica were detected in 73% and 64%. Nine small papillary or invasive transitional cell carcinomas (pTa–pT1) were also incidentally found in individuals from radiation contaminated areas.

Table 6.16. Incidence of urinary bladder dysplasias and carcinomas

Groups	Soil contamination (Ci/km ²)	No. of cases	Dysplasia (%)	Carcinomas		
				total (%)	carcinoma <i>in situ</i> (%)	papillary urothelial carcinoma (%)
1	5–30	73	71 (97)*	53 (73)*	47 (64)*	6(8)
2	0.5–5	58	48 (83)*	37 (64)*	34 (59)*	3 (5)
3	Non-contaminated	33	9 (27) [†]	0 (0)	0 (0)	0 (0)

*Significantly different versus group 3 at $p < 0.0001$ (χ^2 or Fisher's exact probability test). [†]Mild dysplasia.

Additionally, all cases in groups 1 and 2 exhibited proliferative cystitis, i.e. von Brunn's nests, cystitis cystica and squamous and glandular metaplasia, which were frequently combined and had marked features of chronic radiation cystitis rather than simple inflammation. Large areas of sclerosis of connective tissue in lamina propria with less prominent inflammatory cellular infiltration of lymphocytes, histiocytes and plasma cells were typical. Among these lesions, definite new vascularization, sometimes with the development of angiomatoid-like vessels full of erythrocytes and hemorrhage, was detected in 62%, 53% and 6% of groups 1, 2 and 3, respectively. Endothelial proliferation of new micro-vessels in lamina propria was markedly increased in 46% and 34% of cases in groups 1 and 2, respectively. In all female patients, large areas of sclerosis of connective tissue with some inflammatory infiltration and large areas of hypervascularization and patchy hemorrhage were detected in lamina propria. Patterns of chronic cystitis without proliferative changes but with clear cell urothelium and marked inflammatory cellular infiltration in lamina propria were found in patients of group 3. However, hypervascularization and development of angiomatoid-like vessels were not observed.

Unfortunately, we have no means to determine what radiation dose our patients received. However, we obtained results of ¹³⁷Cs measurement in daily urine of the same

patients with BPH in groups 1 and 2; the values are summarized in Table 6.17. Significant elevation of ^{137}Cs levels was found in patients from group 1 (6.47 Bq/L) and to a lesser extent in patients from group 2 (1.23 Bq/L), as compared with group 3 patients (0.29 Bq/L). Our radiometric study showed significant increase of ^{137}Cs in urine of patients from groups 1 and 2, who suffered from BPH and presumably urinary retention, so that radiation exposure of the urothelium would be expected to have been high. Such male patients with BPH could be the group with the highest risk of Chernobyl cystitis. Our female patients with symptoms of chronic cystitis from groups 1 and 2, who were without urinary retention but with increased ^{137}Cs in urine, also demonstrated the same pattern of chronic proliferative atypical cystitis with less frequent mild dysplasia. These data strongly suggest the same pathway for development of urinary bladder lesions in male and female patients dependent on long-term exposure to low-dose IR.

Table 6.17. ^{137}Cs levels in urine of patients

Indices	Groups		
	1	2	3
Number of patients	55	53	12
Soil contamination levels (Ci/km^2) ^a	5–30	0.5–5	non-contaminated
^{137}Cs levels in urine (Bq/L , Mean $\pm$ SD)	6.47 $\pm$ 14.30 ^b	1.23 $\pm$ 1.01 ^b	0.29 $\pm$ 0.03

^aData from Rees et al. [2]. *Uroto associated a References source. Statistica medica 2 Rando J. (1964) A survey of the metabolism of Cesium in man. Br. J. Radiol., 37: 214–217.* ^bSignificantly different versus group 3 at $p < 0.001$ (Steel-type separate ranking test).

^{137}Cs levels in urine of patients

However, synergistic effects of other ecological hazards (chemical pollution and cigarette smoking) in Ukraine, which is acknowledged to be an ecological disaster area, also have to be taken into consideration. The economic and social structures are very poor, and the majority of the Ukrainian population still is getting food from their private vegetable gardens (without any ecological control). Therefore, our patients (many of whom were smokers) living in the radiation contaminated areas could be synergistically affected by various hazards. The critical role of the Chernobyl accident (involving long-lived ^{137}Cs and possibly many other radionuclides, which were not taken into account in our study) is evident from the lack of such specific pathological lesions in analogous patients from Sweden and Austria.

It should be noted that classic descriptions of acute and chronic radiation effects on the urinary bladder do not coincide with the pathogenesis of human urinary bladder injury after long-term, low-dose exposure to IR. We did not observe reactive epithelial proliferation associated with fibrin deposits, fibrinoid vascular changes and multi-nucleated stromal cells, typical changes secondary to high doses of IR.

Pathogenesis of Chernobyl cystitis

Cell cycle regulation.

Cell cycle check points function to maintain genetic stability by providing additional time for repair of DNA damage and completion of events that are necessary for accurate cell division. Some check points, such as the DNA damage G1 check point are dependent on p53, whereas others, such as the decatenation G2 check point, are not [10]. The molecular, genetic and cellular changes that occur in urothelial carcinomas

are numerous and include loss of cell growth regulation. Several genetic alterations in bladder cancer have been linked with inactivation of tumor-suppressor genes [10]. In bladder urothelial carcinomas, one of the most frequently inactivated tumor-suppressor genes is the *p53* gene. Genetic alterations in *p53* occur in ~50% of bladder carcinomas and are associated with high-stage and high-grade urothelial lesions [11]. Spruck *et al.* have suggested the participation of two molecular pathways in urinary bladder carcinogenesis, with *p53* alterations occurring early in CIS and dysplasia before the development of non-papillary invasive lesions but occurring late in papillary urothelial carcinomas [12]. Thus, early detection of *p53* mutations in urinary bladder epithelial lesions (Chornobyl cystitis) may be strongly predictive of future urinary bladder cancer, especially of the non-papillary invasive type [13].

Our pilot studies indicated increased proliferative activity and possible genetic instability, with over-expression of *p53*, proliferating cell nuclear antigen (PCNA) and *mdm2* in the bladder urothelium of exposed individuals with Chornobyl cystitis [14]. The staining intensities of *p53*, PCNA, $p21^{WAF1/CIP1}$ and cyclin D1 were directly correlated with levels of radiation exposure and amounts of ^{137}Cs excreted in the urine by patients of groups 1 and 2. The patients from 'clean' (not radiation contaminated) areas of Ukraine had significantly lower values for all parameters investigated [15].

It has been shown that wild-type *p53* plays an essential role in transcription-coupled repair of DNA damage induced by oxidative stress, IR and some chemical carcinogens. The *p53* protein expression in nuclei of bladder urothelium in both radiation contaminated groups 1 and 2 thus likely represents wild-type *p53* accumulation in response to DNA damage. This speculation is supported by aberrant increases of PCNA, cyclin D1 and $p21^{WAF1/CIP1}$ in the same areas of urothelium, which could be explained by possible involvement of PCNA, cyclin D1 and $p21^{WAF1/CIP1}$ not only in DNA synthesis but also in DNA excision repair. An alternative explanation for the strong *p53* protein expression observed in urothelium with Chornobyl cystitis is that it reflects mutations within *p53* gene.

Biopsied urinary bladder specimens were therefore analyzed for mutational inactivation of the *p53* gene by polymerase chain reaction–single strand conformation polymorphism (PCR–SSCP) analysis. In addition, urine sediments collected over intervals were examined by PCR–SSCP and assays of the ability of human *p53* to activate transcription in yeast [13]. Because human *p53* cDNA polymerase chain reaction products can be cloned directly into the reporter yeast strain by homologous recombination without intermediate bacterial cloning steps, the percentage of positive red yeast colonies accurately reflects the mutant *p53* mRNA content of the starting material. Therefore, the assay can detect mutant *p53* in a minor fraction of cell clones such as those in urine sediments. Mutational analysis of the *p53* gene in DNA extracted from the urothelium of patients living in radiation contaminated areas of Ukraine revealed 9 of 17 cases (53%) to harbor one or more mutations within identical or separated samples [13]. This frequency is similar to values described for human high-grade, invasive urinary bladder cancers [12]. Although base deletions or insertions of the *p53* gene have been found in a certain proportion of human urinary bladder carcinomas [16], all *p53* mutations identified in this study were single-base-pair substitutions. The most striking feature was the predominance of G:C to A:T transition mutations at CpG dinucleotides, especially in codons 158, 245 and 248. Although IR has been reported to cause a variety of types

of DNA damage including single- and double-strand breaks and cross-linking, direct *in vivo* evidence of radiation-induced base-pair substitutions is lacking. Thus, the underlying mechanism might be different from that responsible for the specific mutations observed in this study. In human urinary bladder carcinomas, no specific substitution pattern for the p53 gene has hitherto been described, and there has been no pointer to any specific mutagen [16]. On the other hand, mutational analysis of schistosomal urinary bladder carcinomas (endemic in Egypt) gave results that are consistent with our findings; namely, a high proportion of base-pair changes at CpG dinucleotides (18 of 34; 53%). Chronic urinary infection with *Schistosoma hematobium* is a significant etiological factor in schistosomal urinary bladder carcinomas. Chornobyl cystitis was a common characteristic feature of cases in the present study. Recently, a close relationship between chronic infection and cancer risk has been suggested, with the production of nitric oxide (NO) during inflammatory processes playing a role [17]. It has been shown that NO can produce transitions at CpG dinucleotides by deamination of 5-methylcytosine [17]. In addition, endogenous formation of urinary *N*-nitroso compounds leads to O⁶-alkylguanine formation and G:C to A:T transitions [17].

Two patients of nine with p53 mutations proved to have multiple p53 mutations in the urinary bladder, in line with the report by Spruck *et al.* [12] and Goto *et al.* [18]. Different p53 mutations in independent urothelial lesions (case 16) or in metachronous samples (case 17) indicate that a strong carcinogenic insult may have resulted in multiple transformation events in a large field of urothelium, as demonstrated previously in an animal model. The frequent (9 of 17 cases, 53%) p53 mutations of altered urinary bladder epithelium in patients with Chornobyl cystitis suggest that prediction of induction of urinary bladder carcinoma may be possible.

Concerning the cell cycle transition regulation, it is necessary to note that activation of cyclin D1 and p21^{WAF1/Cip1} occurs in response to activation of wild-type p53. It can be speculated that long-term exposure to low doses of ¹³⁷Cs induces chronic DNA damage in bladder urothelium. An increased level of p21^{WAF1/Cip1} could thus be interpreted as a manifestation of cellular differentiation. p21^{WAF1/Cip1} directly interacts with PCNA but the majority of studies show that it does not inhibit PCNA-dependent nucleotide excision repair. This suggests that p21^{WAF1/Cip1} not only plays a critical role in negatively controlling proliferation but also could be activated in a p53-independent manner. About 75% of our cases in both groups 1 and 2 were cyclin D1 positive. It is possible that some of the observed cyclin D1 over-expression occurred in response to activation of wild-type p53 during radiation-induced DNA damage. The dramatic increase of PCNA staining in 88% of cases in groups 1 and 2 involving not only basal cells but also the suprabasal and superficial layers of bladder urothelium is indicative of increased cell proliferation.

In conclusion, the present finding of significant increases in p53, PCNA, cyclin D1 and to a lesser extent p21^{WAF1/Cip1} expression in the majority of patients with Chornobyl cystitis in groups 1 and 2 chronically exposed to ionizing irradiation, associated with multiple p53 gene mutations at CpG dinucleotides, is suggestive of a deregulated cell cycle transition that leads to increased chance of malignant transformation with the possible development of preferentially high-stage and high-grade bladder carcinoma among the population in Ukraine.

Increased oxidative stress, signaling cascades

Cellular stress is known to be a multifaceted condition generated by free-radical species, hypoxia and other factors that target the transcriptional factor nuclear factor- κ B (NF- κ B) with its two major subunit polypeptides, p50 and p65, and modulate their activity directly or indirectly by activating other signaling cascades. The p38 mitogen-activated protein kinase (MAPK) pathway is also involved in primary signaling activated by inducers of stress, such as IR. Inflammatory cytokines, mediating inhibition of cell proliferation and causing cell death. p50/p65 are the active transactivating species of NF- κ B, responsible for induction of several genes involved in the control of cell proliferation, apoptosis and, hence, oncogenesis.

The pattern of Chornobyl cystitis, including four small incidentally detected pTa tumors in male patients with BPH of the radiation contaminated groups 1 and 2, was associated with strong p53 staining of the urothelial nuclei in the majority of cases (CIS and papillary urothelial carcinomas with severe dysplasia), overlapping with over-expression of the oxidative DNA damage marker, 8-hydroxydeoxyguanosine (8-OHdG), accompanied by elevation of cytoplasmic cyclooxygenase 2 (Cox2) and inducible NO synthase (iNOS). Therefore, our data suggest that the chronic low-dose irradiation could have at least two effects on the urinary bladder urothelium: (i) direct induction of oxidative stress due to irradiation DNA damage as IR is known to be a pure source of reactive oxidative species and (ii) indirect actions with possible consequences for homeostasis and reactions, involving inflammation following enhanced NO production, which triggers a cascade of oncogenic events, possibly initiated by p53 mutations at sites involving CpG dinucleotides [13].

It is necessary to note that chronic infection and inflammation have become well recognized as risk factors for a variety of human cancers. It has been proposed that reactive oxygen and nitrogen species, both formed in infected and inflamed tissues, play roles in the multi-stage carcinogenic process. NO, a potential toxic substance with free-radical properties, is generated from L-arginine by constitutive NO synthase or iNOS.

One of the possible mechanisms underlying enhancement of initiation and promotion in carcinogenesis by oxidative stress is the ability to induce prostaglandin formation catalyzed by the enzyme Cox, which converts arachidonic acid to prostaglandins. Presently two Cox enzymes are known Cox1 and Cox2, an important mediators of inflammation, whose synthesis can be stimulated rapidly by tumor promoters.

The marked increase of 8-OHdG, Cox2 and iNOS expression found in the majority of urothelium samples in groups 1 and 2 is an indicator of oxidative stress in its different manifestations. Murata *et al.* [19] demonstrated that NO induced mutations inactivating p53 by causing deamination of cytosine, leading to G:C to A:T transitions *in vitro*. Our data, showing strong 8-OHdG, Cox2 and iNOS expression associated with p53 mutational inactivation with frequent G:C to A:T transitions at CpG dinucleotides (in 73% of all mutations) with a hot spot in codon 245 in bladder urothelium of Ukrainian male patients with BPH [13], support the hypothesis that NO-mediated DNA deamination was induced by oxidative stress in groups 1 and 2 bladder urothelium.

Over-expression of p38 MAPK, p65 and p50 subunits of NF- κ B by the same cells of urothelium in patients living in the radiation contaminated areas also strongly indicates a pivotal role of oxidative stress. Thus, our results point to low-dose radiation exposure acting on at least two distinct pathways:

- cytoplasmic accumulation of NF- κ B with p65 and p50 subunits as a result of protein synthesis, manifested by nuclear translocation;
- p38 MAPK-dependent transactivation of NF- κ B.

Both of these are known to be required for full activation of NF- κ B-dependent transcription.

Interestingly, the marked activation of angiogenesis in urinary bladder lamina propria observed in all cases of groups 1 and 2 with Chernobyl cystitis was associated with a dramatic increase of p38 MAPK and p65 cytoplasmic and nuclear expression as well as cytoplasmic iNOS and Cox2 in endothelial cells. These findings indicate a critical role for oxidative stress, as well as for the p38 MAPK signaling cascade and for the transcriptional factor NF- κ B in endothelial cell activation, as an important component in the pathogenesis of Chernobyl cystitis. iNOS and Cox2 cytoplasmic expression in capillary endothelial cells of lamina propria, accompanied by the hypervascularization and the development of angiomatoid-like vessels, especially in group 1 patients, confirms the involvement of oxidative stress as a trigger of the angiogenic cascade, which is known to play a major role in human bladder carcinogenesis.

Furthermore, strong H-ras expression was evident in the bladder urothelium of patients from groups 1 and 2 [20]. Numerous cytokines and growth factors can activate the ras gene and H-ras expression may affect cell responses to chemical inducers of oxidative stress. This means that the H-ras expression in our material might reflect not only H-ras oncogene activation in bladder urothelium as an early event of human bladder carcinogenesis but also angiogenesis activated by oxidative stress.

In contrast to our results, it has been recently suggested that oxidative stress might play a key role in the inhibition of tumor initiation by p38 MAPK. Thus, Dolado et al. [21] suggested that p38 MAPK acts to suppress tumor formation by H-ras. p38 MAPK pathway is implicated in suppression of tumorigenesis because it can inhibit cell growth by decreasing the expression of cyclin D, inhibit the activity of Cdc25 phosphatases and engage the p16/Rb and p19ARF/p53 tumor-suppressor pathways.

In conclusion, our findings point to a significant relationship between oxidative stress induced by long-term, low-dose IR in people living >20 years in radiation contaminated (^{137}Cs) areas of Ukraine and the development of Chernobyl cystitis, a possible pre-neoplastic condition in the urinary bladder.

Growth factors

Exposure of cells to a variety of stresses, including IR, induces compensatory activation of multiple intracellular signaling pathways. Change in expression of growth factor receptors such as fibroblast growth factor receptor 3 (FGFR3) and epidermal growth factor receptors (EGFRs) expressed by target cells due to radiation-induced signalling is therefore a question of interest. In our search for new markers of urinary bladder carcinogenesis, we recently were attracted by the FGFR3, a member of the receptor tyrosine kinase family, with heparin glycosaminoglycan as an important cofactor [22]. Recently, FGFR3 mutations were found in >70% of non-invasive papillary urinary bladder carcinomas in humans [22]. The high frequency of FGFR3 mutations in superficial urothelial carcinomas contrasts with the absence of FGFR3 mutations in CIS and the low percentage of mutations in invasive urothelial carcinomas [22].

Recent studies showed that EGFR1 expression is increased in muscle-invasive carcinomas as compared with superficial bladder carcinomas and correlates with poor survival of patients with bladder cancer. Moreover, the ErbB2 proto-oncogene, also called HER2 or EGFR2neu located at 17q21, which encodes a 185 kDa transmembrane glycoprotein (p185HER2/neu) with tyrosine kinase activity, has been shown to be over-expressed in a variety of primary human carcinomas with a more aggressive clinical course, including breast, lung, ovarian and urinary bladder carcinomas [23].

Several signal transduction pathways have been implicated in EGFR-dependent cell survival as it relates to the anchorage-independent state. Primarily, these include the Ras/Raf/MEK/MAPK cascade. Raf-1 is a member of a family of serine-threonine protein kinases termed Raf-1, B-Raf and A-Raf, activated dependent upon Raf-1 translocation to the plasma membrane where phosphorylation occurs. Raf-1 activates MAP/ERK kinase (MEK), which in turn phosphorylates MAPK. Raf-1 protein expression is elevated in human breast carcinoma and it might be responsible for malignant progression. However, we were unable to find studies reporting the presence of Raf-1 protein in urinary bladder carcinomas, except one, related to Raf-1 amplification in urothelial cancers with mutation of the p53 gene.

On the basis of these observations, we designed a study to compare alterations in signaling cell systems in different patient groups (with pre-neoplastic lesions, Chornobyl cystitis and already well-developed urothelial carcinomas) and relationships among three immunohistochemical markers of the growth factor receptors and Raf-1.

Molecular analysis of some growth factor receptors in urinary bladder lesions, described by our group as Chornobyl cystitis (groups 1 and 2), as well as urothelium from analogous patients from so-called clean areas (group 3) with primary urothelial carcinomas, revealed dramatic nuclear and cytoplasmic FGFR3 (isotype unknown) over-expression with a high score of 95% in group 1 cases [24]. Multiple areas of dysplasia and CIS were stained consistently and very strongly. Furthermore, because the FGFR3 gene is known to be expressed both in normal urothelium and in bladder carcinomas, it is likely that the mutant gene has an oncogenic role in bladder carcinoma pathogenesis [22].

We have recently found FGFR3 gene mutations in 30% of cases in the urothelial carcinoma patients (A.Romanenko, K. Morimura, A.Kakehashi, H.Wanibuchi, A.Vozianov, and S.Fukushima, unpublished data), the majority within the spectrum of oncogenic mutations (codon 249 TCC to TGC). Mutations were detected almost equally in both high- and low-grade/stage urothelial carcinomas, in contrast to recent reports of an absence of FGFR3 mutations in CIS and a very low percentage of mutations in invasive (pT2-4) urinary bladder carcinomas [22].

The marked increase of FGFR3 nuclear expression in 95% of urothelia in group 1 patients, associated with dysplasias and CIS, could be strongly related to the early events of the urinary bladder carcinogenesis in people living in the radiation contaminated areas of Ukraine. The majority (70%) of the primary bladder carcinomas in the additional urothelial carcinoma group (also from patients living in the radiation contaminated areas) demonstrated nuclear, membranous and more rarely cytoplasmic FGFR3 expression in both superficial and invasive carcinomas. These findings provide support for the idea that in the case of the presence (induction) of FGFR3 mutations, invasive carcinomas may arise from mutated superficial (pTa-pT1) carcinomas, which might progress from CIS.

It is necessary to note that FGFR3 cytoplasmic and nuclear over-expression was detected in both endothelial cells and fibroblasts of lamina propria of the same patients. Our results might suggest that mesenchymal cells are required for the induction of urothelial cell transformation. Evidence has accumulated that epithelial autocrine cells, constitutively producing and secreting the FGF and expressing FGFR3, have a highly aggressive mesenchymal phenotype [25]. Other studies demonstrated that FGFs can activate FGFRs that in turn can promote tumor cell proliferation in an autocrine fashion by stimulating the growth, survival and migration of stromal cells, including fibroblasts and smooth muscle cells, or, as demonstrated in our work, participating in the induction of tumor angiogenesis [26]. Importantly, the marked activation of angiogenesis in urinary bladder lamina propria in the majority of cases with Chernobyl cystitis was associated with a dramatic increase of FGFR3, EGFR1 and EGFR2neu expression in endothelial cells.

In our studies, EGFR1 and EGFR2neu immunoreactivity was found to be increased in the same cells of the bladder urothelium in group 1. Cytoplasmic over-expression in particular appears significantly associated with the Chernobyl cystitis pattern. This may also be the case for squamous cell carcinoma and pre-cancerous lesions of the lung [27], with prognostic importance for trafficking of EGFR1 between the Golgi apparatus and cell membranes and internalization of EGFR1 after interaction with an EGFR ligand. Moreover, cytoplasmic expression of EGFR has been correlated with a poor prognosis and increased metastatic potential in lung cancers [27].

EGFR and other receptor tyrosine kinases are immediate early response gene products that are activated by IR within minutes, as with their physiological growth factor ligands that cause complex cytoprotective responses including increased cell proliferation, reduced apoptosis and enhanced DNA repair. As repeated radiation exposure can induce a strong cellular proliferative response *in vitro*, evaluation of human response levels after sustained long-term, low-dose IR is clearly of interest.

Cell-cell contact inhibition is considered to contribute to control of cell growth, with involvement of molecules that mediate cell adhesion, such as cadherins. In addition to our finding of significant EGFR1, EGFR2neu and Raf-1 activation in urothelium of group 1 patients, transforming growth factor (TGF)- α over-expression and alteration in E-cadherin- β -catenin complexes in the analogous bladder lesions has been reported [28]. Since cell-cell contact inhibition is an important regulatory mechanism of cell growth, its loss due the MAPK activation could contribute to uncontrolled tumor growth and might correlate with an invasive phenotype. Collectively, our findings suggest that FGFR and EGFR signaling pathways associated with EGFR2neu and Raf-1 activation may contribute to several facets of multi-stage urothelial carcinogenesis, including auto- or paracrine growth stimulation, up-regulation of angiogenesis and stromal remodeling.

DNA damage and repair

Understanding the long-term effects of low doses of IR on living organisms requires identification of critical radiation-induced DNA lesions, measurement of their repair and determination of the consequences of misrepair or non-repair. Our results suggest that increased oxidative stress in the bladder urothelium of the Ukrainian population in Chernobyl radiation contaminated areas is accompanied by marked DNA damage and repair by 8-oxoguanine-DNA glycosylase 1.

Our recent studies showed mutational inactivation of the p53 tumor-suppressor gene, with strong p53 immunoreactivity of urinary bladder urothelium in this group of Ukrainian patients [13]. The p53 gene *in vivo* modulates the base excision repair activity pattern after the oxidative stress generated by IR, suggesting a direct role in housekeeping base excision repair activity, with molecular cross-talk between the p53 protein and the DNA repair machinery. Furthermore, mutant p53 seems to confer higher base excision repair activity in response to oxidative stress, in agreement with our observations of strong base excision repair enzyme expression in areas of urothelial dysplasia and CIS. However, activity is irregular in the background urothelium so that some cells may suffer inefficient base excision repair [29]. In contrast, nuclear over-expression of apurinic/apyrimidinic endonuclease 1 (APE1), a major endonuclease acting on apurinic/apyridinic sites after exposure to IR, appears homogeneous. Recently, nuclear APE1 expression was documented as a facet of increased cellular resistance to oxidizing agents [30], contributing to base excision repair of reactive oxygen species (ROS)-induced lesions. This finding is consistent with the previous observation that APE1 activation after ROS generation is accompanied by adaptive resistance of the cells to ROS [30]. Our data suggest that APE1 is also important for repairing oxidative DNA lesions in human urothelium as a result of long-term, low-dose exposure to IR [29].

The significant increase of nuclear xeroderma pigmentosum A (XPA) protein level evident in urothelium of group 1 patients is indicative of activation of transcriptional coupled repair and global genome repair pathways. Recent studies showed a major role of XPA protein in the early steps of nucleotide excision repair reaction with low-dose (0.2–2 Gy) IR exposure [31]. A marked increase in nuclear 8-OHdG, APE1 and XPA, but not 8-oxoguanine-DNA glycosylase 1, was detected in the endothelial cells of micro-vessels, macrophages and lymphocytes close to the urothelium, also suggesting DNA repair in the bladder propria mucosa. Importantly, all detectable small invasive transitional cell carcinomas in our group 1 patients were mostly negative for 8-oxoguanine-DNA glycosylase 1, APE1 and XPA presumably, indicating the apparent disruption of the base and nucleotide repair machinery in these malignant cells. Whether oxidative damage does not occur in tumors is a question that requires further investigation.

Microenvironment–intercellular communications

Redox homeostatic processes in the microenvironment, involving signaling pathways and interaction between cells and also with the extracellular matrix, could be targets of chronic low-dose IR. Cellular interactions are modulated by cytokines and growth factors, which are known to be multi-functional molecules that orchestrate most aspects of the inflammatory response, eliciting their effects locally or systemically in an autocrine or paracrine manner. One of the TGF family multi-functional cytokines, the TGF- β 1 (TGF- β 1), is known to regulate cell growth and differentiation, tissue remodeling, immune response and angiogenesis. Three major isoforms exist in mammals: TGF- β 1, 2 and 3.

Alterations of cadherin–catenin complexes have also attracted interest as important for progression of many carcinomas in humans. Components of the E-cadherin– β -catenin complex, as well as TGF- β 1 and iNOS, were evaluated in urinary bladder specimens in an attempt to detect molecular lesions of cellular membranes with a possible role in urothelial changes in patients chronically exposed to low-dose IR after the Chernobyl

accident in Ukraine. We thereby documented for the first time that Chernobyl cystitis with foci of urothelial dysplasia, CIS, and fibrosis of the lamina propria, is associated with activation of TGF- β 1, as well as disruption of E-cadherin- β -catenin expression, which might be a result of chronic long-term, low-dose IR exposure.

Whereas excess ROS production clearly damages DNA, low levels might affect cell signaling, particularly, redox modulation. TGF- β 1 is a major extracellular signaling sensor of damage, as it mediates redox homeostasis in cells and contributes to cell-cell communication. It is known as a mediator of tissue response to IR, pointing to a role in orchestrating changes due to oxidative stress. The present results indicate that alteration of TGF- β 1 is a frequent event in background bladder urothelium of group 1 patients, with decrease observed in areas of dysplasia and CIS and little to no immunoreactivity in urothelial carcinomas. Furthermore, we found TGF- β 1 immunostaining to be most pronounced in the lamina propria, in particular, in areas of sclerosis and fibrosis, where production of inflammatory cell cytokines is clearly important. IR appears to be one of a few exogenous agents that can cause TGF- β 1 activation *in situ*.

Evidence has accumulated that gap junction intercellular communication is sensitive to oxidative stress. It was demonstrated that loss of Cx32/gap junction intercellular communication plays a significant role in radiation-induced tumorigenesis of the liver and importantly that Cx32 may also play a role in tumor suppression and/or tumor progression in other tissue types such as lung and adrenal gland [32]. Cx32 was demonstrated to function as a hepatic tumor suppressor in response to radiation-associated mutation events [32]. E-cadherin- β -catenin complexes are required for formation of gap junction intercellular communication, whereas mutations in the β -catenin gene have been described for various human cancers. Our findings indicate that alterations of E-cadherin- β -catenin are frequent in bladder urothelium of people living in radiation contaminated areas. Elevated protein levels of E-cadherin and β -catenin as well as their abnormal cytoplasmic localization detected in the urothelial cells could be the early event in urinary bladder carcinogenesis induced by long-term, low-dose IR exposure. Our results in line with other studies have suggested that oxidative stress causes internalization of E-cadherin from the plasma membrane to the cytosol [28]. Intracellular accumulation of β -catenin might correspond to an increased level of hypophosphorylated β -catenin caused by a mutation, in turn leading to transduction of oncogenic signals and cancer progression. Over-expression may result in the premature reentry of cells into the cell cycle after γ -irradiation-induced DNA damage and thereby promote the accumulation of oncogene mutations and carcinogenesis.

Importantly, aberrant expression of β -catenin and E-cadherin in association with TGF- β 1 up-regulation in lamina propria appears to be essential molecular alteration in pathogenesis of bladder urothelial carcinomas in the environment of continued chronic long-term, low-dose IR exposure, when compared with CIS and urothelial carcinomas which developed before the Chernobyl accident.

Ubiquitination and sumoylation

Recent evidence suggests that the ubiquitin (Ub)/proteasome system may play a critical role in responses to IR, controlling numerous physiological processes including signal transduction, DNA repair, cell cycle progression, cell survival and stress responses [33]. This is in addition to its more established roles in the removal of misfolded, damaged and effete proteins [33]. Ub, consisting of 76 amino acids, is an element within the

Ub/proteasome system requiring initial activation by Ub-activating enzyme E1 in an ATP-dependent reaction. Ub is covalently bound to E1 and then passed to the E2 Ub-conjugating enzyme, where it forms a similar thiolester linkage. Ubiquitinated proteins are then degraded by the 26S proteasome complex [33]. In addition to Ub, there are several Ub-like proteins. One of them, the small Ub-related modifier (SUMO), has been shown to covalently modify a large number of proteins with important roles in many cellular processes including gene expression, maintenance of chromatin structure and signal transduction. Post-translational modification by SUMO is not associated with protein degradation but rather plays role in determining protein localization and activity. In contrast to the Ub system where dozens of E2 enzymes have been identified, Ubc9 is the only known SUMO E2-conjugating enzyme. Many known targets for SUMO1 and Ubc9 (the integral players in sumoylation) are nuclear proteins with important roles in regulating transcription chromatin structure and DNA repair.

Classical examples are the tumor-suppressor proteins p53 and p27^{Kip1} whose function is abolished in many tumors. Since ubiquitination and sumoylation are intimately involved in major cellular biological processes and many effects of IR involve changes in protein stability, it is reasonable to ask what effect these processes have on cellular responses to chronic long-term, low-dose radiation. Therefore, we examined the levels of ubiquitination and sumoylation elements in Chernobyl cystitis induced by long-term, low-dose IR in Ukraine. For this purpose, Ub, SUMO1 protein, the SUMO E2-conjugating enzyme Ubc9, the cell cycle inhibitor p27^{Kip1} and the tumor-suppressor protein p53 were evaluated by immunohistochemistry in a series of radiation-exposed patients and controls. This revealed Ub, SUMO1 and Ubc9 over-expression, with high scores in 96, 96 and 72% of cases, respectively (Table 6.18). Steady-state accumulation of p53 protein in 60% cases was accompanied by loss or severe reduction of p27^{Kip1} protein in 76% cases [34]. Over-expression of Ub, SUMO1 and Ubc9 might be related to the accumulation of the mutated p53, which is not degraded in IR-affected cells or, on the contrary, to the proteolysis of p27^{Kip1} tumor suppressor.

Table 6.18. Immunohistochemical scores for proteins of Ub/proteasome system

Protein/factor	Groups 1 and 2	Group 3
No. of patients	26	24
Contamination levels in soils (Ci/km ²)	0.5–30	NC ^a
Ub	7.4±1.9b,*	1.7±2.4
SUMO1	7.6±1.8b,**	4.4±2.9
Ubc9	6.4±2.4b,*	2.6±1.5
p53	5.1±3.7b,*	0.7±1.0
p27	3.0±2.0b,*	6.5±2.6

Data are mean±SD (immunohistochemical scores). ^aNon-contaminated. ^bSignificantly different versus group 3. *P<0.0001, **P<0.001.

Immunohistochemical scores for proteins of Ub/proteasome system

The Ub/proteasome system may have a critical role in rapid and late responses to IR with radiation-induced (through free-radical damage) impairment of proteasome function, which results in inhibition of proteolysis. Because repeat radiation exposure can induce a strong cellular proliferative response *in vitro*, human response levels after sustained long-term, low-dose IR are of interest.

The tumor-suppressor protein p53 is known to be rapidly turned over in unstressed cells by the Ub/proteasome-dependent pathway and it has been found to be covalently modified by SUMO1 *in vitro* and *in vivo* through direct interaction with the SUMO E2-conjugating enzyme Ubc9. Accumulating evidence suggests an intrinsic role of sumoylation of p53 in stress-related processes, including DNA damage. Therefore, the fact that at least 60% of group 1 cases showed high scores for p53 protein over-expression is of clear interest[34]. Earlier, we reported defective regulation in cell cycling and DNA damage involving p53 and mdm2 expression, accompanied by specific p53 gene mutations with G:C to A:T transitions at CpG dinucleotides and a hot spot at codon 245 in 53% of a series of patients with Chernobyl cystitis [9,13]. Therefore, our findings support the idea that increased p53 protein stability resulting from mutations prevents recognition by the Ub-mediated degradation machinery as well as by sumoylation modifying processes.

Destabilization of another Ub/proteasome target, the tumor-suppressor p27^{Kip1} protein, has also been implicated in the pathogenesis of cancers [33]. Of our cases of Chernobyl cystitis, 36% showed loss or significantly decreased levels of p27^{Kip1} expression in the nuclei and cytoplasm. However, in 44% of cases, we observed predominantly p27^{Kip1} cytoplasmic staining. These observations suggest that decreased nuclear p27^{Kip1} expression may be due to cytoplasmic translocation, known to be a result of mitogenic stimulation and has been claimed to be related to a poor prognosis.

Thus, our study suggests that ubiquitination and sumoylation processes are direct targets of long-term, low-dose IR exposure, leading to unscheduled protein degradation. Up-regulated ubiquitination and sumoylation processes might be an adaptive response to insufficient proteolysis of aberrant p53 and p27^{Kip1} occurring due to long-term, low-dose IR exposure. These results are in line with recent findings that aberrant, unscheduled proteolysis of many cell cycle regulators contributes significantly to tumorigenesis, with increased protein stability as a result of mutations that prevent recognition by the Ub-mediated degradation and sumoylation machinery. If critically damaged cells with aberrant p53 protein escape from elimination, this would mean survival of cells with mutations in an environment of continued long-term, low-dose IR exposure.

Conclusions

Our data support the hypothesis of distinct molecular carcinogenesis pathways for bladder cancer in Ukraine before and after the Chernobyl disaster [35]. The biological effects of chronic low doses of IR and their relationships with chronic inflammation and carcinogenesis have received much attention in the last few years. Series of our recent studies point to a strong relationship between oxidative stress, accompanied by at least two signaling pathways (involving p38 MAPK NF- κ B cascades) and growth factor receptor activation, induced by the IR exposure of people who have lived for ~20 years in radiation contaminated areas of Ukraine, and the development of radiation chronic proliferative atypical cystitis, so-called Chernobyl cystitis, a pre-neoplastic condition in humans (Figure 6.14) [36]. Dramatic increase of iNOS, Cox2 and 8-OHdG expression is associated with the observed chronic inflammation; this may be mutagenic through NO-mediated DNA damage or hindrance to DNA repair and thus potentially carcinogenic, as suggested by p53 accumulation in urothelium of Chernobyl cystitis together with frequent G:C to A:T transitions at CpG dinucleotides in the p53 gene [13]. Inflammation-induced reactive oxygen and nitrogen species cause damage to important cellular components

(e.g. DNA, proteins and lipids), which can directly or indirectly contribute to neoplastic cell transformation. Over-expression, elevated secretion or abnormal activation of pro-inflammatory mediators, such as cytokines, chemokines, Cox2, prostaglandins, iNOS and NO, and a distinct network of intracellular signaling molecules including upstream kinases and transcription factors facilitate tumor promotion and progression. These enzymes may be acting as a 'landscaping tumor promoters' in the bladder urothelium and influence tumor growth according to the landscaping model proposed by Kinzler and Vogelstein (1998). On the other hand, DNA methylation and histone modifications are important epigenetic mechanisms of gene regulation and play essential roles both independently and cooperatively in tumor initiation, promotion and progression. Furthermore, recently, the possible mechanisms by which inflammation can contribute to carcinogenesis were reported to include induction of genomic instability, alterations in DNA methylation and subsequent inappropriate gene expression, enhanced proliferation of initiated cells, resistance to apoptosis, aggressive tumor neovascularization, invasion through tumor-associated basement membrane metastasis etc. Furthermore, significant alteration in regulation of cell cycle transition associated with increased activation of proliferative processes (p53, cyclin D1, mdm2, p21^{WAF1/Cip1} and PCNA protein over-expression) has been documented [15], along with changes in ubiquitination and sumoylation processes in association with aberrant unscheduled proteolysis of many cell cycle regulators [34]. Moreover, DNA repair appears to be an early event in the radiation-induced adaptive response. Exhaustion of the capacity for base and nucleotide excision repair pathways associated with Chernobyl cystitis may be related to the carcinogenic potential of the urothelial lesions.

Bladder urothelium as well as endothelial cells, fibroblasts and immune cells in the microenvironment respond to chronic low-dose IR by activation of FGFR and EGFR signaling pathways in association with EGFR2^{neu}, Raf-1 and stromal TGF- β 1 activation with aberrant expression of the urothelial β -catenin-E-cadherin complexes, which may potentially contribute to several facets of multi-stage urothelial carcinogenesis, including auto- or paracrine growth stimulation, up-regulation of angiogenesis and stromal remodeling. It can be supposed that the microenvironment changes induced by IR, detected in our studies, could promote angiogenesis, remodeling the extracellular matrix to facilitate invasion as well as the progression of pre-existing initiated cells to malignancy. This hypothesis for chronic long-term, low-dose IR action is supported by the emerging concept that elements other than epithelial cells, and mechanisms other than genetic alteration, influence the processes of carcinogenesis. Based on now available IR-induced urinary bladder carcinogenesis markers in patients living in radiation contaminated areas, prediction and early detection of Chernobyl cystitis becomes very important. Moreover, patients from the radiation contaminated areas have to be followed up for many years with the cytological analysis of urine sediment as well as other clinical investigations. The targeting therapy might be used against some genes, growth factors and other important molecules, which are responsible for cell cycle transition, cell-cell communication and signaling cascades. Furthermore, it might be recommended to use some pectines and able to bind to ¹³⁷Cs and other radionuclides, thus eliminating them. Placing chronic low-dose radiation damage at the cellular level into the context of a dynamic multicellular system will provide a better basis for understanding and treatment of Chernobyl cystitis — the first step in a specific category of urinary bladder carcinogenesis in humans.

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CHAPTER 7 CYTOGENETIC EFFECTS

Chernobyl accident significantly complicated ecological situation in Ukraine. Against the background of already existing man-made environmental contamination it promoted the expansion of contact of the great part of Ukrainian population with one of the most powerful universal mutagens — ionizing radiation which induced genome damages at all levels of genetic material (gene, chromosome, genome) in all living being human including. As it is known just radiation-induced genetic disorders may serve as the basis for the appearance and realization both stochastic effects (hereditary diseases, oncological pathology) and some non-stochastic effects with mutational component (multifactorial pathology, congenital malformations).

Thus, one of the main target for ionizing radiation in human (both by their vulnerability and by the medical consequences of irradiation) is genome of somatic and germ cells, specific radiogenic damage of which at chromosomal level can be not only biological indicator and biological dosimeter of radiation exposure (used under nuclear accidents), but the criterion for formation of the relevant risk-groups concerning the realization of radiation-induced pathology.

Taking into consideration above mentioned, selective cytogenetic monitoring above 3000 persons among the groups of high priority exposed to Chernobyl accident factors in Ukraine (ARS convalescents, clean-up workers with different radiation doses, Chernobyl power plant personnel, Shelter staff, evacuees, children and adults living in territories contaminated by radionuclides, self-settlers from 30-km alienation zone) have being conducted by scientists from Medical Genetics Division of RCRM (cytogenetic and genetic monitoring labs) since January 1987 till now.

All types of chromosome aberrations frequency in generally accepted for cytogenetic studies test-system — the culture of human peripheral blood lymphocytes — was determined according to micromethod modified by us [1].

Only chromosome type aberrations — unstable that eliminated over time (polycentrics, ring chromosomes, acentrics — free double fragments and acentric rings) considered as the markers of radiation exposure and stable (abnormal monocentrics formed by symmetric complete and incomplete chromosome translocations, inversions, insertions, interstitial and terminal deletions) that not only almost completely kept following acute radiation exposure, but also accumulated under chronic exposure; chro-

matid type aberration (single fragments, chromatid exchanges) considered as markers of chromosome instability.

Chromosome aberrations were detected mainly with the help of classic conventional cytogenetics and selectively — by differential G-banding analysis and high tech fluorescent *in situ* hybridization of human metaphase chromosomes with DNA probes to whole chromosomes method (FISH-WCP).

The results obtained during the first 10–20 years following Chernobyl accident published in detail in numerous our articles and summarized in 2007 [1] permit to reveal certain regularities as regards targeted cytogenetic effects induced by external and internal irradiation in persons exposed in Ukraine because of the accident.

In majority of examined critical groups directly exposed to Chernobyl accident factors the increased frequency of cytogenetic radiation markers had been kept ~for two decades; their mean group values depended on character, intensity, duration and conditions of irradiation, time following the accident; their widely varying individual values under identical radiation exposure probably were determined by the individual susceptibility of human organism.

In all surveyed groups considerable variability of above-spontaneous cytogenetic effect was found which did not correlate with initial level of aberrations that can be caused by genetic predisposition and/or radiation-induced adaptation of human organism.

The frequency of unstable chromosome aberration gradually decreased over time (but with saving its residual value which elevated spontaneous level, for ~20 years); stable radiogenic markers remained almost at the same level following acute radiation exposure and accumulated under chronic irradiation.

It had been confirmed that even low for human doses of ionizing radiation under prolonged exposure can induce specific chromosome damages in the human peripheral blood lymphocytes and cause more than twice increase of the spontaneous chromosome mutations level. Such initial damages of human genome at chromosome level are not only biomarkers of radiation mutagenic exposure and biodosimeters of absorbed radiation doses, but by causing the death or disturbances of target cells functions (somatic and germ) may be the basis for origin of stochastic and, possibly, some non-stochastic post-radiation effects with genetic component, in particular, multifactorial pathology.

Interindividual variability in frequency of chromosomal aberrations that occur under the identical radiation exposure may serve as indicator both of adaptive capacity and individual sensitivity of human organism to action of mutagenic factors that must be considered under the occupation monitoring of persons working in conditions of chronic irradiation even under observance of hygienic standards.

However data obtained allow to suggest the existence of such levels of radiation exposure below which cytogenetic effects in certain individuals either are not induced, or are induced but can be repaired, or cannot be detected by methods used at present, limiting individual assessment of mutagenic effects induced by low intensity radiation.

Thus, using traditional approaches to evaluation of cytogenetic consequences of human radiation exposure for monitoring of Chernobyl contingents of high priority in first 10–20 years following the accident, dose-related increase in the intensity of human somatic chromosome mutagenesis was shown that against the background of general

environmental and social distress allowed to suggest Chernobyl accident as new harmful ecogenetic factor for Ukraine's population.

The data received, being indicators of total mutagenic load on the observed contingents were in good agreement with the identical results of colleagues from Ukraine, near and far abroad and testify to the danger of human chromosome destabilization induced by Chernobyl accident factors that in the CIS countries is complicated by the general environmental and social distress [2–5].

As well-known, the ability of genome to stable function in constantly changed ecological conditions is one of the most important their properties, which allowed practically faultlessly to reproduce and transmit genetic information in the generations of cells and organisms. However, modern anthropogenic contamination of the environment by different genotoxic agents leads to increase of mutagenesis intensity in human somatic and germ cells, and hence — to genetic instability manifestation of which on cytogenetic level is the elevation of chromosome aberrations frequency (instability of karyotype) and result — increase of the frequency of pathology with mutational component.

Therefore, in so-called late post-Chernobyl period (15–20 years following the accident) the new problem arose — developing and implementation of modern approaches for estimation of radiogenic damage of human genome — determination not so much as the residual targeted mutagenic effects of ionizing radiation in directly irradiated targeted cells (requiring analysis of huge samples of cells) as disgenomic (untargeted) effects — among which leading role for the realization of human radiation injury are different forms of radiation-induced genomic instability — a phenomenon in which the cells accumulated multiple changes over time that promote transition of normal cells stable genome into the unstable one (typical, in particular, for tumor cells) and so-called “bystander effect” [6].

The results of experimental investigations showed that radiation induced mutagenic effect may not only decrease with time due to elimination of unstable chromosomal aberrations by the death of aberrant cells during mitosis. The genomic instability can be transmitted in the form of potential damages of genetic material through few cell generations and realized with a delay *in vivo* or *in vitro* in delayed term following mutagenic exposure (delayed chromosomal instability) [7]. It had been found also that part of non-repaired or misrepaired genome damages in cells of exposed parents can be transmitted into somatic cells of their progeny in the form of various types of chromosomal aberrations (transmissible chromosome instability) [8].

At the cytogenetic level except delayed and transmissible chromosome instability an important role in destabilizing of human genome plays hidden chromosome instability, which manifests as increase of somatic cells chromosomes sensitivity of irradiated persons to the exposure of other mutagens *in vivo* and *in vitro* [9].

For investigation of late untargeted cytogenetic effects of ionizing radiation in persons exposed to Chernobyl accident factors such new approaches was applied for the first time in Ukraine:

- elaboration, modification and approbation of new model system to study “delayed” chromosome instability by two-termed cultivation of human peripheral blood lymphocytes (the standard short-term 48-hour incubation and long-term 144-hour cultivation) allowing to analyze the cells of first and fifth generations, respectively;

- elaboration, modification and approbation of new model system to study hidden chromosome instability using as mutagen-provocateur *in vitro* antitumor antibiotic-radiomimetic bleomycin in final concentrations 0.05 and 5.00 mcg/ml at G₂ phase of mitotic cycle — “G₂-bleomycin sensitivity assay”;
- elaboration and approbation of new model system to study bystander effect in human cells on cytogenetic level by using mixed human peripheral blood lymphocytes culture differed by cytogenetic sex markers and consisted of population of irradiated (*in vitro* or *in vivo*) cells as a source of harmful signal and untreated populations of lymphocytes received from person of the other gender (used as “bystander”).

The groups of high priority with different intensity of radiation exposure due to Chernobyl accident were voluntarily observed.

We used the two-termed cultivation of lymphocytes for cytogenetic examination of two children groups born to fathers for which ionizing radiation considered as occupational (clean-up workers of Chernobyl accident, the dose range from 40 to 670 mGy; first group) or environmental harmful factors (residents of area contaminated with Cs isotopes submitted to so-called “iodine shock” in 1986; second group). Children born to unexposed parents living in relatively clean for radiation situation territory served as a control group.

Generalized results of these studies are presented in Fig. 7.1.

Analyzing the data obtained we tried to assess the contribution of parents exposure (radiation component) into the cytogenetic effect observed in their children and also evaluate the importance of lymphocytes' culture terms for expression of this effect.

Both under the standard short-term and long-term lymphocytes' cultivation significant ($p < 0.01$) acceleration of spontaneous chromosome mutagenesis was observed only in group of children born to irradiated parents and living on territory contaminated with cesium radionuclides. It is possible that living in such area may contribute to destabilization of genome as modifying factor that increases the chromosomes' sensitivity of somatic cells to other mutagens, so far as the radiation doses of these parents could be much lower than the doses of Chernobyl accident clean-up workers.

It should be emphasized that 144-hour lymphocytes' culturing in both groups of exposed children was significantly increased compared with those under short-term cultivation ($p < 0.05$, $p < 0.01$, respectively), which may prove for the realization of delayed chromosome instability.

Increased exchange-type chromosomal aberrations — stable (abnormal monocentrics) and unstable (centric rings) reflects both the reality of transgeneration phenomenon of chromosome instability in the progeny of

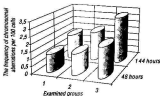


Fig. 7.1. Results of cytogenetic examination of children born to parents exposed and unexposed to ionizing radiation. 1 — children born to unexposed parents (control group); 2 — children born to liquidators of Chernobyl accident; 3 — children born to residents of area contaminated with Cs isotopes.

the first group as well as the destabilization of their genome because of unfavorable ecological situation in the place of residence — in the offspring of the second group.

Taking into account the substantial individual differences even in spontaneous instability of lymphocytes' karyotype under long-term cultivation we analyzed the interindividual dynamics of cytogenetic effects in children born to unexposed parents (control group) and in offspring of irradiated parents in the first and fifth cell generation (Fig. 7.2–7.4).

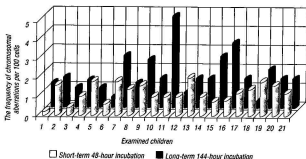


Fig. 7.2 Results of cytogenetic examination of children born to unexposed parents (control group).

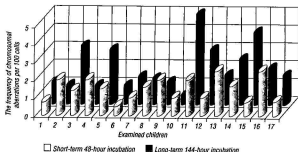


Fig. 7.3. Interindividual dynamics of cytogenetic effects in children born to clean-up workers of Chernobyl accident in the first and fifth cells' generation

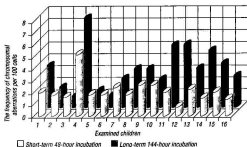


Fig. 7.4. Interindividual dynamics of cytogenetic effects in children born to residents of contaminated territory in the first and fifth cells' generation

In the control group against the background of low spontaneous frequency of chromosome aberrations cytogenetic effect was not changed under both terms of cultivation in almost all examined children, only in two cases significant elevation of cytogenetic effect in 144-hour cultures were seen (cases 11 and 16, respectively).

Significant interindividual variability in the frequency of chromosome aberrations under both terms of cultivation, as well as differences in individual response of the genome for long-term cultivation were observed in both exposed groups.

There was no positive correlation between cytogenetic effects in short-term and long-term cultures. There were individuals with minimum aberrations in the first mitosis and maximum frequency of chromosomes' disorders in the fifth cell generation in all examined groups (No 11, 16 from the control group; No 12, 15 from the group of children born to clean-up workers; No 11, 12, 14 from the group of children born to dwellers of contaminated territory), which reflects the individual peculiarity of spontaneous and / or radiation induced karyotype instability in cell generations.

In children born to clean-up workers in most cases (88%) cytogenetic effect was identical under both terms of lymphocytes' cultivation; in 12% of the examined persons frequency of chromosome aberrations was significantly higher in long-term cultures.

In the offspring of parents exposed at the contaminated territories increased rate of cases (up to 25%) with significantly elevated chromosome instability was found in 5th mitosis, that is two times higher than in children born to clean-up workers of Chornobyl accident.

This effect in 10 years following the accident may be caused by a specific feature of the Polissya area — high coefficient of radiocaesium transfer from soil to plants and its accumulation in food (especially in milk and potato, where its contents more than 5 times higher than the permissible norm), that is by continuation of internal exposure. It cannot also exclude transmissible chromosome instability, which is expressed in offspring of parents exposed to iodine radioisotopes. Just this effect of genome instability transmission through germ cells of irradiated parents to first generation of their progeny

considered nowadays as one of the most urgent problems of radiation genetics, for the resolution of which various methodological approaches, both on chromosome and at the molecular levels, have been developed.

The results obtained have confirmed the data of other authors about the high sensitivity of the child's organism to mutagenic effects of radiation — not only for the induction of primary, but also indirect (transgeneration) chromosome instability.

Hidden chromosome instability expressed as hypersensitivity of human somatic chromosome to the exposure of other mutagens both *in vivo* and *in vitro* plays an important role in destabilizing of human genome.

Estimation of hidden chromosome instability is a new direction of cytogenetic studies that was rather intensively conducted in different cytogenetic laboratories of the world over the past decade mainly to determine the risk of spontaneous or induced oncopathology by chromosome hypersensitivity of peripheral blood lymphocytes to clastogenic exposure of radiomimetic bleomycin considered as the cytogenetic onco-marker [10].

Individual sensitivity of somatic cell chromosomes to testing bleomycin exposure did not depend on human gender and age and was genetically determined, due to certain molecular-genetic mechanisms. As shown in recent years, interindividual variability of cytogenetic effects induced by bleomycin may be linked as with nucleotide polymorphism of the locus SNP A1450G in the gene that encodes bleomycin-hydrolase (BLHX) — a specific enzyme that metabolize bleomycin, as well as with genetic polymorphism of enzymes which determine the effectiveness of the DNA damages repairation [11–13]. Damage of chromosome telomeres plays certain role in causing genomic instability and, hence, increased sensitivity to mutagenic exposure, which in turn can lead to cancer induction or promotion [14]. Started molecular genetic studies (microarray analysis) with regard to identify the genes responsible for susceptibility to oncological pathology, in lymphoblastoid cells' lines derived from individuals with different (high and low) sensitivity to clastogenic bleomycin exposure [15].

The question concerning modification of genetically caused sensitivity of human genome to mutagenic exposure remains still open. Some authors supposed that such modifications can be induced by some environmental factors, but we know only one publication, which showed that the joint action of tobacco smoking and cytomegalovirus infection slightly increased the sensitivity of human peripheral blood lymphocytes to testing bleomycin exposure *in vitro* [16].

It should be noted that currently test "G₂ bleomycin sensitivity assay" was not uniform, that considerably complicated the interpretation and comparison of results obtained by this method. So at an early stage of research we have elaborated and improved model system for studying hidden chromosome instability in human peripheral blood lymphocytes by means of testing mutagenic exposure of bleomycin *in vitro*. It allowed to adapt "G₂ bleomycin sensitivity assay" for detection of individual radiation-induced hidden chromosome instability in human somatic cells.

First we have determined the optimal terms of treating lymphocyte culture by bleomycin (late G₂ phase of mitotic cycle) and appropriate concentrations (0.05 and 5.00 µg/ml), suitable for assessing the sensitivity of somatic human cells' chromosomes *in vitro* to testing mutagenic exposure.

In the next stage of work groups of persons exposed to irradiation of different intensity in anamnesis were formed, background frequency of full spectrum of chromosome aberrations in peripheral blood lymphocytes were estimated and comparative intergroup assessment of radiation exposure intensity by cytogenetic criteria was evaluated.

Results of cytogenetic examination of observations groups (including control) are shown in Fig. 7.5, where all groups are in ascending order of radiation-induced cytogenetic effect — total frequency of cytogenetic indices of radiation exposure — unstable (dicentric and centric rings) and stable (abnormal monocentrics) chromosome aberrations.

As seen from these data the least exposed group by cytogenetic criteria was the group of people worked in 30-km alienation zone in 2006–2009 (chronic radiation exposure of low intensity, mainly external irradiation). Mean-group frequency of radiogenic cytogenetic markers in this group was equal to the control and spontaneous levels.

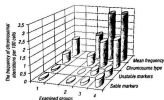


Fig. 7.5. Results of selective cytogenetic monitoring among the groups of high priority exposed to Chernobyl accident factors in Ukraine: 1 — control group; 2 — group of people working in 30-km alienation zone in 2006–2009; 3 — clean-up workers with different radiation doses; 4 — patients recovered from acute radiation syndrome in 1986.

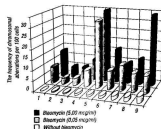


Fig. 7.6. Comparison of individual results of cytogenetic examination persons from control group.

Then followed groups of individuals recovered from ARS (acute radiation exposure of high intensity, mainly external irradiation in 1986) and clean-up workers (prolonged radiation exposure of medium intensity, mainly external irradiation in 1986–1987) in which even in delayed terms following irradiation increased frequency of as residual unstable chromosome markers of radiation exposure as well as primary radiation-induced stable chromosome aberrations had been observed.

At the final stage of research persons from all observed groups were tested using "G₂ bleomycin sensitivity assay" to study the individual sensitivity of peripheral blood lymphocytes' chromosomes to test mutagenic loading and its possible modification by irradiation of different intensity.

The results of such testing are presented graphically in Fig. 7.6–7.9. It is clearly visible both wide interindividual variations of cytogenetic effects induced by testing bleomycin exposure in both used concentrations and lack of positive correlation between background and induced frequencies of chromosome aberrations in all examined groups.

Identification of persons with hypersensitivity to testing bleomycin exposure have been performed similarly to identify individuals with increased sensitivity to ionizing radiation [17] by calculating the coefficient of hidden chromosome instability (K_{hci}) according to simplified by us formula:

$$K_{hci} = M_{hci} / M \quad (1)$$

where: M_{hci} — individual values of chromosome aberrations frequency under testing bleomycin exposure in concentrations 0.05 and 5.00 $\mu\text{g/ml}$;
 M — mean group values of chromosome aberrations frequency under testing bleomycin exposure in concentrations 0.05 and 5.00 $\mu\text{g/ml}$.

It was accepted that cytogenetic effects induced by bleomycin in hypersensitive persons exceeded mean group level of chromosome aberrations and will always be >1 .

The rate of the hypersensitive persons in all examined groups is presented in Fig. 7.10.

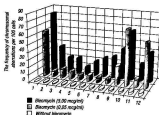


Fig. 7.7. Comparison of individual results of cytogenetic examination persons worked in 30-km alienation zone in 2006-2009.

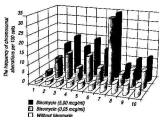


Fig. 7.8. Comparison of individual results of cytogenetic examination clean-up workers with different radiation doses.

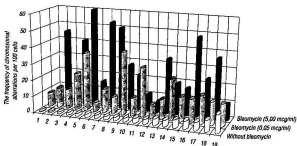


Fig. 7.9. Comparison of individual results of cytogenetic examination patients recovered from acute radiation syndrome

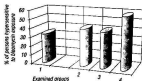


Fig. 7.10. Frequency of hypersensitive persons in groups of high priority exposed to Chernobyl accident factors in Ukraine: 1 — control group; 2 — group of people worked in 30-km alienation zone in 2006–2009; 3 — clean-up workers with different radiation doses; 4 — patients recovered from acute radiation syndrome in 1986.

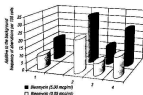


Fig. 7.11. Comparison of cytogenetic effects in groups of high priority exposed to Chernobyl accident factors in Ukraine with the help of “O₂–bleomycin sensitivity assay”: 1 — control group; 2 — group of people worked in 30-km alienation zone in 2006–2009; 3 — clean-up workers with different radiation doses; 4 — patients recovered from acute radiation syndrome in 1986.

When comparing the intergroup sensitivity to clastogenic effect of bleomycin we take into consideration rate of identification of persons with hidden chromosome instability and addition to the background frequency of chromosome aberrations (above-spontaneous level) in each of the examined group which were divided, depending on the frequency of cytogenetic markers of irradiation. The results of this comparison are shown in Fig. 7.11.

As seen from these data, the maximum percentage of persons with hidden chromosome instability (57.9%) was found in patients who had ARS in 1986; in other groups the rate of individuals with increased sensitivity to bleomycin was almost identical and did not exceed 41.6%. However, the maximum addition to mean group frequency of chromosome aberrations (22.00 and 32.34 per 100 metaphases at concentrations 0.05 µg/ml and 5.00 µg/ml of bleomycin, respectively) was observed in workers of the 30-km alienation zone, which may be associated with specific exposure of individuals in the examined groups.

These results confirmed the reality of modification of inherited chromosome susceptibility to the testing mutagenic exposure depending on the intensity of radiation exposure.

Somatic pathology in exposed individuals may be caused not only by direct radiation damage of targeted cells, but also by secondary changes in the untreated cells situated near the irradiated ones – by “bystander effect” belonging to disgenomic (untargeted) mutagenic effects [18]. The existence of this phenomenon was proved under the exposure of both alpha and gamma radiation [19]. It had been shown that signal from irradiated to unirradiated cell may transmit directly through intercellular interaction or indirectly through substances secreted from exposed cells (cytokines, reactive oxygen species) [20]. In the bystander cells increased formation of free radicals, activation of gene expression, induction of mutations, sister chromatid exchanges, chromosome aberrations and development of apoptosis were observed [18–20]. Study of radiation-induced bystander effect is necessary for the prognosis and discovery of mechanisms of the medical consequences of human radiation exposure. Unirradiated bystander cells may acquire induced instability which are considered as preoncological status and stipulated for the importance of its research in human organism exposed to ionizing radiation.

The most important results of mechanisms and patterns study of bystander effect's development were obtained by using experimental models of this phenomenon in conditions *in vitro*. The principal requirements for the creation of such models were the necessity of irradiation only the part of all cells and opportunity to distinguish populations of exposed and unexposed cells. Therefore, modelling of radiation induced bystander effect carried out mainly applying alpha radiation. Such models allow to record the tracks of alpha particles that crossed the cells and damaged them directly. It should be noted that the first radiation-induced bystander effect had been described by H. Nagasawa and J. Little just using alpha-irradiation [21].

The application of X- and gamma-rays for the investigation of radiation-induced bystander effect fulfilled mainly on interspecies hybrid mammalian cell cultures, which allows to distinguish irradiated and unirradiated cell populations [18].

The investigation of bystander effect in human cells requires the application of new methodological approaches. In 2005 we proposed and developed an original model system for the detection and investigation of radiation-induced bystander effect in somatic human cells — "mixed human peripheral blood lymphocytes culture" consisted from irradiated (*in vitro* or *in vivo*) and untreated cells differed in cytogenetic sex markers (XX, Y) and some morphological somatic chromosome peculiarities [22].

By means of such model cytogenetic patterns of induction and persistence of chromosome instability in human lymphocytes as a result of bystander effect under irradiation *in vitro* and *in vivo* at low and high doses were established. Cytogenetic analysis using the differential G-banding metaphase chromosomes staining was performed permitting to reveal the whole spectrum of stable and unstable chromosomal aberrations and correctly determine their level. Damaged chromosomes and points of breaks in accordance with international nomenclature ISCN-2005 were registered [23].

The level of chromosome aberrations in unexposed human lymphocytes cultivated with lymphocytes irradiated *in vitro* in doses 250 mGy and 1Gy was significantly higher than the frequency of aberrations in control mixed cultures of unirradiated lymphocytes ($p < 0.05$; Fig. 7.12). The frequency of chromosome aberrations in the bystander cells elevated with increasing doses of neighboring populations of lymphocytes due to increased induction of chromatid breaks.

The difference between spectra of chromosome aberrations in irradiated targeted cells and intact bystander cells was established. Irradiated targeted cells were characterized by induction of chromosome type damages (deletions, dicentric, centric rings, translocations, inversions), bystander cells contained chromatid breaks that can be consider as the markers of chromosome instability. Radiation-

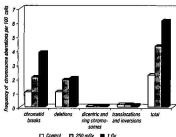


Fig. 7.12. Cytogenetic indices of G-banding analysis in intact cells (bystander) cultivated with lymphocytes irradiated *in vitro*

induced bystander effect had not influence on the induction of stable and unstable markers of radiation exposure in the untreated cells cultivated with exposed lymphocytes that confirmed the legality of the use of targeted cells for retrospective biological dosimetry.

Significant interindividual variability of survived donors both by the level of their sensitivity to radiation exposure in low and high doses and by the intensity of radiation-induced bystander effect was established (Table 7.1).

Table 7.1. Individual frequencies of chromosomal aberrations in the examined donors

Donor	Frequency of chromosomal aberrations, per 100 cells in							
	control culture	unexposed lymphocytes cultivated with lymphocytes irradiated <i>in vitro</i> in doses, Gy			lymphocytes irradiated <i>in vitro</i> in doses (Gy) cultivated with unexposed lymphocytes		lymphocytes irradiated <i>in vitro</i> in doses (Gy) cultivated separately	
		0.00	0.25	1.00	0.25	1.00	0.25	1.00
1	2.00	1.00	4.62	7.26	6.90	29.09	6.40	28.00
2	2.40	3.00	3.33	7.94	16.07	23.33	11.20	24.00
3	2.67	2.00	6.72	6.00	5.80	18.00	4.00	20.00
4	1.43	2.00	3.00	4.38	5.00	20.77	3.41	21.00
5	2.86	2.35	5.00	5.56	14.00	16.13	11.00	16.25
6	2.86	3.00	3.16	4.38	7.00	26.00	8.00	26.00
7	0.80	2.00	4.41	3.48	8.86	25.00	6.40	26.25
8	3.33	3.00	3.18	7.89	6.00	20.90	4.60	22.66
9	2.40	2.00	4.00	7.92	4.86	17.65	2.96	18.42
10	1.00	0.67	5.78	5.22	3.23	22.00	5.36	24.00
Mean	2.15±0.39	2.27±0.36	4.31±0.60	6.13±0.66	6.09±0.87	21.19±1.32	6.09±0.68	22.31±1.55

The frequency of cytogenetic indices identified in human peripheral blood lymphocytes in delayed terms following radiation exposure is the result of integrated response of genome to irradiation and caused by mutagenic effects both in exposed targeted cells and in unexposed bystander cells. Therefore, during the medical control of contingents exposed to uncontrolled irradiation

cytogenetic monitoring must be carried out for the evaluation of targeted and untargeted mutagenic effects induced by radiation in human somatic cells.

Thus, we analyzed the frequency of all types of chromosome aberrations in lymphocytes of Chernobyl accident clean-up workers exposed to ionizing radiation in doses 350–690 mGy and 1.01–2.37 Gy (Fig. 7.13).

It was shown that 19 years following the accident the fre-

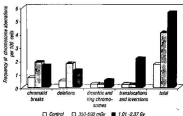


Fig. 7.13. Cytogenetic indices in peripheral blood lymphocytes of Chernobyl accident clean-up workers 19 years following irradiation (G-banding analysis)

quency of cytogenetical markers of irradiation in clean-up workers with official radiation doses 350–690 mGy had no significant difference from control ($p>0.05$) as a result of their elimination in time. However, the mean frequency of chromosome aberrations exceeded the population's one due to damages of chromatid type and deletions ($p<0.05$), indicating an increased level of chromosome instability in their somatic cells. In lymphocytes of Chernobyl clean-up workers exposed to radiation in doses 1.01–2.37 Gy the level of chromosome aberrations exceeded the population's one at the expense of high frequency of all types of chromosome damages and, in particular, — stable and unstable cytogenetic markers of radiation exposure ($p<0.01$).

However, the ability of lymphocytes of Chernobyl clean-up workers (exposed to radiation in doses 350–690 mGy and 1.01–2.37 Gy) to induce bystander effect in the intact human lymphocytes 19 years following irradiation was established (Fig. 7.14).

Clastogenic activity of Chernobyl clean-up workers' peripheral blood as well as increased level of chromatid breaks in it (which are markers of chromosome instability) for many years following the irradiation may be due both to the shift of prooxidant and antioxidant balance as a result of radiation exposure and persistence of radiation induced bystander effect which caused damage of genetic apparatus of the untreated cells.

A question regarding the duration of bystander effect and its ability to be transmitted to future generations of cells remains open to date. The solution of it is important for predicting the development of genomic instability in irradiated human body, because over time both elimination of chromosome damages induced by bystander effect and stabilization of their number or even amplification (at the expense of hidden genomic instability) are possible. In the latter case multiple changes will be accumulated in the unirradiated bystander cells supporting transition of their stable genome to unstable condition, typical for tumor growth.

To confirm the possibility of transfer bystander effect to the following generations of cells cytogenetic parameters in the intact human lymphocytes in the fourth post-irradiation mitosis under the long-term 120-hours cultivation with lymphocytes exposed *in vitro* in dose 0.25 Gy were investigated. The frequency of chromosome aberrations in bystander cells was significantly higher than control parameters received in mixed cultures of untreated populations of lymphocytes ($p<0.05$) due to increased frequency of chromatid type aberrations and had no significant difference in the level of chromosome aberrations in bystander cells in the first post-irradiation mitosis under short-term 48 hours cultivation ($p>0.05$; Fig. 7.15).

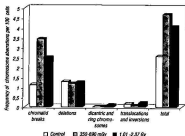


Fig. 7.14. Cytogenetic indices in unexposed bystander cells, under joint cultivation in mixed cultures with clean-up workers' lymphocytes (G-banding analysis)

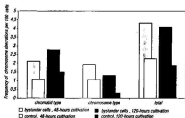


Fig. 7.15 Cytogenetic indices in unexposed bystander cells under long-term 120-hour cultivation in mixed cultures with lymphocytes irradiated *in vitro* in dose 0.25 Gy (G-banding analysis)

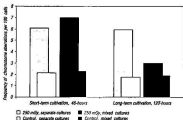


Fig. 7.16 The frequency of chromosome aberrations in human peripheral blood lymphocytes irradiated *in vitro* in dose of 250 mGy under short-term and long-term cultivation in mixed cultures with unexposed lymphocytes (G-banding analysis)

aberrations in the fourth post-irradiation cell division had not statistical difference compared with the first mitosis ($p > 0.05$). However in long-term culture of lymphocytes irradiated *in vitro* in dose 250 mGy the change of chromatid and chromosome types aberrations' ratios because of elevated induction of chromatid breaks (markers of chromosome instability) and elimination of dicentrics (unstable markers of radiation exposure) compared with short-term cultivation were found.

Under standard 48-hour cultivation the impact of the unirradiated cells on the irradiated ones (reverse bystander effect) was not registered, which may be due to lack of time for its realization at the cytogenetic level [24].

The question regarding the mechanisms of radiation-induced bystander effect remains open until now. It is assumed that this effect may be the result of two separate mechanisms: transfer of damages from irradiated cells to their unirradiated neighbor cells through intercellular connections or induction of cytokines or other factors into the environmental cell's medium that may increase the level of active oxygen in the untreated cells.

It is believed that during the induction of bystander effect the mutual influence of irradiated and unirradiated populations of cells can occur. However, in literature there are no data about the results of such interaction under modeling of radiation-induced bystander effect. In our study the "reverse" bystander effect — the positive impact of the unirradiated lymphocytes on the irradiated ones under the joint long-term cultivation had been recorded for the first time. The frequency of chromosome aberrations in cells irradiated *in vitro* and incubated in mixed cultures with intact lymphocytes had no statistically significant difference from appropriate control ($p > 0.05$) and was lower than this value in irradiated long-term cultures under separate cultivation ($p < 0.05$) by reducing the induction of chromatid breaks and increase the elimination of cells with chromosome type aberrations (Fig. 7.16).

Average frequency of aberrant cells and chromosome aberrations

Our results concerning the patterns of bystander effect induction in somatic human cells will allow in the near future to begin further study of the mechanisms of its development with use of the own model system. The results of such investigations will enable to better understand biological processes and discover damages in human cells under radiation exposure, more fully reveal the mechanisms of development and persistence of chromosome instability and induction of carcinogenesis (including the progress of secondary tumors after radiotherapy) in persons exposed to ionizing radiation.

Thus, the use of new approaches to evaluation of delayed untargeted cytogenetic effects as consequences of previous human radiation exposure allowed to reveal the various forms of radiation-induced genomic instability and bystander effect in some priority groups of persons exposed to the factors of Chernobyl accident that can be considered as a risk for realization of diseases with genetic component.

Conclusions

1. The new approaches were proposed in order to investigate various forms of chromosome instability (delayed, transmissible, hidden) and bystander effect and the appropriate model systems were developed allowing to detect and assess untargeted cytogenetic effects in delayed terms after human radiation exposure which are offered as cytogenetic criteria for evaluating of genome stability.

2. Various forms of chromosome instability in somatic cells of persons exposed to ionizing radiation owing to the Chernobyl accident in dose range from 40 to 3400 mGy in delayed terms following irradiation were determined by cytogenetic criteria, by using two-termed cultivation of human peripheral blood lymphocytes; assessment of individual sensitivity of human chromosomes to testing exposure of mutagen-provocateur bleomycin.

3. Modifying influence of ionizing radiation in a wide range of doses on the inherited individual human susceptibility to mutagenic exposure was established.

4. Cytogenetic patterns of induction and persistence of chromosome instability in human peripheral blood lymphocytes because of bystander effect under irradiation *in vitro* and *in vivo* at low and high doses had been defined.

5. The most important task of further scientific investigations is the profound study of the untargeted effects and comparison of primary structural genome damages (at the level of chromosomes or DNA) with realized harmful radioinduced direct (oncological pathology, congenital malformations) and indirect (non-specific violations of health, multifactorial pathology) effects as the consequences of human radiation exposure. Such investigations will enable to get unique data about contribution of radiogenic genomic injuries to realization of delayed medical consequences of human irradiation that finally can allow to develop scientifically based measures directed to minimization of such consequences.

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CHAPTER 8 IMMUNOLOGICAL EFFECTS

The problem of the late effects of low-dose ionizing radiation on the human immune system presents a special interest due to the immune cells radiosensitivity. Studies of the immune function that started in the RCRM in 1987, encountered an experience of world radiobiology. To perform a study in low-dose range there was a need to overcome a line of difficulties related to the necessity of separation the effects of irradiation in a set of several confounding environmental factors, effects of stress, type of exposure, individual radiosensitivity etc.

8.1 FREQUENCY OF IMMUNE DEFICIENCIES IN EXPOSED POPULATION

Long-term changes in the immune system during the first 10 years after the accident included the elements of compensation and impaired regulation of immune function. Compensatory changes were typical for 32% of exposed and were manifested with an increasing number of certain types of T-cells, increased activation and low levels of mutations in the loci of T-cell receptor. Dysregulation changes (37%) included the change in response to neurohumoral stimuli, the instability of immune function, also with the low levels of mutations in the loci of T-cell receptor. Manifestations of cellular immunity deficiency (31%) included the decreased levels of effector cells, preserving the number of T-cell receptor variant cells. Use of the adequate control groups recruited from the not-contaminated areas to the exposed was hampered in connection with lower immunological parameters compared with the reference international figures. At the end of the first 10-year period such difference of control parameters with the international smoothed and became insignificant (Fig. 8.1).

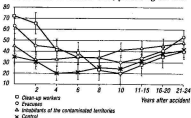


Fig. 8.1. Frequency of the secondary immunological insufficiency in groups of radiation exposed after the Chernobyl accident

Study of the frequencies of the immune deficiencies was continued in a remote period after the accident and was based on investigation of more than 165 000 of exposed from different categories of supervision and of control groups. A frequency analysis has shown reliable growth of frequency, which in a most degree was expressed for clean-up workers of the accident.

Study of correlation with dose and exposure time showed that in clean-up workers with radiation dose above 250 mSv in 13–18 years after the exposure a frequency of steady combined immune deficiency remained elevated (36,7%), as compared to control groups, and become a basis for late radiation effects. In clean-up workers with doses less than 100 mSv, the frequency did not differ from control groups.

In dwellers of the territories contaminated with radionuclides the main factors, that determined frequency of immune deficiency were age, somatic pathology, and other confounding factors.

8.2 IMMUNE FUNCTION IN ACUTE RADIATION SYNDROME SURVIVORS

Early period after irradiation

The study of the immune system of people affected by the accident, since the cessation of clinical manifestations of the acute radiation syndrome led to conclusions about the connection between these manifestations of separate clinical syndromes of ARS and their complications such as nervous system injuries, toxemia, and others. It was found that the immunological criteria to be proposed for detection of morphological changes in the nervous system and that the threshold was determined during irradiation of 2 Gy. The first study, which was held at the Institute of Biophysics, USSR Ministry of Public Health showed deviations in the immunoglobulins content, phagocytosis, nonspecific resistance. A significant reduction of anti-infectious immunity at the acute stage was evidenced by an increase of the number of pathogens colonies in the skin auto-microflora test [1]. Studies of patients with ARS grade 1–3 in the RCRM were launched in one year after exposure [2]. These studies are continuing today. The number of patients under follow-up is in Table 8.1.

Table 8.1. The number of patients who suffered in 1986 from ARS, under immunological follow-up

ARS grade	Number of ARS 1986 survivors under immunological follow-up by years			
	1996–1991	1992–1996	1997–2001	2002–2006
ARS-NC*	96	82	55	56
1	36	30	27	21
2	39	32	27	18
3	12	12	4	4
Total:	183	156	103	99

*non-confirmed ARS, see chapter 4.

The period of recovery after irradiation was characterized as an acquired combined radiation-induced immune deficiency with inhibited T-cell immunity, failure of nonspecific resistance mechanisms.

The changes of basic indices of leucocytes and lymphocytes counts for ARS convalescents within a supervision periods are resulted on Fig. 8.2.

The changes of indices have wavy character, with predominance of cytopenia in the first years, and lymphocytosis in the remote period.

In this period analysis showed the dependence of immunological parameters on disease severity. Gradual development of radiation injury compensation on average, stretched in time by 3–5 years and was characterized by wavy oscillations of surface antigens expression related to the helper-inducer function, mitogen response, increased expression of pan-B and activation antigens. In the early years after an accident a decline of T-cell counts was registered in 73% examined. Detailed description of the immune function in patients during the recovery after the ARS is described in the "Immune System" section of the monograph "Acute radiation syndrome" (1998) and other publications [3–5].

Dynamic study was conducted between 12–24 months, 25–36 months, 36–48 months after the accident, and showed overall satisfactory rate of normalization of cellular and humoral immunity (Table 8.2). The blood formula of patients with ARS-1 recorded significant decrease of lymphocytes to $1,69 \cdot 10^9/L$ in the period of 12–24 months after exposure, then the number of lymphocytes and monocytes increased. The highest inhibition of cellular immunity occurred in people of younger age.

In the recovery period a marked decrease in expression of antigens associated with helper-inducer function, increased presentation of cell surface pan-B and B-blast as well as activation antigens (CD3⁺Dr; CD4⁺8⁺, CD22⁺ CD23⁺ subsets) were demonstrated. However, after 48 months of monitoring a temporary normalization was detected in the numbers of CD4⁺8⁺ and CD8⁺57⁺ cells (Table 8.3). CD38⁺ cells content, which are involved both into cytotoxic and activation reactions, was increased. It should be noted the discrepancy in the first years of changes of CD57⁺ and CD15⁺16⁺ cells, identified as highly active natural killer cells. The variations could be explained by an existence of a certain period of heterogeneity in cell recovery after radiation injury. As for the increased proportion of active T-lymphocytes, it may reflect a non-specific reaction to a number of factors as radiation, the accompanying chronic pathology etc. An increased number of B-cells bearing the late differentiation markers such as surface immunoglobulins of IgG class suggests in favor of the last assumption. A strong tendency of increased counts of CD4⁺8⁺ and CD1⁺ among peripheral blood cells was noted during the whole first period. Normally this phenotype is attributed to the intrathymic stages of differentiation. Their appearance in a peripheral blood, in the absence leukemoid transformation could be interpreted as a manifestation of reparatory reactions in the immune system.



Fig. 8.2. Frequency of the basic changes in leucocytes and lymphocytes counts in ARS survivors, subjected to immunological follow-up.

Patients with such symptoms during the subsequent follow-up have been followed to identify possible immunopathologic processes.

Table 8.2. Dynamics of immunological tests depending on the ARS grade

Immune parameters	Stat. parameters	Cellular immunity indices in dependence on the ARS grade and period after an irradiation					
		ARS grade 1		ARS grade 2		ARS grade 3	
		12-24 months	24-36 months	12-24 months	24-36 months	12-24 months	24-36 months
WBC, 10 ⁹ /L	M	5.98	5.58	5.04	5.01	6.45	5.28
	±m	0.50	0.23	0.58	0.36	2.35	0.57
Lymphocytes, 10 ⁹ /L	M	1.97	1.69*	1.48	1.80	2.23	1.75
	±m	0.14	0.10	0.14	0.17	0.59	0.27
E-RFC, 10 ⁹ /L	M	0.98	0.83	0.87	0.97	1.16	0.69
	±m	0.10	0.06	0.12	0.13	0.55	0.77
E-RFC, resistant to theophylline, 10 ⁹ /L	M	0.51	0.52	0.52	0.53	0.47	0.39
	±m	0.04	0.04	0.09	0.08	0.14	0.11
E-RFC, theophylline-sensitive, 10 ⁹ /L	M	0.23	0.25	0.20	0.22	0.60	0.12
	±m	0.04	0.05	0.05	0.05	0.42	0.09
E-RFC, theophylline-stimulated, 10 ⁹ /L	M	0.05	0.07	0.002	0.20*	0.00	0.26*
	±m	0.04	0.03	0.002	0.09	0.00	0.02
IgA, g/L	M	1.51	1.45	1.79	1.28*	1.83	1.85
	±m	0.08	0.06	0.15	0.09	0.23	0.05
IgG, g/L	M	15.60	13.27*	15.48	15.28	14.23	8.90
	±m	0.83	0.51	0.85	1.18	3.98	1.90
IgM, g/L	M	1.34	1.20	1.14	0.93	1.28	0.89
	±m	0.08	0.09	0.12	0.11	0.60	0.25

WBC — white blood cells; E-RFC — E-rosette forming cells (mainly T-lymphocytes); IgA, IgG, IgM — immunoglobulins of different classes; * — significant differences, $p < 0.05$.

Table 8.3. Relative content of T- and B- cells in the period of the immune system reconstitution after the acute radiation syndrome

Study groups	Years after irradiation	A percentage of lymphocytes among subpopulations (M±m)				
		CD3*19-	CD3*HLA-DR*	CD3*4*	CD4*8*	CD3*19*
Control	7-13	67.39±1.58	6.93±1.22	41.84±1.50	29.77±1.29	7.90±0.66
	7-8	67.78±2.46	8.89±1.99	36.63±2.52	30.26±2.17	6.79±0.68
ARS-NC [†]	9-10	70.16±1.16	10.85±1.31*	40.32±1.35	33.99±1.99*	9.12±0.45
	11-13	66.15±1.73	6.83±1.28	39.25±1.74	29.90±1.68	8.95±0.57
	7-8	65.01±4.65	6.72±3.39	40.17±4.33	26.74±2.97	7.48±1.36
ARS grade 1	9-10	67.26±1.57	7.99±1.25	38.06±1.76	33.59±1.93	8.85±0.97
	11-13	63.71±2.02	4.07±0.67*	37.56±1.71	28.61±0.85	10.80±1.44*
	7-8	65.17±2.59	10.02±2.15*	35.41±1.87*	33.40±1.40*	10.63±1.21*
ARS grade 2 & 3	9-10	65.86±1.64	10.96±1.81*	35.60±1.29*	33.52±1.47	10.16±0.77*
	11-13	62.83±2.33	5.21±0.68	33.34±1.85*	31.88±1.64	10.22±1.00*

[†] — non-confirmed ARS, see chapter 4; * — significant differences with the control group, $p < 0.05$.

A complex of methods, including DNA-cytometry, automated fluorescent activation assays to mitogens and different antigens, blast transformation, ^3H -thymidine incorporation assay, index of migration inhibition was applied for the estimation of immune function in the ARS recovery period (table 8.4).

Table 8.4. Immune cells response to activation stimuli in ARS survivors in 24-36 months after the irradiation

Cell proliferation induction	Stat. parameters	Percentage of positive cells in tetraacycline incorporation test	Blasts percentage in transformation test	Index of stimulation in ^3H -thymidine test	Index of migration inhibition
PHA	M	25.21*	14.73*	41.34*	0.668*
	sm	1.24	1.34	2.52	0.043
Cortisol	M	4.80	2.60*	8.17*	0.897
	sm	0.81	0.23	0.86	0.031
Staphylococcal antigen	M	19.61*	13.96	27.22*	0.690
	sm	0.33	2.72	1.90	0.048

* — statistical difference with $p < 0.05$ as compared to control group

DNA cytometry study showed that majority of unstimulated lymphocytes were presented by G_0+G_1 cells. The amount of hiperdiploid cells was lower in comparison to control and other groups of clean-up workers with lesser doses (Fig. 8.3-8.5). A biological value of this effect in the recovery of immune system after a radiation injury is ambiguous. The decline of mononuclear cells number in the radiosensitive phases of cell cycle both could represent effects of dose and have a protective and adaptation value at system level.

Cell activation studies demonstrated high spontaneous expression of activation T-cells antigens, and a decline of the mitogen-induced reactions, that was typical for ARS-NC and ARS grade 2-3 patients. These conformities repeated at the study of percent content of cells (Fig. 8.6, 8.7).

Study of CD4^+ cells expressing IL-2 receptor, showed an increase of their number

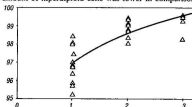


Fig. 8.3. Percentage of peripheral blood G_0+G_1 mononuclear cells in ARS survivors according to the grade of disease (1-3)

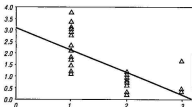


Fig. 8.4. Percentage of peripheral blood S-phase mononuclear cells in ARS survivors according to the grade of disease (1-3)

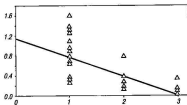


Fig. 8.5. Percentage of peripheral blood G_2+M mononuclear cells in ARS survivors according to the grade of disease (1-3)

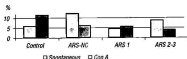


Fig. 8.6. Percentage of $CD3+HLA-DR+$ cells after concanavalin A stimulation



Fig. 8.7. Relative amount of $CD4+25+$ lymphocytes after concanavalin A stimulation.

Table 8.5. Distribution of ARS patients by cytogenetic doses

ARS grade	Doses of irradiation in 1986 (Sv)		
	Mean	Minimum	Maximum
ARS-NC	0.53	0.25	1.43
ARS-1	1.75	0.46	4.0
ARS-2	1.87	0.27	3.40
ARS-3	5.60	5.40	6.0

after exposure (Fig. 8.8). In convalescents of ARS grade 2-3 increased numbers of TCR-mutant cells were shown 20 years after exposure and more (Fig. 8.8).

In population of $CD4+$ cells two groups were analyzed: $CD4^+8^-$ cells were determined in a reduced number with increasing of absorbed dose. The $CD4^+8^-$ cell counts were increased and differences between the groups of exposed over 0.25 Gy with a group of persons exposed in occupational limits, were significant. This subset was described

in ARS-NC patients. In ARS grade 1-3 survivors the spontaneous indices of $CD4^+25^+$ cells did not differ from control; the same was shown after Con A stimulation.

Analysis of the immune function parameters was performed in relationship to the dose of exposure. The doses in ARS survivors, who were subject to immunological studies is presented in the Table 8.5.

Dose dependency was observed for $CD3^+DR^+$ cells and to a lesser extent for $CD4^+$ lymphocytes. Reduced expression of CD3 receptor was crucial for the formation of response to exposure and the rate of immune system recovery.

Cell progeny of T-lymphocytes variant by T-cell receptor (TCR-mutants) have a low density of functional receptor-active surface. In studies of patients with ARS survivors and clean-up workers exposed at low dose, we have determined a correlation between the number of TCR-mutants and proliferative activity, in response to mitogens — PHA and Con A; and biologically active compounds as cortisol, adrenaline, 5-hydroxytryptamin. Despite the negative selection of TCR-mutants *in vivo* due to the half-life of 2 years, the effects remained registered 10-15 years

earlier in different immunodeficiency's and was known that transition through the stage of the simultaneous expression of these antigens was one of the stages of cell differentiation in thymus, so likely that $CD4^+8^+$ cells belonged to subpopulation recently migrated from the thymus.

Reserves of proliferative potential of immune cells after the ARS seem to be satisfactory to support the general tendencies of recovery at the early period after exposure. The compensatory cellular proliferation and bone marrow restitution were mediated, at least, by three factors – a speed of cell division, cell loss and cell cycle time. Cell cycle time is shorter and it seems to be one of the important factors of immune cells response modification and possibly present a preparatory reaction to the next exposure. At shorter cycle time between divisions a lesser dose can be accumulated, that is a necessary factor limiting subsets radiosensitivity on the whole and effective negative selection of mutant cells.

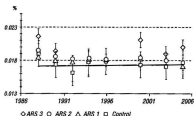


Fig. 8.8. Mean numbers of TCR-variant lymphocytes in ARS survivors divided by degree of disease during the follow-up. A black solid line marks control values

8.3 LATE PERIOD AFTER IRRADIATION

In the late period the group parameters of the major regulatory subpopulations of T cells had a tendency to normalization. The number of T cells did not differ significantly from control values in all patients; after ARS grade 1 even a tendency to increasing the number of $CD3^+$ cells was shown (Fig. 8.9). Noteworthy that after ARS grade 1 the restitution of total T cells counts was due to $CD4^+$ subset, whereas after ARS grade 3, the restoration of the $CD4^+$ cells number did not occur and the average remained significantly lower (Fig. 8.10).

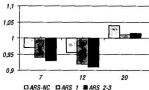


Fig. 8.9. Dynamics of mean peripheral blood $CD3^+$ cell counts in ARS survivors, depending on disease grade. Data are presented as a ratio to control values, taken for 1

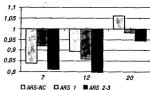


Fig. 8.10. Dynamics of mean peripheral blood $CD4^+$ cell counts in ARS survivors, depending on disease grade. Data are presented as a ratio to control values, taken for 1.

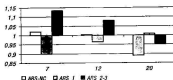


Fig. 8.11. Dynamics of mean peripheral blood CD8⁺ cell counts in ARS survivors, depending on disease grade. Data is presented as a ratio to control values, taken for 1.

CD8⁺ cells number varied according to other regularities (Fig. 8.11). If after ARS grade 1 and ARS grade 3 there was a gradual reduction in the number of cells that becomes significant within 20 years after exposure, in the ARS grade 2 convalescents we have recorded the opposite trend. The analysis is being continued.

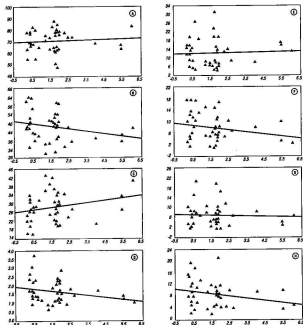


Fig. 8.12. Regression analysis plots of the dose-effect dependencies for main immune cell subsets in ARS survivors 20 years after radiation exposure. X axis — dose (Sv), Y-axis — cell percentage.

To study the influence of dose on immunological indices in a late period after high dose irradiation the analysis was conducted in 56 ARS survivors (ARS-NC — 21; ARS grade 1 — 22; ARS grade 2 — 10; ARS grade 3 — 3). The results of the performed regression analysis showed that in a late period some dose effect tendencies have been continued to occur — the negative correlation between dose vs. $CD4^+$, $CD19^+$, $CD3^+$ HLA-DR $^+$ cell counts and $CD3^+4^+/CD3^+8^+$ ratio (Fig. 8.12 B, D, E, H). The dependency with $CD8^+$ cell counts was of more complicated type (Fig. 8.12 C) with a general tendency of increase with dose. For the basic indices of humoral immunity no dose dependence was determined, with an exception of increased IgA concentration.

Despite the complexity and diversity of the immune system reactions the results of the ARS patients follow-up indicate at the remote period after exposure a certain exhaustion of the cell subsets compensatory abilities, still depending on the radiation dose. However, any results could be influenced by positive selection, i.e. a possibility to analyse data in survivors. It was demonstrated for peripheral blood mature cells as well as for progenitor cells. For a subset of $CD34^+$ cells in total a significant correlation was found and r -value reaches -0.48 . This conclusion was confirmed by the analysis of the number of the $CD34^+117^+$ subset of stem cells and $CD123^+$ early progenitor cells, shown in Fig. 8.13 (A, B, C).

Similar results were got for expression $CD117$ and $CD123$ antigens, which matter in forming the immune response.

8.4 CONFOUNDING FACTORS

Influence of a radiation factor on the human body causes a number of adaptive-compensatory reactions, the most important of which are changes in the central and autonomic nervous system, metabolism, hormonal regulation. Changes in the immune

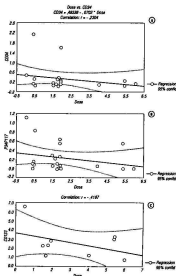


Fig. 8.13. Regression analysis plots of the dependence between the number of early haemopoietic progenitor cells and a dose of irradiation in ARS survivors 20 years after the exposure.

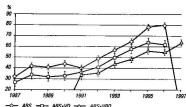


Fig. 8.14. Dynamics of CD3⁺ lymphocyte counts changes in ARS survivors due to the presence of accompanying vascular dystonia (VD) and vascular brain disorders (VBD)

system can be regarded as inseparable part of this ongoing processes of adaptation, which are registered even in the range of large doses. Mechanisms of neuron-immune interactions are described below. The results of follow-up of ARS survivors are shown in Fig. 8.14., and suggest that the presence of concurrent neurological disorders during the first years after the exposure resulted in impaired recovery of T-cell compartment.

Further, this effect could not be traced due to the high prevalence of vascular brain pathology and associated psycho-neurological disorders in that group of patients.

8.5 IMMUNE FUNCTION AFTER THE MIDDLE AND LOW DOSE RADIATION EXPOSURE

Until now, no clear determination exists of ionizing radiation dose, which should be considered as low. Experts in the field of acute radiation pathology have defined low dose as the maximal dose that does not cause a specific response, such as bone marrow hematological syndrome, so is below 1 Gy. In nuclear emergencies such dose was considered as 0.25 Gy, which was used earlier as a dose limit for emergency radiation workers of Chernobyl in 1986 and in Fukushima in 2011. However, this limit, providing some safety from the view of radiation protection, is hardly substantiated with the regards to the low-dose effects described in the immune system during the last twenty-five year period. Other authors have defined as a low dose an absorbed dose of 0.1 Sv or less in a short period of time, or 0.5 Sv for a long time [6]. Similarly, from a standpoint of immunology it has been proposed to consider as a low dose such that did not cause significant changes in the immune system – 0.04–0.05 Gy [7]. The ICRP defines low doses, as less than 100 mSv. In the most recent UNSCEAR documents the low doses are defined as the doses of 200 mSv or less, and dose rates as 0.1 mGy·minute⁻¹ or less for radiation with low LET value (UNSCEAR, 2006). In the latter report an up-to-date evaluation the effects of ionizing radiation on the immune system is presented. The UNSCEAR considers the immune system as a modifier of cancers risks and concludes an impossibility at a current stage to get a clear answer, whether immune function is enhanced or weakened in the range of low doses and how it changes the ability of a human body to resist cancer. At the same time it is recognized the accumulation of substantial new data on nontargeted and late effects of low doses.

Experimental data also exist in mice on the activation of immune function under chronic influence of low dose gamma-radiation. In contrast, an excessive damage to the immune response is described in mouse-models autoimmune pathology under the

influence of chronic low-dose radiation. It remains unclear how these data can be extrapolated to humans including not only purely cellular response to radiation, but also changes of the microenvironment. The UNSCEAR suggests as a key direction the stem cell research with the use of modern analytical approaches, cell kinetics and the micro-environment. So far, according to international experts, the existing data on the impact of radiation in low doses on the immune system, and on dependencies of «dose-effect» type are not consistent and clear [8].

8.6 EARLY PERIOD OF RECOVERY AFTER IRRADIATION

Investigation of ionizing radiation effects after low-dose exposure in the early period was performed in several groups irradiated with different range of doses and duration of exposure — the clean-up workers of the Chornobyl accident, evacuees from Prypyat and 30-km zone of Chornobyl, adults and children living at the territories contaminated after fallout, staff of the 30-km zone of Chornobyl. These results are summarized in the monographs «Chornobyl disaster» (1997), «Chornobyl nuclear power plant-Slavutych: Medical aspects» (1996), the second volume of the monograph «Health effects of the accident at the Chornobyl nuclear power plant. (vol.2: Clinical aspects of the Chornobyl disaster (1999), «The effects of low and very low doses of ionizing radiation on human health» (2000), «Chornobyl: The Exclusion Zone» (2001), and other publications [9–11]. In the monograph of 1999 the influence of ionizing radiation on the neuro-immune interaction during recovery of the immune system was discussed. The present section considers the dose and temporal patterns of changes in immune function.

During 1987–1990 period study group included 3010 clean-up workers of 1986–1989 (Table 8.6).

Methodological approach and limitations of the early period of study

Research approach for study of the low doses effects included ensuring the homogeneity of study groups (exposed vs. comparison vs. controls), the same level of medical follow-up for the elimination of the screening effect, using, whenever possible, one source of information on radiation doses. For analysis mainly official doses specified in the Ministry of Public Health Form N-1 were used. Restrictions for radiobiology research include the lack of previous data on the analysis of the immune function and the uncertainty of official doses. For control groups the difficulties consisted in inability to select individuals, free from irradiation and concurrent diseases, according to the age characteristics and lifestyle. For control group the available opportunities were used. We examined healthy volunteers — residents of Kyiv, who were blood donors, have not participated in clean-up activities and not living on contaminated territories. Individual dose of external radiation and thyroid dose for residents of Kyiv from the control group were not considered. Group of comparison included the rural population of the Poltava region. While conducting research in parallel with the IAEA group (1990) the proposed approach of international group to inspect the near located settlements with different levels of radioactive contamination was applied. Analysis of the clean-up workers immune function data at that time was conducted by groups in accordance to limits of exposure

approved by the USSR Ministry of Public Health. Clean-up workers irradiated in doses less than 0.05 Gy were selected as an internal comparison group (Table 8.6).

The Chernobyl experience shows that recovery of the immune system depended on the dose of exposure. At low dose a dose dependence was set for CD3⁺DR⁺ cells and, to a lesser extent, for CD4⁺ lymphocytes. The correlations were demonstrated between the number of TCR-variant cells and proliferative activity, in response to PHA, Con A, cortisol, epinephrin, 5-hydroxytryptamin.

Table 8.6. Percentage of main subsets of lymphocytes in Chernobyl clean-up workers in the recovery period of 24-36 months after the exposure in dependence on the dose of irradiation

Cells by CD	Percent of cells which expressed antigens, depending on the dose of irradiation, Gy					
	0.0-0.04		0.05-0.24		0.25-1.0	
	M	sm	M	sm	M	sm
CD7 ⁺	64.8	3.6	56.2*	3.9	49.7*	4.4
CD5 ⁺	44.2	4.5	33.7*	5.0	33.9*	2.7
CD3 ⁺	55.4	2.2	48.1	2.6*	41.6*	4.7
CD3 ⁺ Dr ⁺	46.5	2.8	30.6*	3.9	27.5*	5.1
CD3 ⁺ Dr ⁺	5.8	0.6	11.7*	1.6	10.0	4.2
CD4 ⁺ 8 ⁺	36.3	2.3	32.9	6.8	20.5*	4.1
CD4 ⁺ 8 ⁺	1.1	0.2	1.3	0.2	3.7*	0.4
CD4 ⁺ 8 ⁺	23.5	3.5	18.5	3.2	25.2	4.1
CD38 ⁺	13.0	2.6	13.9	1.3	16.2	1.9
CD11b ⁺	11.5	1.4	16.2*	0.8	18.1*	2.1
CD16 ⁺	11.0	1.3	8.5	1.1	10.2	1.3
sIg ⁺	10.6	0.14	17.1*	1.0†	17.3*	0.92
CD19 ⁺	8.3	0.5	10.2	0.9	12.6*	0.5
CD3 ⁺ Dr ⁺	7.9	2.0	13.1	2.1	9.2	2.4
CD23 ⁺	10.9	2.0	5.7*	0.6	8.1	1.6

*signif/corr difference, $p < 0.05$

The decrease was demonstrated of the mean counts of the T-lymphocyte main subsets (Fig. 8.15). Group values showed dose dependency, however a decrease in number of cells that expressed the earliest T-cell CD7 differentiation antigen was the smallest. The number of cells that express CD5 antigen, changed in parallel to content of CD3⁺HLA-Dr⁺ lymphocytes, but not CD3⁺HLA-Dr⁺ cells. The cells of CD4⁺8⁺ phenotype were determined in a reduced number with increasing the absorbed dose. Number of CD4⁺8⁺ cells was increased and group values difference to a comparison group was significant in doses exceeding 0.25 Gy.

Studies have shown heterogeneity of cytotoxic cell response. The number of cells that expressed CD38, had not changed and tended to increase. Designated was a threshold type of the changes of cytotoxic CD11c⁺16⁺15⁺ lymphocytes. In patients with doses of 50-250 mSv the number of these cells was declining, at higher doses — not different from normal. Marked reduction was demonstrated for CD8⁺57⁺ and CD8⁺57⁺ lymphocytes.

According to the results of DNA flow cytometry an instability of cells traffic through the different phases of the cell cycle was observed (Fig. 8.16). In the range

of doses up to 250 mSv 24–36 months after the exposure an increased numbers of mono-nuclear cells were shown in S- and G_2 +M phases. The growth of cell population heterogeneity was demonstrated by increased values of mean-square deviation and coefficient of variation indices. After irradiation at doses greater than 250 mSv, the presence of nonreplicative DNA synthesis with blocking the cell cycle and G_2 - or G_1 -block was demonstrated. The frequency of changes also depended on some threshold: G_2 -block was determined at doses above 250 mSv, G_1 -block — at doses exceeding 1 Sv.

The registered variability could result from a different sensitivity to irradiation and recovery efficiency of various subsets of T- and B-lymphocytes. It was found that reducing the number of T-cells that express CD5 was not accompanied by activation processes and the increasing of cells in S- and G_2 -M phases. In the CD57⁺ population with a high density antigen an accumulation of cells that entered the proliferative cycle was registered at doses lower than 250 mSv, and at doses exceeding this threshold the effect was not observed. The slg⁺ B-cells differed from the other by an elevated number of lymphocytes being in the proliferative cycle. At higher doses the increased average numbers of B-cells were accompanied by increasing of cells number in G_2 and M-phases, without changing the number of cells in S-phase.

In staff of the 30-km zone of Chernobyl working by a shift method a higher content of cells in the phase of DNA synthesis and mitosis was detected (Table 8.7). An accumulation of cells in S-, G_2 - and M-phases of the cycle was characteristic for NK and B-cells, mean cell cycle parameters of T lymphocytes did not differ from control. The identified patterns could provide evidence of differences in cell lineage radiosensitivity, and presence of compensatory and adaptive type reactions of NK cells. B-lymphocytes, to our opinion, are in a state of activation, as evidenced by increased expression of differential antigen, surface immunoglobulin and promotion of cell cycle. These findings are consistent with those obtained by ³H-thymidine incorporation assay. More extensive

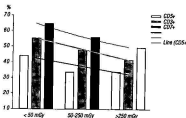


Fig. 8.15. Dose dependences of CD5⁺ T-cell counts through 24–36 months after irradiation (n=2322)

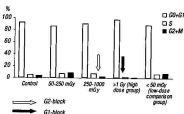


Fig. 8.16. Lymphocyte distribution by the phase of cell cycle in Chernobyl clean-up workers and staff of the exclusion zone (low-dose comparison group) 24–36 months after irradiation (n=338).

intake of ^3H -thymidine dominated in examined with the higher absorbed doses. Age and gender analysis have not shown any reliable differences.

Table 8.7. Peripheral blood lymphocytes DNA synthesis by ^3H -thymidine intake in staff of 30-km Chernobyl exclusion zone 36 months after start of work by shift method

Staff distribution by external doses (%)		Intensity of DNA synthesis (number of impulses) after stimulation of PHA in concentrations		
base-line	from base-line to upper professional limits	Statistical parameters	50 µg/ml	100 µg/ml
50.0	50.0	M	6.918	10.548
		sm	1.270	1.287
42.8	57.2	M	53.603	79.139
		sm	5.436	5.408
40.0	60.0	M	105.785	158.690
		sm	11.921	12.743
16.7*	87.3*	M	208.705	287.541
		sm	30.861	20.451

*significant difference, $p < 0.05$.

Study of exposed shows that the effectiveness of recovery mechanisms of cell subsets depended on the dose stepwise, which was likely due to the different sensitivity of compensatory mechanisms. Data on some contributive mechanisms revealed at the early period are presented in Table 8.8. Thus, the release of the immature $\text{CD4}^+\text{8}^+$ T-cells to the peripheral blood with concomitant decreased number of mature $\text{CD3}^+\text{4}^+$ cells became a substantial factor in the dose range above 250 mSv ($p < 0.02$). T-cell activation, by contrast, was depressed. Expression of CD25 antigen (interleukin-2 receptor) was increased, starting in the dose range from 50 mSv, but not accompanied by increased expression of T-cell class II major histocompatibility complex antigens, which was an essential factor of activation and promotion of cells to G_2 and M phases of cycle. Last recovery mechanism seems to value as a defensive and compensatory, at doses greater than 1 Sv.

Table 8.8. Variation of some T-cell indices in Chernobyl clean-up workers

Immune parameters	Index of parameter change as a ratio to control at different dose ranges			
	<50 mSv	50–250 mSv	250–1000 mSv	>1 Sv
Increase of $\text{CD4}^+\text{8}^+$ lymphocytes number in peripheral blood	1.22	1.44	4.11	2.5
Number of activated CD3^+ HLA-DR $^+$ lymphocytes	0.34	0.67	0.59	0.92
Number of CD25^+ cells	0.86	2.45	1.56	0.76

8.7 MODIFICATION OF IMMUNE FUNCTION AT LOW DOSES

Although the majority of clean-up workers especially in younger age groups provided sparse information on the diseases before clean-up and the clinical hematological counts were in a normal range the immunological analyses in the majority had not been performed before. After the exposure the different rates of the immune system recovery

were observed in individuals with pathology of the early period. We performed a comparative analysis of the immune system restoration in the period from 6 to 32 months after irradiation in 872 clean-up workers. In healthy T-cell counts increased during this period from 38.2% to 50.3%, while in patients with pathology of the digestive tract — to 42.1%, vegetative-vascular disorders — to 41.8%. In a series of further studies a number of humoral factors modifying the immune response were revealed, as cortisol, epinephrine, 5-hydroxytryptamin, leu-enkefalin, interleukins (IL) 1, 2, 4, 10, alpha- and beta-endorphins, VEGF. In clean-up workers with cerebrovascular diseases and Th₂ type of response domination, the *in vitro* interleukins 4 and 10 lead to paradoxical activation of Th₁-cells. The oploid receptor agonists in doses up to 0.5 Gy inhibit the IL effects, and after doses higher than 0.5 Gy a potentiation effect was shown. Modifying influence of metabolites of arachidonic acid was shown: an inclusion of 12-HETE to lymphocyte membranes was associated with high CD4⁺ and low CD8⁺ counts, and the association of high content of 12-HETE with a low number of cytotoxic and natural killer cells, that can act as a factor for promotion of cell transformation [12].

As a result of the follow-up immune function studies it was, therefore, possible to formulate the main factors in the range of low doses effects:

- radiation-induced non-lethal damage of cells (proliferation of functionally defective offspring; spreading effect due to humoral factors);
- modification of the immune response (neuro-immune factors, lipid metabolism accompanying pathology);
- system adaptive response (transition to the radioresistant cell cycle phases; threshold type of dose-response recovery reactions including immature cells; nonspecific activation).

8.6 EFFECTS IN DOSES WHICH DO NOT EXCEED THE PROFESSIONAL LIMITS

The results of the last low-dose studies testify the necessity in critical revision of some for years not changeable positions. At first, the presence of adaptive response demonstrates that cells recognize an ionizing radiation, as a stress stimuli and answer to it at genetic and epigenetic levels, including the changes of genes expression. These changes can result in violation of normal processes as well as in an increased resistance to the next irradiation. The quantitative gene studies supported the suggestions on a possible protective influence on the development of remote effects of irradiation. Thus, a bridge overturns between the action of extremely low doses and tumor induction while a nature of this interconnection remains disputable.

In several experimental studies an attempts was done of modelling the chronic low-dose exposure using the cell lines from which more frequent used were fibroblasts. On human tumor lines Hela S3, Hela SB and Jurkat a linear response was demonstrated during 48 hours after irradiation in a dose of 0.5 Gy with low intensity 3.75 mGy/min., with the involvement of caspase-3 [13]. A linear specificity of answer during 48 hours after irradiation was set, which was related to low activity of caspase-3. These studies served as a basement for planning long-term study on the influence of radiation exposure on tumor and normal cells. Such research has been performed with a 1¹/₂

year cell culture exposure to gamma-radiation inside the Chernobyl "Shelter" and to a radionuclide mixture from the fallout to the areas adjacent to Chernobyl reactor (so called "Red Forest"). The study data show higher adaptation potencies of the normal immortalized cells in comparison with tumor lines [14]. These results were used at the implementation of the regulations of professional health control for staff of the Chernobyl exclusion zone.

To study in humans the initial changes caused by low-dose exposure we have conducted a comparative analysis of immune function parameters in 255 construction workers of new Chernobyl confinement at entry to the radiation zone, within 1–3 and 4–6 months. Personnel was exposed to low-dose chronic irradiation during shifts to the exclusion zone followed by two-week periods of no exposure. Doses of irradiation were provided by the RCRM Department of Dosimetry (head- prof. I. Likhtarev) and in all inspected were in professional limits (Table 8.9).

Table 8.9. The doses of irradiation of inspected personnel

Type of exposure	Individual doses, mSv:	
	minimum	maximum
External	2.1	13
Internal	0.2	1.1
Total	2.4	13.8

Basic parameters included T-cellular immunity and natural killers: CD3, CD4, CD8, CD16, CD45, CD56, HLA-DR, TCR-variant cells; B-cells: CD19, HLA-DR, humoral immunity — immunoglobulins IgA, IgM, IgG, circulatory immune complexes, specific immunity — HBsAg, antibodies to HCV, HSV 1+2, cytomegalovirus (CMV) and *Toxoplasma gondii*. In 34 subjects the

CD34⁺ stem cells and early progenitors were studied together with FAS antigen, regulatory proteins p53, bcl-2; local cellular immunity — phenotype of broncho-alveolar lavage cells.

During first 3 months of irradiation no increase of radiogenic mutations number was found in the locus of T-cell receptor. At the later periods (4–6 and 12 months) an increase was demonstrated of the mean group values. No dose dependency was identified. In 3 subjects with the incorporation of ¹³⁷Cs a five-fold increase of TCR-CD4⁺ cells count was shown. Studies of a possible relationships between the dose of internal and external irradiation have not demonstrated any substantial results except of a "dose-effect" type of the T-lymphocytes counts changes (Fig. 8.17). However, all the counts remained within the normal limits.

For a number of parameters the differences were detected on a group level with the absence of dose dependencies. The decreased apoptosis, increase in the number of NK-cells and monocytes as well as lymphocyte activation in peripheral blood and in broncho-alveolar lavage, increase the number of CD34⁺117⁺90⁺ early hemopoietic precursors were found. An increased expression of nuclear antigen PCNA, anti-apoptotic bcl-2 activation in the test with Con A and the reduced p53⁺ CD95⁺ cell fractions were demonstrated with the increased expression of CD117 antigen (C-kit) on early progenitors and ZAP-70 on T-cells. CD117 gene encodes a transmembrane receptor tyrosine-kinase that is essential for early hemopoiesis. These results coincide with data showing the signal quality of antibody and antigen receptors depending on tyrosine phosphorylation-based activation (ITIM) and SH2-tyrosine-kinases. Inhibition of protein tyrosine kinase-ITIM-receptor mechanism is a common strategy to control cell activation and other hemopoietic

cells. Increased expression of tyrosine kinases on-stage differentiation of T-lymphocytes may enhance the activation process.

Protein-tyrosine kinase ZAP-70 with mass of 70 kDa is involved to the control of the early stages of signal cascade system of T-cell receptor. Phosphorylation of ZAP-70 is connected with stimulation of CD3 receptor. ZAP-70 is usually expressed on thymocytes, mature T-cells and natural killers, its absence results in the syndrome of a severe combined immunodeficiency with blocking of T-cells development at the stage of CD4⁺CD8⁺ thymocytes. Such process could result in an increase of CD4⁺8⁺ cell counts in peripheral blood described in radiation-exposed.

There is experimental evidence for a link between tyrosine kinases and early hemopoietic progenitors. Intrathymic injection of progenitor cells to ZAP-70 deficient SCID mice leads to increased numbers of peripheral T cells, increase the percentage of naive T cells and expansion of the T-cell receptor repertoire. It is likely that the registered changes as activation of T-cells with increased expression of CD117 and ZAP-70 present a single complex that reflects the response to radiation in a specified low-dose range. One key element is the 1,2-diacylglycerol (DAG), which together with Ca²⁺ and phosphatidylserine activate protein kinase C. DAG hydrolysis is necessary for the synthesis of eicosanoids, including 12 HETE, which regulate T-cell function and is involved in radiation aging. Joint research of the Institute of Biochemistry NAS of Ukraine, the RCRM and the Laboratory of Biochemistry and Pharmacology U352, INSERM (France) demonstrated the association of free 12-HETE content and its inclusion in the membrane of lymphocytes CD3⁺8⁺ with a threshold value of 250-300 mSv at the period of 15 years after exposure [12]. Possibly a reduced DAG hydrolysis switches the changes at doses below the specified threshold, however the threshold could be influenced by the intensity of the immune function recovery. The result is the balance between an increased anti-tumor control and preservation of mutations by reducing apoptosis. The endpoint with a regard to multi-stage tumor induction process remains at present unclear.

The study shows that immune system reacts to radiation exposure in doses not higher than the professional limits by a set of differentiation and activation changes including an increase of number of early hemopoietic progenitors, cytotoxic cells activation, enhanced expression anti-apoptotic proteins and decline in efficiency of apoptosis. These changes are related and could be explained by increased expression of protein-tyrosine kinases at the different stages of differentiation.

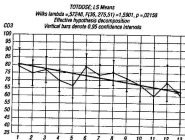


Fig. 8.17. Dose dependence of CD3⁺ lymphocytes count of "Shelter" object personnel: x-axis — radiation dose (mSv), y-axis — percent of CD3⁺ cells

The revealed certain dependencies, except of the relationship between dose of exposure and CD3 antigen expression, correspond to the experimental data, obtained in cell lines, that allowed to forecast adaptation of immune function of personnel and existence of possible threshold value of 100 mSv. Possibility of changes in balance of mutation rates vs. anti-tumor control could depend at this range of doses on the influence of additional factors.

8.9 EFFECTS IN THE IMMUNE SYSTEM AT THE REMOTE PERIOD AFTER IRRADIATION

Research on the late effects in the immune system included the quantitative estimation of radiogenic mutations, studies of immune function and influence of confounding factors. From radiation markers at the late period we have studied somatic mutations in Glycophorin A (GPA) and T-cell receptor (TCR) loci.

GPA is present on erythrocytes in amount $5 \cdot 10^5$ per cell, is coded at 4q and it is determined as two allelic forms, according to M and N blood groups. Unlike variant lymphocytes, the red blood cells variant by GPA, do not pass selection *in vivo*, GPA mutation does not change life-span of red blood cells and is not associated with any clinical changes. Genotoxic effect is registered from erythroblast stage, it can be a marker of damage of early progenitors of red blood cells, including the polypotent stem cell. The assay was performed with analysis of 10^6 cells by two-color cytometry in Chernobyl clean-up workers, who were heterozygous by MN. Cells were stained with M-specific monoclonal antibody (moAb) 6A7B and N-specific moAb BricCF1. Detailed protocol was published previously. A satisfactory correlation was shown with the results of other methods of biological dosimetry. In healthy individuals from control group studied subsequently from 3 to 5 times a mean frequency of mutations (M+SD) by variant hemizygote N0-erythrocytes was (12.3 ± 4.8) per 10^6 cells. This data are compatible with results obtained in the RERF. In Table 8.10 the results of comparative analysis of official doses by physical dosimetry with doses by dicentric and GPA-doses are presented.

Table 8.10. Results of comparative analysis of mean doses calculated by GPA test, dicentric and physical dosimetry

Dosimetry method	Median dose of groups, Gy				
	M	Minimum	Maximum	$\pm$ SD	$\pm$ T
Official dose	0.43	0.21	1.42	0.33	0.09
Dose according to the method of chromosomal aberrations	0.46	0.000	1.50	0.40	0.11
Dose by definition GPA-mutants	0.47	0.19	1.10	0.32	0.09

Studies of the individual parameters have shown weak correlations between the number of mutant erythrocytes, official radiation dose and number of dicentric (Fig. 8.18). Survey of Chernobyl 30-km exclusion zone staff showed the increased frequency of mutations up to 8,9–23 per 10^6 cells. The method is enough high informative for determining exposure, or biological indication. The limitation of this assay was

a possibility to study only MN-heterozygote individuals, who represent from $1/4$ to $1/3$ of population.

Research of mutations in a locus of T-cell receptor/TCR gene showed that for Chernobyl clean-up workers with the doses of more than 500 mSv the dose dependent effects in the immune system were observed even through 15 and more years. In a period after 2000 the number of variant cells also exceeded mean figures for control group, but a difference was not reliable (Fig. 8.19).

Contrary to other radiation markers like GPA or chromosome aberrations the experimental studies with cloning of variant TCR-cells and analysis of their progeny have demonstrated relationship of TCR mutations with impaired immune function [15]. We conducted the analysis of correlative dependences between the number of variant T-cells, content of CD3⁺ cells and expression of CD3 receptor. In a late period such dependence was not found, with an exception of group of patients who have survived after grade 3 acute radiation syndrome. At chronic irradiation in doses which not exceed professional limits, such dependencies were not shown. However, in workers with increased variant TCR-lymphocyte counts a number of T-cells was significantly lower but within the normal range.

Studies of the immune system performed in patients at the late period after exposure have demonstrated stable radiation-induced changes of cellular and humoral immunity, that can contributive to the onset and progression of somatic pathology; the possibility of the use of immunocorrection and immunomodulation for prophylactic

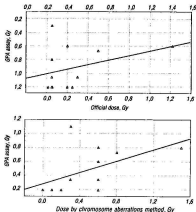


Fig. 8.18. Individual relationship between GPA and cytogenetic dose: x-axis — dose by chromosome aberrations method, Gy; y-axis — GPA assay data, Gy

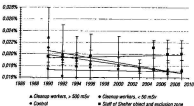


Fig. 8.19. Changes in number of TCR variant lymphocytes in exposed at low doses

reasons in exposed with acquired immune deficiency; the contributory role of CMV, Epstein-Barr virus (EBV) as well as hepatitis B and C viruses in the groups of persons with the formed immunological violations, determination of nosotropic value of transmitter of viruses of hepatitis B, C, cytomegaly in modification of immune function. Important part of studies was research of the mechanisms of radiation-induced leukemia and myelodysplastic syndrome.

Follow-up studies of the immune function in patients with vegetative dysfunctions (VD) of early period and cerebrovascular diseases of the later period after radiation exposure bridge the neurological disorders and changes in the immune system. In patients with VD in the period of recovery after radiation damage the violations of neuro-humoral regulation and expression of receptors for neuropeptides, messengers of neural signals and hormones, and hypersensitivity to brain tissue antigens were shown. Attempts were made to describe a post-irradiation systemic dysregulation of homeostasis, with a key role of the immune system in the formation of neuro-immuno-endocrine abnormalities.

In a late period such relationship is less obvious. According to studies of immune-mediated brain pathology a set of pathways can be proposed including:

- 1) the secondary subset changes associated with disorders of the nervous-humoral regulation;
- 2) cytotoxic mechanisms with a switch of antigen-dependent and natural killer reactions, apoptosis through Fas (CD95) and Fas-ligand (CD95-L);
- 3) hypersensitivity immune complex and reagin type antibody and delayed type cellular reactions with an increase of hemato-encephalic barrier permeability;
- 4) an impaired lymphocyte regulation of a nervous system function by a release of lymphocytic neurotropic substances, accumulation or, to the contrary, decrease of consumption by lymphocytes of different neuropeptides (beta-endorphins, Leu-enkefalins, S-100 and others) due to receptor changes.

8.10 IMMUNE FUNCTION IN PATIENTS WITH CEREBROVASCULAR DISEASES (CVD) EXPOSED TO IONIZING RADIATION AS A RESULT OF THE CHORNOBYL ACCIDENT

The existing level of knowledge allows considering the contributive role of the immune function changes of the late period after irradiation to the cerebrovascular pathology pathways. Therefore, it is also actual taking into account an interaction between the immune and nervous systems. Encephalopathy ("radiation encephalopathy", "discirculatory encephalopathy"- DER, "demyelination" encephalopathy) according to medical statistics is one of the most common disorders of a late period after irradiation and one of the most frequent diagnosis for establishing a causal connection of disease with a radiation factor. At the same time, a nature of this disorder is not identified, and many researchers do not recognize it as an effect of irradiation [2]. In some studies it was depicted as a result of a direct brain tissue radiation damage [6], or as an indirect effect due to damage of vascular circulation [1]. Development of the brain pathology as a result of CVD and vegetative dysfunctions, and the substantial vasculatory changes revealed in encephalopathy patients provide support to a latter assumption.

Study of dose dependencies of the immune system parameters was conducted in CVD patients with available dose estimates. We used official data on doses from the SRU and doses from Chornobyl NPP dosimetry shop. Analysis of immunological parameters depending on the time of irradiation showed that at acute exposure and time after exposure greater than 10 years, the statistically significant differences were determined depending on the dose, not the time. Doses, the effect of which could be detected at this period were 0.25–0.35 Gy. However, for chronic exposure of staff of the 30-km exclusion zone, the major factor was the duration of work activity in the zone, and the threshold dose was reduced. Examination of clean-up workers with CVD has demonstrated in 42.3% of cases the decreased CD3⁺ and CD4⁺ cell counts in peripheral blood. Depression of cellular immunity was associated with the preservation of the increased variant TCR-cell counts.

The study of dose dependency and irradiation time showed that in 23.2% of patients in 11–13 years after exposure a stable depression of T-immunity was shown with a high degree of correlation of CD3⁺HLA-DR⁺ cell counts with radiation dose. The majority of these workers were exposed over 400–500 mSv. The second group included clean-up workers of the same period exposed below 250 mSv with prevalence of activation reactions and positive correlation between radiation dose and number of CD3⁺DR⁺ B-cells, the concentrations of circulating immune complexes and IgA. The significance of these changes in the formation of immunopathological syndromes of late period after irradiation was not definitively established.

Mean subset counts in groups of clean-up workers with CVD divided with a regard to dose of exposure are presented at Table 8.11.

Table 8.11. Percentage of main peripheral blood lymphocytes subsets in clean-up workers with cerebrovascular diseases 10–13 years after radiation exposure

Dose, Sv	Statistical parameters	Cell counts (%) by subset					
		CD3 ⁺ 19 ⁺	CD4 ⁺ 8 ⁺	CD8 ⁺ 4 ⁺	CD3 ⁺ DR ⁺	CD3 ⁺ 16 ⁺ 56 ⁺	CD3 ⁺ 16 ⁺ 69 ⁺
Over 0.5	M	61.91*	37.31*	26.52	4.04*	3.18*	7.12*
	±m	1.3	2.4	2.9	0.3	0.3	0.2
Below 0.5	M	64.92	35.96*	33.97*	5.7	5.49	9.48*
	±m	2.2	4.3	2.8	0.7	0.9	0.2
Control	M	69.87	48.03	26.16	7.25	7.26	15.10
	±m	2.4	3.1	2.9	0.9	0.7	0.7

*Difference to control with $p < 0.05$

Presented data show the existing significant differences related to a dose of exposure. In patients with doses over 0.5 Sv a decrease was demonstrated of the CD3⁺ cell counts due to a lesser number of CD4⁺ cells and also CD3⁺DR⁺ cells. In clean-up workers with doses below 0.5 Sv a similar tendency was accompanied by an increased number of CD8⁺ cells. The number of cytotoxic T-cells and the NK-cells was decreased in both groups. B-cells have shown absence of substantial changes. The number of CD19⁺ cells in exposed with doses below 0.5 Sv was $6.7 \pm 0.5\%$, over 0.5 Sv — $8.19 \pm 0.6\%$, in control — $8.45 \pm 0.17\%$; for HLA-DR⁺ B-cells the counts were accordingly $8.78 \pm 0.9\%$; $10.48 \pm 0.87\%$; $9.89 \pm 0.34\%$. Absolute counts have confirmed the aforementioned regularities (Table 8.12).

Table 8.12. Absolute counts of main peripheral blood lymphocyte subsets in clean-up workers with cerebrovascular diseases 10–13 years after radiation exposure

Dose, Sv	Statistical parameters	Lymphocyte counts ($\times 10^9/L$):					
		CD3 ⁺ 19 ⁺	CD4 ⁺ 8 ⁺	CD8 ⁺ 4 ⁺	CD3 ⁺ DR ⁺	CD3 ⁺ 18 ⁺ 56 ⁺	CD3 ⁺ 18 ⁺ 56 ⁺
over 0.5	M	1.27	0.79*	0.57	0.09	0.08*	0.16*
	$\pm m$	0.06	0.04	0.06	0.01	0.01	0.02
below 0.5	M	1.32	0.77*	0.70*	0.12	0.14	0.18*
	$\pm m$	0.07	0.05	0.04	0.02	0.01	0.02
Control	M	1.45	1.00	0.54	0.14	0.13	0.29
	$\pm m$	0.08	0.08	0.05	0.02	0.02	0.03

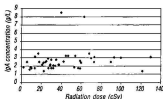
*difference to control with $p < 0.05$ 

Fig. 8.20. Dot plot of the IgA concentration.

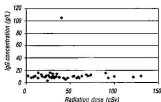


Fig. 8.21. Dot plot of the IgG concentration.

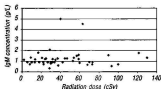


Fig. 8.22. Dot plot of the IgM concentration.

No relationship was revealed between radiation dose and the serum immunoglobulin concentrations (Fig. 8. 20–8.22).

The presented data show a presence of substantial changes of cell subsets in clean-up workers with CVD at the late period from 10 to 13 years after radiation exposure. At the group levels these changes have a maximal difference from the control parameters in patients with higher doses. These changes are consistent with the described at the period of recovery after radiation exposure and with a confidence could be referred as resulting from the radiation-induced changes of immune system of the early period. At the same time no substantial changes were found in humoral immunity showing its recovery and lesser influence of humoral mechanisms.

At the period of 15 to 20 years after exposure we have studied the immune function and influence of chronic somatic pathology in clean-up workers exposed to ionizing radiation. Research was carried out in 306 workers with doses exceeding 50 mSv (50–770 mGy) and the most common types of chronic disease of a late period (chronic obstructive pulmonary disease, chronic hepatitis, CVD). Analysis of immuno-

logical parameters have demonstrated that in acute exposure at the period after exposure greater than 10 years, the statistically significant differences depend on dose, not time. The threshold dose, the effect of which remains in the formation of long-term effects in patients with CVD, is 0.25 Sv. However, in chronic exposure at the 30-km zone, the major factor is the length of stay in the zone, and the threshold dose is reduced.

In the long-term period some changes were registered in immunity with a predominance of activation reactions, which probably indicate damage to the immune mechanisms. A substantial share of CVD pathways was a formation of hypersensitivity reactions, which were confirmed by cell activation by 5-100 protein and other brain antigens.

Study of the endorphines effects on lymphocyte proliferation was performed in 55 male clean-up workers of Chernobyl accident exposed at the doses range of 0.25–3.8 Sv (aged 36–69 years) and diagnosed CVD in comparison with 30 non-exposed CVD patients and 25 healthy volunteers. Physiological concentrations of α - and β -endorphins intensify the spontaneous and mitogen-dependent proliferation of T-lymphocytes (Fig. 8.23, 8.24). Intensifying effect of the opiate peptides was being blocked by naloxone. Obtained data demonstrated the unidirectional opiate action, however the β -endorphine

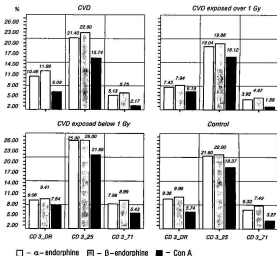


Fig. 8.23. Percentage of subsets of activated CD3⁺ cells after 18-hour culture with Con A and endorphins in radiation-exposed CVD patients in comparison with non-exposed patients and healthy controls

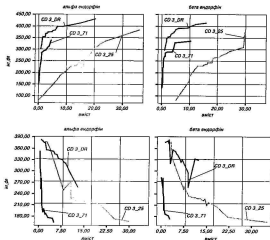


Fig. 8.24. Dependency plots of the fluorescence intensity of lymphocyte activation markers on T-cells of clean-up workers with doses 1–3.8 Sv after incubation with endorphins

effect dominated. The detected effects of endorphines were shown both among donors and patients (exposed to ionizing radiation at 0.25–0.9 Sv levels and non-exposed). The irradiation higher than 1 Sv resulted in disturbance of the endorphine reception by the T-cells with blockage of the influence of naloxone. That confirmed the presence of radiation effects in opiate regulation of the lymphocyte proliferation.

8.11 APOPTOSIS AND TELOMERE LENGTH

Modern achievements of radiobiology, molecular biology and genetics, allowed to offer few theories in relation of biological senescence of cells which partly complement each other, and partly cause contradiction. Molecular-genetic theories describe genome changes at aging as programmed, or – as a result of accumulation of mutations. We have studied apoptosis as one of the system mechanisms of stability of cellular populations and telomere length in forming of cell response to ionizing radiation. Shortening of telomeres is regarded as one of the important stages in cell senescence [16, 17, 18]. Telomere shortening after irradiation could decrease proliferative abilities and thus influence the elimination of cells containing double strand breaks (DSB) and, possibly, dicentric chromosomes, mediate p53 pathways. A result could be an error-prone recovery of immune cells. To the contrary, experimental studies have shown that adding of

telomerase to the genome of fibroblasts allows to elongate the life cycle [19]. Research on relative length of telomeres (RTL) was conducted in Chernobyl clean-up workers and staff of the Chernobyl 30-kilometer exclusion zone by flow-FISH assay. The analysis was performed in a FACS Calibur flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA) using the FL1 channel for detection of fluorescein signal and the FL3 channel for propidium iodide. No compensation was set on the instrument. List mode data from 10^4 cells in each experiment were collected and analyzed using CELL-Quest software (Becton Dickinson). The telomere fluorescence signal was defined as the mean fluorescence signal in G_0/G_1 cells after subtraction of the background fluorescence signal (i.e. FISH procedure without probe). The relative telomere length value was calculated as the ratio between the telomere signal of each sample and the control cell line (K562) with compensation for the DNA index of G_0/G_1 cells. This compensation was performed in order to "normalize" the number of telomere ends per cell. On Fig. 8.25 an RTL analysis is illustrated by flow cytometry in the "Dot plot" mode. Sample after hybridisation with peptide nucleic acid (PNA) probe-FITC, show a significantly higher fluorescent FL1 signal (Fig. 8.25 B), than base-line/autofluorescence signal from the cell samples, without PNA probe (Fig. 8.25 A).

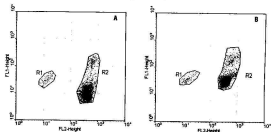


Fig. 8.25. Dot plot of the relative length of peripheral blood lymphocytes telomeres: A — dot-plot analysis: FL1/FL2 (hybridization cells without PNA); B — dot-plot analysis: FL1/FL2 (hybridization cells with PNA); R1 — experimental cells, R2 — control cells (K562 line)

Data are presented in Table 8.13. A tendency of decrease of the RTL is demonstrated in staff of the exclusion zone ($p < 0.05$). However the lowest RTL, in comparison to a control group is characteristic for clean-up workers of 1986–1987.

Table 8.13. Relative telomere length in peripheral blood lymphocytes of Chernobyl clean-up workers 1986–1987 and staff of the exclusion zone exposed to chronic irradiation at professional limits

Group	Parameters of telomere length		
	RTL, % (M $\pm$ m)	Relative ratio to control	p
Control	18.6 $\pm$ 0.73	1.0	
Clean-up workers	13.2 $\pm$ 0.69	-0.29	<0.001
Staff of the exclusion zone	15.5 $\pm$ 0.85	-16.6	<0.05

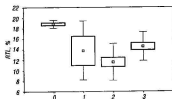


Fig. 8.26. Relative telomeres length in peripheral blood. x-axis — study groups: 0 — control; 1 — ARS survivors at the late period after exposure; 2 — Chernobyl clean-up workers at the late period after exposure; 3 — exclusion zone staff exposed under professional limits; y-axis — RTL (%)

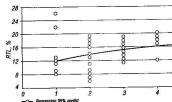


Fig. 8.27. Distribution of individual relative telomeres length indices in the study groups, depending on character of irradiation. x-axis: 1 — ARS survivors; 2 — clean-up workers; 3 — exclusion zone staff; 4 — control group; y-axis — RTL (%)

in thereby for the damage of DNA. Tp53 as oncosuppressor occupies one of key places in adjusting of cellular answer for the damage of DNA, by authors conducted research of lymphoblast lines of human: TK6 (wild type of Tp53) and WTK1 (mutant Tp53). For both lines a dose dependent 2.5-fold increase of telomerase activity was found one hour after X-ray irradiation in a dose of 4 Gy, while for dose interval from 0.1 Gy to 1 Gy an induction of telomerase activity was determined only for the TK6 line. Two weeks after an irradiation in TK6 and WTK1 cells a lengthening of telomeres was registered +1.3 kb, in comparison to the non-irradiated cells. In other study the telomere length was bound to enhanced chromosome radiosensitivity [21]. From other data the long telomeres, but not activity of telomerase are protective for proliferation potential at radiation exposure [22, 23].

Obtained data (Table 8.13; Fig. 8.25, 8.26) show the shortening of telomere length in humans without oncologic diseases exposed to low-dose irradiation, unlike the results in tumor cell lines. Possible causes include apoptosis, age and other contributing factors. The mean age in all studied groups was over 50 and did not differ significantly (Fig. 8.29).

Mean values for the studied groups (Fig. 8.26) demonstrate the decreased RTL in study groups with different doses of exposure.

Taking into account age heterogeneity of group data we present results of cross-correlation analysis of individual indices of RTL by the study groups showing a decline of the RTL with dose (Fig. 8.27).

The analysis of changes in RTL in staff of exclusion zone (Fig. 8.28) shows some dose dependency at dose levels under professional limits. We continue this study to obtain an extended statistics.

Thus, our study has demonstrated a decline of the RTL with increase of irradiation dose and the maximal effect in a late period after radiation exposure below 1 Sv. In ARS survivors a marked individual variation was detected. In existing studies there is no consensus on this subject. Neuhof et al. [20] conducted experimental research of telomerase activity and it was suggested to regard it as a radiation induced function, responsible

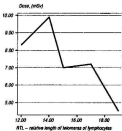


Fig. 8.28. Relative telomeres length in peripheral blood cells of the Chernobyl exclusion zone staff depending on the dose of irradiation.

A general tendency of shortening telomere length is similar to the described by other groups (Savalli et al. in 2009) (Fig. 8.30).

Correlation dependencies between the RTL and the dose of irradiation in a combined group of clean-up workers of 1986–87 and ARS survivors standardized by age have shown a tendency of decrease in telomere length in a dose interval between 100 and 500 mSv and a marked decrease in doses over 500 mSv (Fig. 8.31). However, a separate analysis at the interval below 250 mSv has shown some unexpected results (Fig. 8.32). The importance of these findings have to be further evaluated.

Thus, research of relative telomeres length in PB cells of different groups of humans exposed to ionizing radiation has demonstrated:

- a general tendency of shortening telomeres at the late period after radiation exposure as well as in a chronic exposure in doses not higher than 20 mSv;

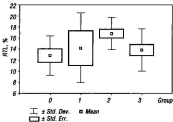


Fig. 8.29. Mean age of study groups. x-axis — groups of investigated: 0 — control group; 1 — patients in the remote period after ARS; 2 — participants of Chernobyl accident clean-up works; 3 — staff of exclusion zone; y-axis — age (years)

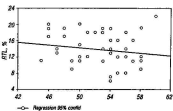


Fig. 8.30. Diagram of dispersion with a regressive line of the relative telomeres length and age in radiation-exposed humans. x-axis: age, years; y-axis — RTL (%)

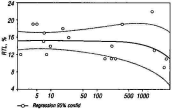


Fig. 8.31. Diagram of dispersion with the regressive line of dependence of relative telomeres length on the dose of irradiation. x-axis — dose (mSv), y-axis — RTL (%)

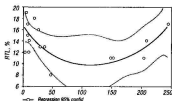


Fig. 8.32. Diagram of dispersion with the regressive line of dependence of relative telomeres length on the dose of irradiation in the range of 0–250 mSv. x-axis — dose (mSv), y-axis — RTL (%).

- the same tendency of decrease of telomeres length with age as in non-exposed groups of population;
- a tendency of RTL decrease became significant at doses over 500 mSv. Further studies have to validate these findings.

Study of apoptosis was performed using flow cytometric estimate of CD95⁺ fraction and Annexin-V assay (Fig. 8.33).

The results were different for radiation-exposed groups, both in an early period and in 20 years after the irradiation. Positive correlation was set for the group of the exclusion zone staff. The Pearson coefficient of correlation (r) was 0.64 ($p < 0.04$) (Fig. 8.34).

In a group of Chernobyl clean-up workers of 1986–1987, as a result of similar analysis of relationship between apoptosis and the age on exposure no statistically reliable dependence was observed, with low correlation — $r = -0.42$; $p < 0.21$. However, it should be noted that character of this dependence was negative, unlike a control group and group of the exclusion zone staff.

So, in the studied group a decline in annexin⁺PI⁺ cells numbers was shown with age, which can testify to some changes in cell entry to the apoptosis and as a result to violation of processes of elimination of damaged, or transformed cells in the remote period after irradiation. Results are presented on Fig. 8.35.

Correlation analysis was performed to reveal possible dependence between apoptosis changes and a radiation dose in the exclusion zone staff. The results are presented on Fig. 8.36. In this case we find correlations similar to those, as age and level of

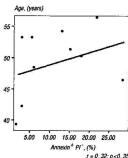


Fig. 8.33. Diagram of dispersion with a regressive line of the percent of annexin⁺PI⁺ cells and age for the persons of control group.

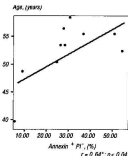


Fig. 8.34. Diagram of dispersion with a regressive line of the percent of annexin⁺PI⁺ cells and age of the exclusion zone staff.

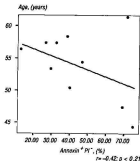


Fig. 8.35. Diagram of dispersion with a regressive line of the percent of annexin⁺PI⁻ cells and age of Chernobyl clean-up workers of 1986–1987

early apoptosis – the number of apoptotic cells grows accordingly with the increase of dose. Result is statistically unreliable, however Pearson coefficient high enough $r=0.62$.

Relationship between the processes of the programmed cellular death and relative telomere length was studied depending on the group of exposed. Results are illustrated on Fig. 8.37. The opposite changes were demonstrated with a combination of a decline of the RTL index with increased annexin⁺PI⁻ cells fraction. The maximal differences were typical for a group of Chernobyl clean-up workers of 1986–1987. Such tendency, as to our mind, is logical, because such factor, as telomere shortening could initiate activation of the programmed cell death – apoptosis. Our study is also in consistence with experimental findings showing that the telomerase-negative cells with short telomeres reach radiation-induced senescence after a lesser number of cell divisions, than cells with long telomeres.

In the last years considerable progress was attained both in the study of the phenomenon of apoptosis and in understanding of molecular mechanisms of its realization and regulation [25, 26]. A key role of Tp53 and p53 protein is elucidated in this process as well as of its target genes, like BAX and BCL-2 [27, 28]. Our data demonstrate a

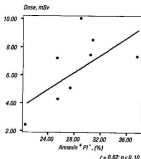


Fig. 8.36. Diagram of dispersion with a regressive line of the percent of annexin⁺PI⁻ cells and dose of irradiation in the exclusion zone staff

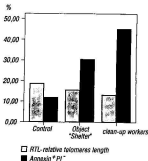


Fig. 8.37. Mean values of the relative telomeres length and Annexin⁺PI⁻ cells number in study groups

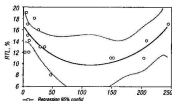


Fig. 8.32. Diagram of dispersion with the regressive line of dependence of relative telomeres length on the dose of irradiation in the range of 0–250 mSv. x-axis — dose (mSv), y-axis — RTL (%).

- the same tendency of decrease of telomeres length with age as in non-exposed groups of population;
- a tendency of RTL decrease became significant at doses over 500 mSv. Further studies have to validate these findings.

Study of apoptosis was performed using flow cytometric estimate of CD95⁺ fraction and Annexin-V assay (Fig. 8.33).

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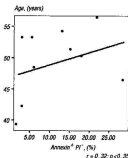


Fig. 8.33. Diagram of dispersion with a regressive line of the percent of annexin⁺PI⁺ cells and age for the persons of control group.

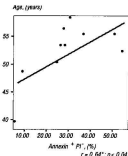


Fig. 8.34. Diagram of dispersion with a regressive line of the percent of annexin⁺PI⁺ cells and age of the exclusion zone staff.

rate. For clean-up workers study period was distanced from the moment of exposure and doses were 10–15-fold higher. Encountering the correlations between the telomeres length and shortening with parameters of immune function (data is presented below) and a revealed presence of the adaptation reactions of the immunity under professional limits, it is possible that the RTL changes to be transient. Continuing studies of this group could lead to new data. Gene regulation by *TERT*, *TRF1*, *TRF2* and other genes and telomerase activity quantification are needed. The possible significance of the RTL and apoptosis changes as a protective mechanism of suppression of cell transformation is to be further evaluated.

It is known, that the cases of different infectious diseases, autoimmune processes and tumors become more frequent with age. Possibly, it is partly conditioned by the age-old defects of the immune system. Connection of such wide spectrum of dependency upon age pathological processes of immune system resulted in a suggestion, that the senescence of the immune system can limit life-span [26]. However, in spite of a number of experimental and clinical studies, which testify the exhaustion of the immune system with age, an existing knowledge seems to be not enough for explanation of all the senescence pathways [26, 29].

Radiation cell aging and senescence are influenced by a set of cellular and humoral immunity parameters. It is possible that narrowing of the reserve response possibilities could result in a declined ability to react on stress, including radiation. Changes are different due to cell maturity, i.e. for stem cells, as a rule, a sustained function is reported in old age, though with mild proliferation changes [30]. Most of the changes are demonstrated at the post-thymic stage, including decrease of T-cell function at a senescence [31], decreased T-cell counts, and clear difference in the relative T-cell subsets counts [32].

A performed study of the RTL and subsets of immune cells has shown a negative correlation between the percent of CD3⁺ cells and the RTL (Fig. 8.43).

At senescence a relative increase of cytotoxic suppressor T-cells and diminishing of the CD4 helper cells compartment are described. Functional defects of the cellular immunity also correlate with decrease of the CD4⁺ subset [21]. Diminishing of T-helpers amount and function of cellular immunity are accompanied by the increased production of antibodies and autoimmune reactions [29]. The results show the different type

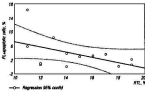


Fig. 8.42. Diagram of dispersion with a regressive line of the RTL and percent of annexin V-PI⁺ cells. x-axis — RTL (%), y-axis — annexin V-PI⁺ cells count (%).

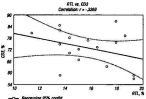


Fig. 8.43. Diagram of dispersion with a regressive line of the relative telomeres length and percent of CD3⁺ cells.

of correlation dependencies between the RTL and subsets of T-cells. An increase of the percent of CD4⁺ cells is related to an increase of the RTL index (Fig. 8.44). Negative correlation is demonstrated between the relative amount of cytotoxic lymphocytes and the RTL index. The same was shown for the antiviral immunity (Fig. 8.45).

Changes of humoral immunity were contradictory. However, consensus exists on the fact that senescence is associated with the presence of different antibodies, especially antibodies against nuclear antigens. Proofs are got, that senescence influences an intensity of antibody production by activated B-cells [21]. In our study correlations were set between the concentration of immunoglobulin IgM (g/L), circulatory immune complexes and the RTL in the staff of exclusion zone. Results are shown on Fig. 8.46, 8.47, 8.48.

Immune and the haemopoietic systems are closely connected, having an unique descendance from the hemopoietic stem cells. Both play a key role in a body defence, providing antitumor control and response to different pathogens. However, the age changes of hemopoiesis are much milder than of immunity. The decline of immune function of T-cells is associated with such factors as stem cell defects, replicative exhaustion of clonal proliferation [30, 33]. Late period after radiation exposure in Chornobyl clean-up workers of 1986–1987 is characterized by increased counts of the most primitive CD34⁺

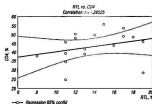


Fig. 8.44. Diagram of dispersion with a regressive line of the relative telomeres length and the percent of CD4⁺ cells.

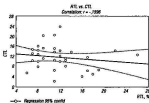


Fig. 8.45. Diagram of dispersion with a regressive line of the relative telomeres length and the percent of CD3⁺16⁺56⁺ cytotoxic lymphocytes.

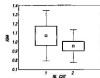


Fig. 8.46. Mean immunoglobulin M (g/L) concentrations in personnel with the RTL of 15.0 and higher (1) and personnel with the RTL less than 15 (2).



Fig. 8.47. Mean concentrations 4% circulatory immune complexes in personnel with the RTL of 15.0 and higher (1) and personnel with the RTL less than 15 (2).



Fig. 8.48. Mean concentrations of 7% circulatory immune complexes in personnel with the RTL of 15.0 and higher (1) and the RTL less than 15 (2).

progenitors. On Fig. 8.49 a positive correlation is illustrated between the level of early CD34⁺38⁻ cells and the RTL with a high degree of significance ($r=0.70$). The excess numbers of the early progenitors that are not followed by increased numbers of cells at the next differentiation stages seem to be an unfavorable factor, possibly related to a probability of malignant transformation.

The fluorescent hybridization *in situ* with a multicolor automated flow cytometric detection and analysis on current time is most rapid and the most sensitive method of quantitative determination of middle or median length telomeres. A method is developed less than 10 years ago, all its possibilities are not studied in detail; it can be used for the study of different cell subsets – granulocytes, naive T-cells, memory T-cells, B-cells, natural killers. Lately a method is applied for research of diseases with the innate gene anomalies [34].

With application of these technologies it was set that telomeres length of granulocytes in patients with aplastic anemia correlates with answer for immunosuppressive therapy, and in patients with chronic myeloid leukemia – with time to progress of disease [35, 36]. There also exist some interpopulation differences. In lymphocytes the telomere length decreases in a higher degree, than in granulocytes. The highest indices were shown for a young age. Differences could be related to telomerase activity. Telomerase-deficient mice and cells show reverse correlation between telomere length and radiosensitivity, but it is not the same for telomerase-proficient cells.

On three immortalised fibroblast clons with different telomeres length, derived from the same cell line (cen3tel4: length the terminal fragment of restriction 13 kb; cen3tel5: 10 kb, and cen3pcl6: 4 kb, that is less than level in senescent fibroblasts), after gamma-irradiation the absence of difference in LD50 and induction of chromosomal aberration was shown, that testified to the stable role of telomerase activity in the aspect of radiosensitivity [37]. The most researches in humans are devoted to a prognostical value of telomeres in sensitivity to chemotherapy, prognosis of oncologic pathology or radiosensitivity. On HEp-2, eight cellular lines of hepatocellular cancer and five breast cancer lines a reverse correlative dependence between the radiosensitivity and length of telomeres was demonstrated by the Southern blot and cell survival assay [38]. Research of genotoxical effect of ionizing radiation in a group of 181 healthy volunteers of the same age group a revealed reverse connection between the telomere length and radiation-induced micronuclei: a selected sub-group of persons with short telomers was characterized by the highest micronuclei counts [39]. It supports the experimental data and shows possibility of use the telomeres length as a biomarker of individual chromosomal instability under ionizing radiation exposure. In a group of staff of the exclusion zone we have performed analysis of dependency between telomere length and cytogenetic parameters. Cytogenetic studies were conducted in the laboratory of cytogenetics

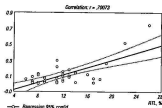


Fig. 8.49. Diagram of dispersion with a regressive line of the RTL (x-axis) and the percent of early CD34⁺38⁻ cells (y-axis).

of the Institute of Experimental Radiology of the RCRM, and authors are grateful to prof. M. Pilinskaya and her staff Dr. S. Dibsky, Dr. O. Dibskaya and Dr. O. Shemetun for presented cytogenetic data. Correlations were shown between the RTL and aberrant cell counts, single and pair acentric fragments (Table 8.14).

Table 8.14. Results of correlation analysis of telomeres length and chromosomal aberration

Cytogenetic indices	Coefficient of correlation with RTL (r)
Aberrant cells, (%)	-0.61
Total aberrations /100 metaphases	-0.48
Single acentric fragments, (%)	-0.98
Pair acentric fragments, (%)	0.99

The revealed correlations were limited to the most widespread types of aberrations. Especially interesting data were obtained on the single and pair acentric fragments. These information is much similar to the obtained before [39, 43, 44]. It should be noted that an effect is registered at the low-dose interval. At the same time no correlation was shown for the aberrations most characteristic for radiogenic chromosome changes like dicentric and centric rings, as the counts were under the assay sensitivity limits.

Conclusions

The performed study show a presence of the immune system cellular reactions after radiation exposure in a wide range of doses starting from high doses inducing radiation injuries to low doses and dose rates, which are determined both in the early and in the remote period. The results allow to expand knowledge from the experimental data to humans, and testify the leading role of radiation factor in induction of the effects in immune system in exposed due to Chernobyl accident, and primarily — clean-up workers.

Unlike the experimental data of irradiation the cell lines, the cellular effects in human are modulated not only by a bystander and other non-targeted effects but by a set of confounding factors, from which the most important to our opinion are neuro-immune interaction and lipid metabolism especially the arachidonic acid metabolites. A substantial part of effects is of an adaptive type, like cells transition to the radioresistant phases of cycle with "early" G_1 - and "late" G_2 -blocks; dose-dependent entry of the immature T- and B-cells to peripheral blood and nonspecific activation.

At the same time both in recovery period and in a remote period the radiation induced unlethal cell damage was demonstrated, including a differentiation of early progenitor cells to a functionally defective progeny. At exposure in the professional limits a set of activation changes at the different stages of differentiation of immune cells was shown with an increased genome instability, which was revealed by an increase of non-replicative DNA synthesis, accumulation of S- G_2 -M cells and enhanced expression of antigens of proliferating cells. The radiobiologic role of changes of apoptosis has to be understood in future.

A necessity is to fill the gap and find the link between the changes of immune function at low doses and formation of the late stochastic tumor effects as well as the non-tumor pathology of the late period.

The obtained findings enable the elaboration of strategies of staff selection and follow-up under low-dose exposure. First examples of such approach could be the staff of the 30-kilometer exclusion zone and Chernobyl „Shelter“. On the basis of the experimental data an adaptation to ionizing radiation in doses up to professional limits is supposed for healthy individuals, while at latent tumor processes an increase of genotoxic effects could be possible. It confirms the necessity of the thoroughful staff entry checks to the radiation zone. Our data on reverse dependencies between an apoptosis and telomere length are confirmed by the experimental studies [38], in which it was shown that a chemical suppression of telomerase, as a factor which promotes renewal of telomeres length leads to enhanceable apoptosis. So, in humans there is a relationship between violation of apoptosis, telomere shortening, parameters of cellular immunity and radiogenic somatic mutations due to ionizing radiation. At present the dependencies were described between the relative telomere length, dose of irradiation over 500 mSv, age and some types of chromosome aberrations. Future analysis is needed in humans expanding the studies on the role of gene regulation at the late period after low-dose exposure.

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CHAPTER 9 THE SIGNIFICANCE OF PERSISTENCE VIRAL INFECTIONS

Multiple studies of the effects of the 1986 Chernobyl NPP accident paid close attention to the potential problem of increases in oncological diseases, i.e., thyroid tumors first of all, and of non-tumor pathologies of the cardiovascular, bronchopulmonary, digestive, and urogenital systems in exposed clean-up workers and non-NPP individuals living in the vicinity of the plant [16]. As a dose-effect ratio was not strictly evident in many of the documented cases, our own investigations focused on the potential for non-radiation-based components, i.e., a presence of persisting infections, to contribute to the onset/progression of any observed changes in incidence of the various pathologies [1].

There are numerous publications in the literature about virus-induced oncogenesis in which the influence of viral infection on genomic instability, which is a hallmark of most human cancers, is discussed [2]. Common fragile sites are preferential targets for human papilloma virus (HPV)-16 integrations in cervical tumors [3]. When maintained as an episome, HPV-16 DNA can disturb centrosome homeostasis and subvert genomic integrity of the host cell during early stages of the viral infection. The two HPV-encoded oncoproteins, E6 and E7, can independently induce chromosomal abnormalities [26]. The fragile histidine triad (*FHIT*) gene, which is frequently lost in many cancers, has been identified as a candidate tumor suppressor gene at chromosome 3p locus 14.2 [14]. Alterations of *FHIT* were found in several kinds of viral-associated tumors including hepatitis C virus (HCV)-associated hepatocellular carcinoma [3].

Viruses or their products also cause modification sites on chromosomes that are known to look like non-staining gaps. The *CKR5* structural gene mapped to human chromosome 3p21 gene codes for the chemokine receptor 5 (CKR5) protein that serves as a secondary receptor on CD4⁺ T-lymphocytes for certain strains of human immunodeficiency virus (HIV)-1 [5].

The link between viral infections and some somatic diseases is also under intense examination. There are data that cytomegalovirus (CMV) may contribute to atherosclerotic events, peripheral occlusive artery disease, type 1 diabetes, and/or rheumatoid arthritis [6]. The association between CMV infection and subsequent development of allergy, specifically allergic asthma, has been known since the late 1970s [7]. HCV

infection is associated not only with liver diseases, but also numerous hematologic, renal, dermatologic, rheumatic, and autoimmune disorders, including arthralgia, arthritis, vasculitis, Sicca syndrome, myalgia, and fibromyalgia [8]. The persistence of HCV in lymphocytes causes their oligoclonal and monoclonal proliferation, the appearance of non-organ-specific autoantibodies, and leads to autoimmune type II mixed cryoglobulinemia and development of several types of indolent non-Hodgkin's lymphomas [9].

Our laboratories have been involved in examining the problem of persisting infections for almost 20 years. This interest was sparked by a 1993 cooperative study (under the supervision of Professor H. Yoshizawa from Hiroshima University, Japan) of HCV infection prevalence among blood donors at the Kyiv Municipal Blood Center [10]. Building upon these and other earlier investigations, the studies presented here reflect our most recent investigations of the potential links between somatic and non-somatic diseases and persisting infections among individuals (clean-up workers, residents of nearby radiation-contaminated territories, and power plant workers) who had suffered from the Chernobyl NPP accident in 1986 and thereafter.

Materials and methods

Patients

Patients were investigated during their hospitalization in the in-patient clinic at RCRM. Persons who were included in the CER of the RCRM were also investigated in the context of their biennial clinical-laboratory surveillance visit(s). The Ethics Commission of the RCRM approved all study designs used herein; patients were entered into the study only after they had provided informed consent and full medical histories of any past/ongoing tumor- and/or non-tumor-based (i.e., somatic) diseases.

The following groups of patients were recruited for these studies: convalescents of ARS, $n=118$ (mean age 49.3 [± 0.82 (SE)] years); Chernobyl NPP clean-up workers, $n=1017$ (mean age 47.6 [± 0.57] years); dwellers of radiation-contaminated territories in the Ukraine, $n=578$ (mean age 46.5 [± 0.37] years); and NPP workers, $n=122$ (mean age 42.2 [± 0.73] years). For comparison purposes, a group of patients with different somatic diseases, and who had not been exposed to ionizing radiation (IR; $n=367$, mean age 44.8 [± 1.17] years) and the earlier blood donors of Kyiv Municipal Blood Center ($n=41,021$, mean age 39.7 [± 1.17] years) were employed as controls.

In addition, for analyses of any relationship(s) between infection (past/ongoing) status and IR exposure history, patients with chronic lymphoproliferative disorders (294 with chronic lymphocytic leukemia (CLL) and four with stable large granular lymphocytosis LGL) were also recruited and studied. The diagnosis of CLL and LGL was based on clinical history, lymphocyte morphology, and immunophenotypic criteria. The stage of CLL was established according to classifications of Rai [11] and the International Workshop on Chronic Lymphocytic Leukemia [12]. CLL patients were divided into two groups according to IR exposure: the first group included 63 IR-exposed CLL patients and the second group (the nosologic control group) consisted of 233 IR non-exposed CLL patients.

The group of IR-exposed CLL patients included 48 clean-up workers, 10 inhabitants of nuclide-contaminated areas, and five evacuees, who were observed in the Department of Hematology of RCRM during June 2004–December 2008. Information about do-

ses for the clean-up workers was available from the SRU. Among the clean-up workers, estimated doses ranged from 25.0 to 120 cSv among those who were on-site the earliest; doses for those who arrived later in 1986 and then over 1987–1989 ranged from 0.14 to 9.9 cSv. Accumulated doses (since 1986 to the diagnosis of CLL; range 0.23–2.12 cSv) in the residents of contaminated areas were calculated on the basis of the data on ^{137}Cs soil contamination density (mean age dose typical for a given level of local contamination) and information on measurements of radioactive Cs in the body at the CLL diagnosis. The control (IR-non-exposed) group included CLL patients who were observed in the Department of Hematology and Bone Marrow Transplantation of RCRM (129 patients) and the Department of Hematology of Poltava Regional Hospital (102 patients) during the same period without special selection of patients.

Blood sampling and preparation of blood plasma for antibody analyses

As part of any given patient's routine diagnostic protocol, fasting blood (10 mL) from the cubital vein was collected (in the morning) into polypropylene tubes containing heparin (Terumo Corp., Tokyo, Japan). The blood was mixed with the heparin by gentle rocking (at least five times) and avoiding foaming. The cellular elements were then removed by centrifugation at $200\times g$ for 10 min. The interval between the collection and centrifugation of the blood did not exceed 30 min. The resultant plasma was then carefully recovered and aliquoted into small Eppendorf tubes (at least two tubes/each patient). The tubes were marked, logged, and then placed in a -20°C freezer (Liebherr-Hydraulikbagger GmbH, Kirchdorf, Germany) until analysis.

Confirmation of serum anti-HCV and anti-CMV antibodies

Anti-HCV (IgG) and/or anti-CMV (IgG and IgM) antibodies in an isolated serum sample were assessed using commercially available enzyme-linked immunosorbent assay (ELISA) diagnostics kits (Sanofi Diagnostique Paster, Marnes-La-Cosquette, France; RPC Diagnostic Systems, Nizhniy Novgorod, Russia; DiaProf Ltd., Kyiv, Ukraine; Vector-Best, Novosibirsk, Russia; Diagnostic Systems Laboratories (DSL), Webster, TX) and following manufacturer's instructions.

Immunophenotyping of peripheral blood mononuclear cells

Sub-populations of peripheral blood lymphocytes were quantified using two-color laser flow cytometry. The differential tagging of cells was performed with monoclonal antibodies of the Leu series obtained from Becton Dickinson (San Jose, CA) using standard protocols and following manufacturer's instructions. All data were then analyzed using the software provided by the manufacturer.

PCR analyses of CMV DNA presence in cell samples

The presence of CMV DNA was investigated in the fraction of peripheral blood mononuclear cells by polymerase chain reaction (PCR). In brief, DNA was extracted from the cells using a sorption method. PCR of the samples was then performed using kits from DNA-Technology JSC (Moscow, Russia) and the following regimen: 5 cycles of 94°C for 90 s, 94°C for 50 s, 67°C for 45 s, and 72°C for 30 s, then followed by 40 cycles of 94°C for 20 s, 67°C for 20 s, and 72°C for 20 s. The resulting PCR products were then electrophoresed over 1.5% agarose gels and visualized using standard ethidium bromide staining.

PCR analyses of IgHV genes in CLL cell samples

The rearrangements of *IgHV* were studied according to the BIOMED-2 consortium rules [13] as described previously [14]. In brief, total RNA was extracted from peripheral blood and/or bone marrow cells using guanidine isothiocyanate–phenol–chloroform extraction according to Chomczynski and Sacchi [15]. Total cellular RNA (1–5 µg) was reverse-transcribed to cDNA using MolV reverse transcriptase enzyme (Applied Biosystems, Branchburg, NJ) and random hexamers as primers. The cDNA was amplified in a single multiplex PCR bearing six *IgHV* framework 1 primers combined with an *IgJH* consensus primer as designed by the BIOMED-2 consortium. All reactions were carried out in a 50 µL final volume containing 10 pmol of each primer, 200 nM dNTPs, 1 U AmpliTaq Gold, and 10×PCR buffer II (Applied Biosystems). The cycling conditions were: pre-activation at 94°C for 7 min; 35 cycles of 94°C for 45 s, 60°C for 45 s, and 72°C for 45 s; and, a final 10 min cycle at 72°C.

PCR products were then analyzed on a 1.5% agarose gel and visualized with ethidium bromide staining. Products were spin column-purified using a PCR purification kit (Promega, Madison, WI) and sequenced directly using forward and reverse primers in an automated DNA sequencer ABI-310 (Applied Biosystems) using Big-Dye Terminator Cycle Sequencing Reaction Kit (Perkin Elmer, Foster City, CA). The closest germ line *IgHV* gene for each B-CLL *IgHV* sequence was assigned using current databases, for example, IgBlast (National Center for Biotechnology Information, Bethesda, MD), IMGT (International Immunogenetics Database) [20], and JoinSolver [25]. The length of the HCDR3 was calculated, followed, established IMGT criteria. Sequences reported in this study can be found in GenBank database under accession numbers: EF091900–EF091931, EF175384–EF175440, EF407822–EF407849, EF441744–EF441761, EF583659–EF583683. In some cases with homologous *IgHV* sequences, Ig variable light κ or λ chains (IgV κ and IgV λ) were amplified according to the BIOMED-2 protocol.

Statistical analysis

Data from all groups were analyzed using multivariate analysis of variance (MANOVA) to evaluate the relationship between group and presence/absence of past/ongoing infection with the viral agent(s). All statistical analyses were conducted using the SPSS 11.0 software package (SPSS, Chicago, IL), general linear model. Data about ages of the investigated persons are presented as their mean±standard errors (SE). The chi-square test for categorical variables was used to compare characteristics between different subgroups of patients. All *p* values are two-sided, and significance was set at $p \leq 0.05$.

9.1 HCV INFECTION IN PERSONS SUFFERED DUE TO THE CHORNOBYL NPP ACCIDENT

The presence of anti-HCV antibodies was assessed in 965 clean-up workers, 114 convalescents of ARS, 578 dwellers of radiation-contaminated territories of Ukraine, and 87 NPP workers. Samples from 41,021 blood donors at the Kyiv Municipal Blood Center were examined for comparison. As given in Table 9.1, a very significant ($p < 0.0001$)

greater prevalence of HCV infection was found in all groups of individuals who suffered from the Chernobyl accident as compared with that among the Kyiv blood donors.

Table 9.1. Presence of anti-hepatitis C virus (HCV) serum antibodies in individuals in the indicated populations

Groups of observed persons	Persons with anti-HCV antibodies		
	total*	men*	women*
Blood donors (n=41,021; ♂=28990; ♀=12031)	1417 (3.45)	1145 (3.95)	272 (2.26)
Clean-up workers (n=965; ♂=791; ♀=174)	#218 (22.59)	#169 (21.36)	#49 (28.16)
Convalescents of ARS (n=114; ♂=106; ♀=8)	#31 (27.19)	#28 (26.41)	#3 (37.5)
Dwellers of radiation-contaminated territories (n=578; ♂=273; ♀=305)	#65 (11.24)	#37 (13.55)	#28 (9.18)
NPP workers (n=87; ♂=64; ♀=23)	#22 (25.28)	#19 (28.68)	#3 (13.04)

*First value given is actual number of subjects with the antibodies; value in parenthesis is the calculated percentage among the respective population analyzed. #Reported value was significantly different ($p < 0.0001$) from that in the blood donor (i.e., IR-non-exposed) persons.

In a separate PCR analysis of the HCV found in the blood samples from HCV⁺ subjects, a prognostically unfavorable 1b genotype of HCV — one associated with frequent development of cirrhosis — was the most prevalent form found (i.e., incidence of 86.92%) among all the subgroups analyzed (PCR analyses were performed at the Department of Hygiene, in the School of Medicine of Hiroshima University, Hiroshima, Japan, under the supervision of Professor H. Yoshizawa).

How and why these individuals became HCV⁺ were also of interest to our investigation. For this purpose, groups of 199 male clean-up workers (71 persons received blood transfusions) and 140 male IR-non-exposed persons (75 persons received blood transfusions) were studied. In both groups we found a strong association ($p < 0.001$) between the presence of anti-HCV antibodies and prior blood transfusion(s). Among persons who received blood transfusions, HCV seropositivity was significantly higher ($p < 0.001$) in the group of clean-up workers (39.4%) as compared with among the IR-non-exposed persons (14.3%). No significant differences were found among individuals who had not received blood transfusions (12.2% versus 6.7%; $p > 0.05$).

The studies here also sought to determine, in part, if there was any association be-

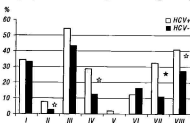


Fig. 9.1. The frequency of some somatic diseases in clean-up workers as related to hepatitis C virus (HCV) seropositivity. I: coronary heart disease; II: acute myocardial infarction in anamnesis; III: essential hypertension; IV: thyroiditis/goiter; V: type 1 diabetes; VI: type 2 diabetes/impaired glucose tolerance; VII: chronic hepatitis; VIII: chronic gastritis. Differences between HCV⁺ and HCV⁻ patients are significant at $p < 0.05$ (white star) or $p < 0.001$ (black star).

tween persistence of HCV in these patients and selected somatic diseases. The data here showed that in a group of clean-up workers (specifically, 368 patients without malignant diseases), HCV infection was associated with active chronic hepatitis (32.4% versus 11.0% among clean-up workers without anti-HCV serum antibodies; $p<0.001$), chronic gastritis (40.9% versus 27.4%; $p<0.05$), acute myocardial infarction in anamnesis (7.6% versus 2.6%; $p<0.05$), thyroiditis, and nodular goiter (26.6% versus 12.5%; $p<0.01$), thrombocytopenia (4.8% versus 0.7%; $p<0.05$), lymphadenopathy (14.3% versus 4.9%; $p<0.05$), and hemorrhagic vasculitis (2.8% versus 0.4%; $p<0.05$) (Fig. 9.1). Thus, a significant prevalence of HCV infection among these IR-exposed persons appears to unfavorably influence their health in many manners.

9.2 CMV INFECTION IN PERSONS THAT SUFFERED DUE TO THE CHORNOBYL NPP ACCIDENT

As compared with the control group of IR-non-exposed persons, male subjects in each of the various groups of individuals who suffered from the Chernobyl accident (except for the dwellers of radiation-contaminated areas) had a significantly greater incidence of CMV infection as determined by the presence of anti-CMV IgG antibodies (i.e., values ranging from 79.4% to 85.6% versus 69.2%; $p<0.05$) (Table 9.2).

Table 9.2. Presence of anti-cytomegalovirus IgG serum antibodies in individuals in the indicated populations.

Groups of observed persons	Persons with anti-CMV antibodies		
	total*	men*	women*
IR-non-exposed persons ($n=367$; $\sigma^2=162$; $\varphi=205$)	254 (69.2)	114 (70.37)	140 (68.29)
Clean-up workers ($n=505$; $\sigma^2=428$; $\varphi=77$)	[#] 401 (79.4) $p<0.001$	[#] 342 (79.9) $p<0.01$	[#] 59 (76.62) $p<0.05$
Convalescents of ARS ($n=118$; $\sigma^2=113$; $\varphi=5$)	[#] 101 (85.59) $p<0.001$	[#] 96 (84.95) $p<0.004$	5 (100)
Dwellers of radiation-contaminated territories ($n=115$; $\sigma^2=61$; $\varphi=54$)	89 (77.39)	48 (78.68)	41 (7.92)
NPP workers ($n=122$; $\sigma^2=98$; $\varphi=24$)	[#] 98 (80.32) $p<0.01$	[#] 80 (81.63) $p<0.05$	18 (75)

*First value given is actual number of subjects with the antibodies; value in parenthesis is the calculated percentage among the respective population analyzed. [#]Reported value was significantly different (at indicated p value) from that in the IR-non-exposed group.

Specifically, the level of significance of these differences between the male IR-non-exposed persons and the male clean-up workers, ARS convalescents, and NPP workers were $p<0.001$, $p<0.001$, and $p<0.05$, respectively. In addition, both the clean-up workers and ARS convalescents had significantly more frequent laboratory signs of active CMV infection as determined by positive PCR measures for detection of viral DNA (i.e., 14.3 and 16.2%, respectively, versus 5.51%; $p<0.05$) (only male persons were included in this analysis). As a consequence of repeated viral activation, we estimated that there has also been likely a presence of high titers of anti-CMV IgG antibodies in the serum of the vast majority of these exposed subjects. Lastly, a correlation between IR-absorbed

dose and the level of CMV seropositivity was found within the ARS convalescents group (i.e., $r=+0.3244$; $P=0.028$).

In all groups of observed subjects (a total $n=1017$), the presence of anti-CMV IgG antibodies was associated with somatic diseases (76.86% versus 49.11% among persons without chronic somatic diseases; $p<0.001$), especially with chronic gastritis, bronchitis, and different types of arthritis (Fig. 9.2). Furthermore, a simultaneous existence of bronchopulmonary and digestive

system diseases was typical for the CMV⁺ patients comparing with CMV⁻ patients. Such co-morbidity was observed in 13.19% of the CMV⁺ patients versus 4.16% of the CMV⁻ patients among the IR-non-exposed subjects ($p<0.05$) and in 36.84% versus 11.24% of patients in a group of clean-up workers ($p<0.01$).

The repeated reactivation of CMV significantly impaired the course of HCV infection (note: this analysis was performed in group of 105 HCV⁺ clean-up workers). The clinical signs of active hepatitis (e.g., liver enlargement, elevated alanine and asparagine aminotransferase activities, and extrahepatic symptoms) were observed in 44.4% of HCV⁺CMV⁺ patients, but only in 21.1% of HCV⁺CMV⁻ patients ($p<0.05$). In a one-way layout analysis, the presence of anti-CMV antibodies ($r=0.3418$; $p<0.0001$), masculine gender ($r=0.3553$; $p<0.0001$), serological signs of viral hepatitis B in anamnesis ($r=0.2896$; $p=0.003$), and an increase in absolute CD8⁺ T-lymphocyte counts (only in CMV⁺ patients, $r=0.5745$; $p=0.005$) were the major risk factors for prediction of active HCV-associated hepatitis. It is possible to suppose that CMV may potentiate any hepatotropic action of HCV by the influence of the former on the liver parenchyma or by causing increases in the activity of cytotoxic T-lymphocytes.

Because there are some data that indicate that persistent CMV activation can trigger and maintain the clonal expansion of large granular T-lymphocytes [16], we also examined a small group ($n=4$) of clean-up workers with LGL. In all of these patients, substrate cells were found, which belonged to the class of mature post-thymic T-lymphocytes (i.e., CD3⁺, CD8⁺, CD4⁺, CD16⁺, and/or CD57⁺). A presence of CMV in blood mononuclear cells was found in two of these patients (by PCR). One of these patients who had stable LGL over the last 4 years (WBC $4.1-6.1 \times 10^9/L$, with a differential count of 56-68% lymphocytes, and 85-87% of these cells had a CD3⁺CD8⁺CD16⁺ phenotype); after intensive anti-CMV therapy (i.e., anti-CMV IgG, ganciclovir), the patient's blood cell profile was completely restored and the patient displayed an improved general condition (i.e., no signs of chronic infections and episodes of febrile fever).

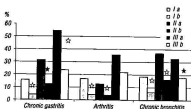


Fig. 9.2. The frequency of some somatic diseases in the observed patients appears to depend on cytomegalovirus (CMV) seropositivity. Group I = IR-non-exposed persons (Ia: CMV⁺, Ib: CMV⁻); Group II = clean-up workers (IIa: CMV⁺, IIb: CMV⁻); and Group III = ARS convalescents (IIIa: CMV⁺, IIIb: CMV⁻). Differences between CMV⁺ and CMV⁻ patients are significant at $p<0.05$ (white star) or $p<0.001$ (black star).

9.3 CLL CELLS DISPLAY HOMOLGY OF THEIR IG RECEPTORS WITH ANTIVIRAL OR ANTIMICROBIAL ANTIBODIES

Over the previous decade, CLL has been the subject of increasingly intensive research. Several research groups identified so-called homologous CLL cases that expressed immunoglobulins (Igs) composed from stereotyped combinations of heavy-chain variable (IGHV), diversity (IGHD), and joining (IGHJ) genes and very close heavy-chain complementarity-determining region-3 (HCDR3) [31]. Most of the CLL clusters were also characterized by specific Ig light kappa (κ) or lambda (λ) chain gene usage [17]. Together with a biased repertoire of Ig genes [18], a similarity of CLL Ig sequences and antibodies against autoantigens and microbial/viral antigens [19] and some epidemiological data [20] has led to the hypothesis that there is a possible role of viral/microbial antigens in the development of CLL.

In the studies here, we analyzed Ig gene rearrangements in 294 CLL sequences from observed patients (63 of them who suffered from the Chornobyl NPP catastrophe) and identified 46 cases presenting a homology with antibody sequences of known specificity [5]. No differences were found between sequences from IR-exposed and IR-non-exposed CLL patients. Among the 46 CLL cases, 26 evidenced a HCDR3 homology with antibacterial or antiviral Ig clones, namely, anti-PPS antibodies against *Streptococcus pneumoniae* polysaccharides (12 cases), Igs that recognized capsular polysaccharide of *Neisseria meningitidis* (three cases), anti-staphylococcal protein A Ig clones (two cases), rotavirus-specific Ig clones (four cases), anti-rabies Igs (four cases), and anti-herpes simplex virus type 1 (one case). Additionally, 20 Ukrainian CLL cases demonstrated HCDR3 homology with autoreactive clones. These included with homologies to: Igs in rheumatoid arthritis patients (five cases); anti-cardiolipin antibody (six cases); Igs peculiar to systemic lupus erythematosus (anti-Sm; five cases); CLL Ig with autoantibody activity (two cases); Ig from a bone marrow transplant recipient with graft-versus-host disease (GVHD; one case); and Ig from B-cell lymphoma secreting anti-Pr2 erythrocyte autoantibodies (1 case; see Table 9.3).

Table 9.3. Ukrainian CLL cases showing HCDR3 homology with immunoglobulins (Igs) of known specificity.

IGHV,IGHD,IGHJ,mutation status, patient,IgBlast number	HCDR3 Amino acid sequence	Antigen specificity
3-30-3*01_2-2*01_6*02_UM_AY906636	-EVQDINVVPAAI-YYYYYGMDV	
UA1-69*01_2-2*01_6*02_UM_D70_EF583653	-G-6DIWVPAEY-YYYYYGMDV	
UA1-69*01_2-2*01_6*02_UM_D94_EF091906	PGPGQINVVPAAY-YYYYYGMDV	
UA1-69*01_2-2*01_6*02_UM_D3_EF175390	-EVQDINVVPAAI-YYYYYGMDV	anti-PPS Ig
UA1-69*01_2-2*01_6*02_UM_C17_EF175388	-EVQDINVVPAAI-YYYYYGMDV	
UA1-69*01_2-2*01_6*01_UM_E94_EF175420	-EVQDINVVPAAI-YYYYYGMDV	
UA1-69*01_2-2*01_6*01_UM_F89_EU433873	-EVQDINVVPAAI-YYYYYGMDV	
3-33*01_3-3*01_4*02_UM_D0535056	AG-SD-YDPWSGYPPYFDY	
UA4-30-4*01_3-3*01_4*01_UM_E16_EF583678	VGPSSGYDPWSGYPPYFDY	anti-PPS Ig

IGHV/IGHD/IGHJ_mutation status_patient_kj/seq number	HCDR3 Amino acid sequence	Antigen specificity
3-48*03_2-21*01_4*02_UM_AY909680	DRTYCGGDCYGPYFDY	anti-PPS Ig
UA3-48*03_2-21*02_4*02_M_D76_EU814967	DMAYCGGDCYSGFFDY	
3-33*01_3-10*01_4*02_UM_DQ322780	PQLWFGEIN-YYFDY	anti-PPS human antibodies
UA3-11*01_3-10*01_4*02_UM_F85_EU433870	DTLWFGEFHAYYFDY	
3-11*03_3-3*01_5*02_UM_DQ322854	DTRPYDFWSGYYP-NWFDP	anti-PPS Ig
UA3-11*01_3-3*01_5*02_UM_G17_EU667604	--DRYDFWSSGYIGNWFDP	
UA4-61*01_3-3*01_5*02_UM_E53_EF091926	HRGDYDFWSSGYYPNWFDP	
3-33*01_6-19*01_6*02_UM_AF431051	DREWLYYYYGMDV	anti-PPS Ig
UA3-33*01_6-19*01_6*02_M_P8_GU358673	DPVWMIYYYGMDV	
1-18*01_3-22*01_6*01_UM_AF115133	--ADYYDSSGYYY-DYIIYYGMDV	anti-Neisseria meningitidis Ig
UA4-4*02_3-22*01_6*02_UM_D36_EF583668	ASGNYYDSSGYYWNNYYYYGMDV	
UA3-11*01_3-22*01_6*03_UM_F34_EF175439	APKNYYDSSGYYWNNYYYYYMDV	
UA4-59*08_3-22*01_6*02_UM_D15_EU814956	--RPLYDSSGYYVMNYYYYGMDV	
3-30-3*01_3-22*01_4*02_UM_AB021532	AR-MYYDSSGY-SFDY	anti-Staphylococcal protein A Ig
UA4-59*01_3-22*01_4*02_UM_F35_EF175440	AR-YYDSSGY-YFDY	
UA4-8*01_3-22*01_4*01_UM_E90_EF175423	VKEYYYDSSGYYGFY	
3-30*03_2-6*02_6*02_UM_EF177969	DHRYCTGGVCYDSYYYGMDV	Rotavirus-specific Ig
UA1-2*04_2-8*01_6*02_UM_F74_EU33868	PSFYCTNGVCYTDYIIYYGMDV	
UA1-2*02_1-26*01_6*02_UM_AY944713	VYSGSYPIIIYYGMDV	Rotavirus-specific Ig
UA1-2*02_1-26*01_6*02_UM_E70_EF091912	PYSGSYPIIIYYGMDV	
UA1-2*02_nd*01_6*02_UM_G19_EU667802	LYSGSYPIIIYYGMDV	
3-23*04_5-5*01_6*02_UM_AY944710	--ADTAMVIIIIYYGMDV	Rotavirus-specific Ig
UA3-43*01_5-5*01_6*02_UM_F24_EF407840	GMVDAMVIIIIYYGMDV	
3-49*04_3-3*01_6*02_UM_AY941852	DRDGTYYDFWSSGYPPPIYYGMDV	anti-rabies Ig
UA1-69*01_3-3*01_6*02_UM_E92_EF583675	DWGTTYYDFWSSGYFSP-YGMDV	
1-24*01_5-24*01_6*02_M_Y17784	MKDGYNKVVIIIIYYGMDV	anti-herpes simplex Ig
UA1-69*13_5-24*01_6*02_UM_GU358657	EGDGYNYGVIIIIYYGMDV	
1-69*06_3-16*02_3*02_UM_AF460965	GGNYDYTWGSYRSNDAFDI	anti-cardiolipin Ig
UA1-69*01_3-16*02_3*02_UM_D34_EU814958	GGDYDYTWGSYRPNDAFDI	
UA1-69*01_3-16*02_3*02_UM_E77_EF091909	GGDYDYTWGSYRPNDAFDI	
UA1-69*01_3-16*02_3*02_UM_D89_EU814960	GGAYDYTWGSYRPNDAFDI	
UA1-69*01_3-16*02_3*02_UM_D73_EF175395	GGGYDYTWGSYRSNDAFDI	
UA1-69*01_3-16*02_3*02_UM_P16_GU358676	GGNYDYTWGSYRTNDAFDI	
UA1-69*01_3-16*02_3*02_UM_D97_GU358670	GGDYDYTWGSYRSNDAFDI	

IGHV, IGHJ, IGHJ_mutation status, patient IgBlast number	HCDR3 Amino acid sequence	Antigen specificity
1-3*01_3-10*01_3*02_M_AY393651	ASGRFWQSRG-DAFDI	rheumatoid arthritis Ig
UA3-21*01_3-22*01_3*02_M_B78_EF175385	VSGRNYDSSGSDAFDI	
1-89*06_6-6*01_4*02_M_AY393652	GQSSSSSRFPDY	rheumatoid arthritis Ig
UA3-74*01_6-6*01_4*02_M_E22_EF441750	EEGSSSLR-PFDY	
5-51*03_6-13*01_4*02_UM_AY393191	RADTSSWYYFDY	rheumatoid arthritis Ig
UA4-34*01_6-13*01_4*02_M_E49_EF441754	GSDGSSWYYPDF	
4-34*01_3-3*01_4*02_UM_X54441	GGSV-LRFLEWLLYPADY	
UA1-69*06_3-3*01_4*02_UM_G12_EU667599	SGE-LRFLEWLLSADFDY	rheumatoid arthritis Ig
UA1-69*01_3-3*01_4*02_UM_F10_EF175426	EGSRPLRFLWLL---FDY	
1-69*13_2-2*01_6-13*01_UM_U86795	TDIVVPAAMIYYYYGMDV	CLL cells, autoantibody activity
UA1-69*01_6-13*02_UM_E41_EF175412	VQGSAAAYENYYYYGMDV	
UA1-69*01_2-2*01_6*01_UM_F3_EF175425	TLIEVPAWYTIYYYYGMDV	
4-31*03_3-9*01_6*02_UM_U80127	DGGN--YDILTGYRADYYGMDV	BMT recipient with GVHD
UA3-21*01_3-9*01_6*02_UM_E85_EF407825	DGGSLYDILTGYSLYYGMDV	
1-18*01_2-15*01_4*02_M_X15611	APGYCSGGGQYRGDDY	B-cell lymphoma secreting anti-Pr2 erythrocyte autoantibodies
UA4-34*01_2-15*01_6*02_M_F20_EF583679	GSGYCSGGGQYGGLDV	
AY942901	DFEYHYESSGY---PYYYGMDV	anti-rabies Ig
3-33*01_3-22*01_6*02_UM_Z46379	DN-YYYDSSGY---YYYYGMDV	
UA1-2*04_3-22*01_6*02_UM_G2_EU433681	DIGHYYDSSGYSYPYYYYGMDV	anti-Sm Ig
UA3-30-3*01_3-22*01_6*02_UM_E73_EF175415	DTTYYYDSSGY---YYYYGMDV	
UA4-59*01_3-22*01_6*02_UM_D62_EF091925	GVGDYYDSSGY---LHYYYGMDV	
3-23*01_4-17*01_3*02_UM_AJ305181	GQYGDYSFAFDI	
UA3-33*01_4-17*01_3*02_UM_E13_EF407846	GVPGDYVMAFDI	anti-Sm Ig
UA3-30-3*01_4-17*01_2*02_UM_F42_EF407832	GPRGDYVSSFDL	

Discussion

On the basis of our observations of several different groups of individuals who suffered during and after the Chernobyl NPP catastrophe, as well as a control group of persons without any previous direct exposure to IR, a high prevalence of HCV and CMV infections among the afflicted groups was established.

It is well-established that preceding blood transfusions are considered one major reason for HCV seropositivity in epidemiologic studies of disease (i.e., HCV) incidence. The data from the current studies confirmed this phenomenon. Nevertheless, even if

transfusions were a major source of infection, it is known that after the initial infection, HCV may be completely eliminated in ≈ 17 –25% of the affected subjects. By comparing persons who received blood transfusions relative to IR exposure, these studies revealed that there was a significantly higher ($p < 0.001$) frequency of anti-HCV antibodies present among clean-up workers than in IR-non-exposed individuals. Based on our previous studies that revealed considerable disturbances in T-lymphocyte-mediated immunity among Chernobyl clean-up workers — and especially among ARS convalescents [21], it is possible to suggest that this state of immunomodulation may be one reason for the high level of HCV infection among these individuals—regardless of whether or not the initial infection was due to a transfusion or not.

Our data concerning the prevalence of anti-HCV serum antibodies in the clean-up workers and ARS convalescents were in agreement with data from Japanese investigators who had earlier concluded that radiation exposure may accelerate the progress of chronic liver disease associated with HCV in atomic bomb survivors [22]. Recently, it was noted that the nonstructural protein 3 of HCV interacts with the ataxia-telangiectasia-mutated (ATM) protein and, in turn, delayed dephosphorylation of phosphorylated forms of ATM [23]. These same investigators also described that this HCV protein also impairs the efficiency of DNA repair and renders infected cells more sensitive to DNA damage after IR exposure [23]. This interaction may contribute to the observed HCV infection-associated pathogenesis among the IR-exposed patients here and in the Japanese studies. Specifically, among the group of HCV⁺ Chernobyl clean-up workers monitored here, some diseases (e.g., chronic hepatitis, chronic gastritis, thyroid diseases, thrombocytopenia, and acute heart attack in anamnesis) were found to have occurred with greater frequency in comparison with that among HCV⁻ clean-up workers.

From the studies here and those of other investigators, some mechanisms to explain the potential for interactions between CMV and IR have been revealed as well. For example, it was demonstrated (in different experimental models) that CMV gene expression was increased by exposure to oxygen radicals or after irradiation [24]. This may be a major reason for the observed increases in CMV seropositivity and repeated CMV reactivation among our Chernobyl clean-up workers and ARS convalescents. The increases in CMV infectivity status (likewise HCV) were also found to be positively associated with increases in the occurrence of somatic diseases, especially chronic gastritis, bronchitis, and different types of arthritis among the CMV⁺ patients.

Studies here and elsewhere have also noted a significant homology between IGHV sequences in the lymphoid cells of CLL patients with antiviral and antimicrobial antibodies. Recently, Lanemo Myhrinder et al. [19] revealed that several CLL Igs reacted with bacterial components (*S. pneumonia* polysaccharides and phosphorylcholine) as well as molecular structures exposed on apoptotic cells (e.g., vimentin, proline-rich acidic protein-1, and oxidized LDL). These findings led to the suggestion that viral and bacterial infections in synergy with self-antigens or apoptotic cells could drive CLL. Among the CLL patient examined here, similar findings of CLL Igs that react with viral and bacterial components were proof that infections — even almost a quarter of a century after the nuclear accident — may contribute to CLL pathogenesis.

In summary, it appears that persistent viral infections, especially when accompanied by exposure(s) to IR, can be quite deleterious. The worst-case scenario, where each

of these factors have occurred/are ongoing, is one that appears to result in a modified (i.e., aggravated) clinical course of somatic diseases and/or to promote malignancies (i.e., CLL) among these hosts. Nevertheless, even under these seemingly "dire conditions," the persisting infections can be transformed into "steerable/manageable constituents" with antiviral treatments, even if the patient had been exposed earlier to an irradiation. In those cases, the patients can be medically steered to an eventually vastly improved general condition.

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CHAPTER 10 MEDICO-DEMOGRAPHIC CHANGES AFTER CHORNOBYL CATASTROPHE

10.1 BASIC RADIO-ECOLOGICAL AND SOCIAL- DEMOGRAPHIC CONSEQUENCES OF CATASTROPHE

The explosion of the Chornobyl nuclear reactor, which happened on 4/26/1986, led to the release into the environment large quantities of radioactive substances. They spread almost all over the globe. Irradiation after the passage of radioactive cloud and the formation of radioactive fallout has undergone more than two billion inhabitants of the planet. The largest fall-out was formed in Europe [1, 2]. According to these data it should be recognize that the consequences of the explosion led to global environmental pollution and exposure of billions people of the planet. The main radionuclides, which formed the contamination and exposure on a global scale, were radioactive isotopes of iodine and cesium. The first surrounded people about two months, while the latter formed a loss of soil and the environment for many decades. Strontium, plutonium, americium are also in fall-out in the near zone of the accident.

For the protection of the population living in the 30-km zone around nuclear power plant from the effects of radiation only in Ukraine 91.2 thousand people were evacuated for two months. After 1991 in the country almost 3.4 million people were attributed to the victims of the catastrophe, who needed social, medical and radiation protection. More than 2 million citizens forced to live in contaminated areas

Chornobyl catastrophe had a major impact on living conditions, nutrition and employment of people. Evacuated from the 30-km zone did not return to their homes, they have lost housing, property, work, family ties. According to our generalizations, providing them with new housing and employment lasted almost two years. This area (now it is the exclusion zone) for hundreds of years has become unfit for human habitation and economic activity.

At attributed in 1986 to contaminated Polisske, Ivankiv, Narodychi, Luhyny, Kozets, Ripky and Chernihiv districts (so-called strict radiation control areas) various restrictions modes of life, labor and agricultural production, management livestock, forestry and so on were introduced as radiation protection measures. Consuming of products locally produced and received in individual farms was prohibited. A feeding

by clean delivered products was supposed. Children were transferred to the organized free-of-charge feeding in preschool establishments and schools.

However, these measures by lack of compliance did not give positive results [3, 4].

Therefore, the USSR Ministry of Public Health Board approved on 21.10.1988 (protocol № 30-1) radiological concept of permanent safe living of the population within the territories of the Russian Federation, UkrSSR and Byelorussia, which were subjected to radioactive contamination as a result of the Chornobyl accident (the so-called 35-rem concept). According to it contaminated settlements (they were 686 in the USSR) were divided into three groups.

In the first group people could be exposed for a lifetime to dose up to 35 rems (437 settlements with a population of 181.1 thousand people), in the second group population could be exposed for a lifetime dose of 30–50 rems (141 settlements, 43.9 thousand persons) and in the third group people could be exposed for a lifetime in doses above 50 rems (108 settlements with a population of 22.2 million people).

In the first group of settlements the life and employment of the population had no limitations. In the second group restrictions could be abolished by a centralized set of the complex land improvement works and transfer of private cows to clean fodder. In the third group while maintaining all existing at the time protective measures and restrictions proposed dose limit could be exceeded and people were obliged to resettle in clean areas. In the UkrSSR in this group 65 settlements were assigned with 14,777 families of dwellers. Later in Ukraine their number was increased up to 86.

Government decision on resettlement was adopted in 1990 and it was planned for 1991–1992. Taking together with the independent departure of the inhabitants it caused desertation of large territories in Zhytomyr, Kyiv, Rivne and Chernahiv regions.

After independence, Ukraine had entered the national criteria for assessing levels of contamination and population exposure doses. They essentially differed from what were in former USSR.

Implementation of the voluminous work on the catastrophe consequences liquidation and of the social, medical and radiation protection of victims began. However, it has not yielded positive results and effects of the catastrophe in 25 years is not eliminated [5, 6].

Over the past 11 years such indices as the radiation contaminated areas (RCA), farmland and forests and the extent of contamination have not changed (Table 10.1).

The departure of the victims to clean districts continued, after 1992 the number of such persons culminated almost up to 73 thousand. At the same time, for 18 years in the country the victims were not completely resettled from the zone unconditional (mandatory) resettlement to the clean areas. And as was shown above, at an prohibition of constant residing in this zone, the number of the inhabitants even grew.

That is, according to listed indices for last decade no positive shifts in overcoming consequences of catastrophe has taken place. Till now 12 regions, 73 administrative districts and 2293 settlements (Table 10.2) are considered as radioactive contaminated. It requires a continuation of measures on protection of people and territories and liquidation of consequences of catastrophe as a whole.

Table 10.1. Dynamics of the basic radio-ecological and social-demographic consequences of Chernobyl catastrophe in Ukraine

Parameters	Years	
	2000	2010
The area of the radiation contaminated territory with density of ^{137}Cs fall-out 37 kBq/m ² and more	53454 km ² (4.8% of territory of the country)	53454 km ² (4.8% of territory of the country)
The area of the radiation contaminated agricultural land	4.6 millions hectares (12% of the total area)	4.6 millions hectares (12% of the total area)
The area of the radiation contaminated forests	25,357 km ² (40% of the total area)	25,357 km ² (40% of the total area)
Scales of radioactive contamination	12 regions	12 regions
	73 districts	73 districts
	2,293 settlements	2,293 settlements
Total number of evacuated, moved and independently gone away from zones, in 1986–2009, persons	155,964	More than 164.7 thousand
Persons to obligatory resettlement in 1990–1992 from them were not resettled	43,984	43,984
	9,666	1,985

Table 10.2. The distribution of the radiation contaminated settlements on areas and zones of radioactive contamination in accordance with applicable regulations

Regions	Number of the settlements by zones				
	1–4	1	2	3	4
Vinnitsia	89	0	0	0	89
Volyn	166	0	0	166	0
Zhyomyr	734	7	63	301	363
Ivano-Frankivsk	5	0	0	0	5
Kyiv	559	69	20	33	437
Rivne	342	0	1	273	68
Sumy	9	0	0	0	9
Ternopil	10	0	0	0	10
Khmelnitskiy	9	0	0	0	9
Cherkassy	105	0	0	4	101
Chernihiv	254	0	2	61	191
Chernivtsi	14	0	0	1	13

1 – exclusion zone, 2 – zone of unconditional (mandatory) resettlement, 3 – zone of voluntary guaranteed resettlement, 4 – zone of the strengthened radio-ecological control.

The results of a last of dosimetric passportization [7] testify that nowadays only 814 settlements can be referred as radioactive contaminated, including 76 remaining in the zone 1, 44 — in the 2nd zone, 357 — in the zone 3, and 337 — in the zone 4 (Table 10.3).

At the 25th year after catastrophe living conditions and employment of RCA residents not yet fully meet the requirements of radiation safety.

Table 10.3. Number and distribution of the settlements by parameters of doses in 1997–2007

Year	The passport of an irradiation dose, mSv per year			
	<0.50	0.50–0.99	1.00–4.99	≥5.00
1997	1350	359	442	10
1998	1338	375	441	7
1999	1368	384	400	9
2000	1415	299	441	6
2001	1445	311	392	5
2002	1471	317	372	3
2003	1538	334	289	2
2004	1551	405	207	0
2005	1749	298	116	0
2006	1799	294	72	1
2007	1368	246	57	1

Table 10.4. Retrospective average irradiation dose per person of the population in zones of radioactive contamination of Zhytomyr and Kyiv regions in 1991–2005, mSv per year

Years	Zones		
	2	3	4
1991	3.3±1.73	1.4±0.71	0.5±0.28
1992	2.7±1.39	1.2±0.57	0.4±0.23
1993	2.3±1.13	1.0±0.45	0.36±0.19
1994	2.0±0.95	0.9±0.38	0.34±0.17
1995	1.7±0.80	0.7±0.52	0.26±0.15
1996	1.5±0.70	0.6±0.50	0.24±0.13
1997	1.3±0.58	0.6±0.23	0.24±0.13
1998	1.21±0.50	0.52±0.24	0.23±0.12
1999	1.09±0.43	0.49±0.22	0.21±0.11
2000	1.0±0.40	0.44±0.20	0.20±0.10
2001	0.94±0.38	0.41±0.18	0.20±0.10
2002	0.91±0.35	0.4±0.17	0.20±0.40
2003	0.90±0.32	0.4±0.17	0.2±0.40
2004	0.5±0.19	0.2±0.23	0.08±0.06
2005	0.5±0.19	0.2±0.23	0.05±0.04

Almost the same limit were set for the zone of the strengthened radio-ecological control.

In connection with changes of land ownership in the country in the settlements of RCA for many years was being maintained a high level of unemployment. In addition, in rural areas almost all the work are being carried out in conditions of increased radioac-

Thus, according to the current legislation, the residing of the population, receiving of agricultural and other production, food products that meet acceptable levels of radioactive substances, or which is inexpedient to use by ecological conditions are impossible at the lands of zones restricted and unconditional (mandatory) resettlement.

In the settlements of these areas there are virtually no social, living and medical facilities. Areas of zones guaranteed voluntary resettlement and strengthened radio-ecological monitoring require the realization of measures of radiating protection and others special interventions, directed to limit further exposure and to ensure normal economic activity. In the zone of guaranteed voluntary resettlement are prohibited:

- construction of new and expansion of the working enterprises, not directly related to providing radio-ecological, social protection of population and conditions of its life and work;
- any activity, which worsens a radio-ecological situation; the use of nature, which does not meet the requirements of the norms of radiating safety;
- applying pesticides without special permission of the relevant bodies of the Cabinet of Ministers of Ukraine;
- involving pupils and students to works, which may negatively affect on their health.

tive contamination. Residents of areas subjected to pollution (Table 10.4) continue to be exposed to radiation.

Calculations made by us based on the data [17] show (Table 10.4, 10.5) that most dwellers of high radiation zone 2 have undergone to the highest irradiation and in whom the maximum accumulated dose value by 2005 had exceeds the law for life (70 mSv).

As it is seen from the data of Table 10.6 in 2009 317,467 persons including 87,986 children under the age of 18 years, yet continued to be subjected to radiation doses of more than 0.5 mSv per year [15]. Thus 986 of them can receive an additional dose of radiation more than 5 mSv per year and 135,621 — more than 1 mSv per year.

The country's long economic crisis that is seen by the example of the dynamics of gross domestic product (GDP) size negatively influences on living conditions, labor and food of the RCA dwellers (Fig. 10.1). Therefore, funding of programs to protect people and liquidate the consequences of catastrophe is not complete and causes resentment of affected citizens [5, 6].

In recent years, the liquidation of catastrophe consequences was conducted according to the State Program of Overcoming Consequences of the Chernobyl Catastrophe (SPOCCC) in 2006–2010 [9]. Unfortunately, it was not implemented in full [10, 11].

Using the results of our sociological pool of 2009 of managers and professionals concerned to overcoming the effects of Chernobyl catastrophe, dealing with implementation in 2005–2009 under the national legislation and SPOCCC we found that:

- defined by applicable legislation measures of radiation, medical and social protection of RCA inhabitants were not executed fully and in most areas of funding varied from poor to a minimum up to the complete absence in a number of directions;
- according to experts mind, from 23 required by law countermeasures 9 were "almost not performed" (up to 10 points), 5 were performed "below average" (11–40 points),

Table 10.5. Total irradiation doses of the population of radiation contaminated zones of Zhytomyr and Kyiv regions accumulated for 1986–2005, mSv

Radiation contamination zones	Total accumulated irradiation dose, mSv			
	minimum	median	maximum	average
2	19.3	60.1	149.0	64.8±27.7
3	8.4	22.6	49.2	24.6±9.4
4	1.6	8.4	30.5	9.08±5.0

Table 10.6. The distribution of the population living on the RCA and able to receive from the Chernobyl fall-out additional irradiation dose more than 0.5 mSv per year, in 2009

Average dose per year, mSv	Aggregate number, persons	Children at the age up to 18 years, persons
>5	986	319
1 – <5	135621	32758
0.5 – <1	179874	54909
Total	317467	87986

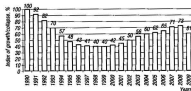


Fig. 10.1. Size of a gross domestic product in Ukraine, as% to 1990

3 were of "medium level" (41–60 points), 5 — "more than average" (61–80 points) and 1 was "good" (81–90 points);

- the dispersal of funds and lack of protection measures stipulates that at present there is no settlement belonging to contaminated, which would be removed from the zone of contamination;
- not fully implemented soil-conservation actions that could help to reduce radiation risks, namely: liming of contaminated agricultural land, introduction of high doses of fertilizers, creation of meadows and improvement of contaminated meadows and pastures, distribution of fodder with radioprotective properties;
- a shortage of devices, materials and experts for the realization of the radiation control of soil, waters, air, agricultural raw material, food products and wood production, definition irradiation doses; absence of mutual understanding between local authority and the population of contaminated areas. To fully meet the requirements of Permissible levels-2006 it is needed urgently to supply radiological laboratories of villages, districts, and department of sanitary-epidemiological service with modern modern spectrometer hardware and devices.

Experts have identified as priority countermeasures:

- exclusion of contaminated food consumption, production of clean milk in individual farms, improved control over the environment, ensuring compliance with radiation safety standards, the introduction of new standards for food, build a new shelter for the 4th block and afforestation of technologically contaminated land;
- opposition to the self-migration in settlements of zones alienation and unconditional (obligatory) resettlement, from which residents had been relocated;
- reduction of radiation risks to the health of every citizen of RCA and the population as a whole;
- immediate restoration of countermeasures for the prevention of internal exposure of critical settlements population;
- prevention of public exposure when staying in the forest and use of wild berries and mushrooms;
- implementation of information programs for different categories of dwellers on contemporary problems of RCA.

The results of the specialists and administrators pool were generalized in "Analytical studies of performance in the years 2005–2009 measures of radiation, medical and social protection of dwellers of contaminated by the Chernobyl catastrophe areas in Ukraine," which in January 2010 was sent to the Ministry of Ukraine of Emergencies and Affairs of Population Protection from the Consequences of Chernobyl Catastrophe, Ministry of Public Health of Ukraine, Academy of Medical Sciences of Ukraine, the Verkhovna Rada of Ukraine Committee on Environmental Policy, Natural Resources and Elimination of Chernobyl Catastrophe Consequences and the National Commission on Radiological Protection.

This information was offered to take into account during the performance in 2010 of the SPOCCC and in developing new program for the next period. General strategy to overcome consequences of catastrophe on RCA in remote period should be directed to elimination of the basic risk forming factor — radiation.

10.2 DEMOGRAPHIC SITUATION IN UKRAINE AND ON THE RADIATION CONTAMINATED TERRITORIES

In Ukraine, the study of medical-demographic situation in connection with the Chernobyl catastrophe began in the early months after the reactor explosion and all the years have been and remains an integral part of scientific developments in the study and evaluation of medical and radiological consequences. In Ukraine they covered almost all areas, which according to national laws were defined as radioactively contaminated.

Population size, migration, the number of abortions, birth, babies, stillbirths, deaths and the distribution of their sex, age and cause of death, fertility, mortality, infant mortality, overall mortality, population losses, life expectancy and various factors affecting their implementation (standards of living, levels of contamination with radionuclides, radiation dose) were assessed. Study, including control area, has covered almost 22 million people. Period of observation was mainly since 1979, the most radioactively contaminated Zhytomyr, Kyiv, Rivne and Chernihiv regions and their districts were studied particularly careful.

Information fundamentals of our research was established automated control system of database management of medical and demographic consequences of the Chernobyl catastrophe (ACS DB DEMOSMONITOR) [13]. The research results are reflected in international publications [14], monographs [15, 16], national reports, materials of parliamentary hearings, information and reference materials of the Cabinet of Ministers of Ukraine for overcoming the consequences of the Chernobyl catastrophe and statistical reference books [17].

In the absence of wars and epidemics the population of Ukraine reduced dramatically rapidly — from 800 to 250 thousand people per year just in the last decade (Fig. 10.2). Since 1991 the country lost nearly 6.5 million people.

In a demographic situation some improvement became positive. For last years it has grown almost in 1,5 times. At the same time the mortality is kept on a high level. From 15,4 ‰ in 2000 it has amount to 16,6 ‰ in 2006. Last years it's is. That should be estimated as positively and it is possible to expect a preservation of this tendency in the future.

Positive in the demographic situation is a slight improvement of birth rate (Fig. 10.3) In recent

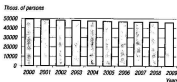


Fig. 10.2. Average annual population of Ukraine in 2000–2009, thousand persons. 2009 — as on 01.01.2010

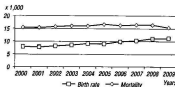


Fig. 10.3. Birth rate and mortality of the population of Ukraine in 2000–2009, per 1,000 population

years it has grown almost 1.5 times. At the same time at a high level of mortality is stored. From 15.4 ‰ in 2000, it reached in 2006 16.6 ‰. In subsequent years some decrease was marked. This trend should be evaluated positively and is expected to be maintained in the future.

As it is seen from the data of this figure, the mortality prevailed over births rate since 1991. This circumstance was the main cause of depopulation in the country. The number of children deaths under 1 year grew. In 2000–2006 on average 4216 babies died for the year, in 2007–2008 — 5083. However, the level of infant mortality varied within the range of 9.5–11.9 per 1000 live births and has a downward trend (Fig. 10.4). But these levels are high enough and point to trouble with infant mortality in the country.

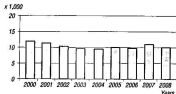


Fig. 10.4. Infant mortality in Ukraine in 2000–2008, per 1,000 live births

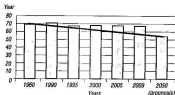


Fig. 10.5. Average life expectancy at birth in Ukraine, years

Negative trends in health status of citizens caused low average life expectancy in the country (Fig. 10.5). It dropped from 71 years in 1990 to 67 years in 2009. According to forecasts, the reduction will continue till 2050.

Comparative data (Fig. 10.6) show that if in Japan, Western Europe and Cuba after 1990 average life expectancy level increased, in Ukraine it was reduced. It is predicted that by 2050 the gap in the level indices of comparable countries reached nearly 30 years.

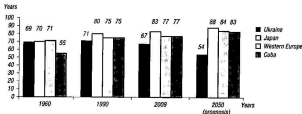


Fig. 10.6. Average life expectancy in the countries of the world, years.

In the most affected regions (Zhytomyr and Kyiv) in recent years the demographic situation essentially did not differ from national parameters. Thus, the birth rate in this regions in 2000–2008 was even slightly higher (Fig. 10.7).

Mortality was higher (Fig. 10.8): in Ukraine its average level for 2000–2008 was 16.0‰, in Zhytomyr region it was 17.8‰, and in the Kyiv region — 17.4‰. But this differences were statistically not significant ($t=1.2$ and 0.35).

Infant mortality in regions did not exceed national levels (Fig. 10.9).

Data differentiated by zones of radioactive contamination are important for assessing the demographic consequences of Chernobyl catastrophe. As it is shown in Fig. 10.10, in 1991–2005 the population losses in the areas of contamination were more pronounced than in areas of their location. According to our estimates, in 1991–2000 52–98% loss of population zones 2 and 3 was caused by migration outflow of population (organized and voluntary resettlement). In subsequent years the cause of such losses was the prevalence of deaths over births.

In the radioactive the contaminated districts, in particular in zones 2 and 4, the population is elder on age, than in other territories of the country

In contaminated areas, especially in zones 2 and 4, the population is older than in the rest of the state (Fig. 10.11).

In 1992–1999 increased levels of birth rate in zones 2 and 3 was noted (Fig. 10.12). They occur in the most active period in the resettlement from the affected to clean area and, in our opinion, it was connected with a desire to have advantages in obtaining housing and employment in new settlements.

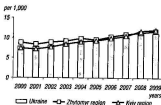


Fig. 10.7. Birth rate in Ukraine, Zhytomyr and Kyiv regions in 2000–2009, per 1,000 population

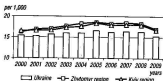


Fig. 10.8. Mortality of the population in Ukraine, Zhytomyr and Kyiv regions in 2000–2008, per 1,000 population.

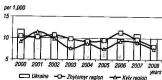


Fig. 10.9. Infant mortality in Ukraine, Zhytomyr and Kyiv regions in 2000–2008, per 1,000 live births

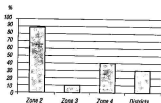


Fig. 10.10. Loss of the population depending on the category of radioactive contamination zone in 1991–2008, %.

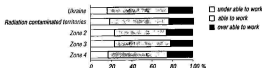


Fig. 10.11. Population structure by major age groups according to zones of radioactive contamination on 01.01.2008, % (calculated according to the State Statistics Committee of Ukraine)

At the same time in all the studied areas significantly ($p < 0.05$) increase in mortality was noted with an annual growth rate $+0.19\%$ in Ukraine, $+0.78\%$ — in zone 2, $+0.33\%$ — in zone 3, $+0.89\%$ — in zone 4 (Fig. 10.13). Mortality depend on the category of radioactive contamination zones and the level of average individual dose of radiation regulated by the legislation.

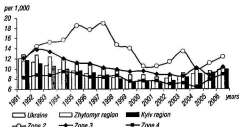


Fig. 10.12. Birth rate of the population in Zhytomyr and Kyiv regions and investigated districts of zone 2, 3, 4 in 1991–2006, per 1,000 population.

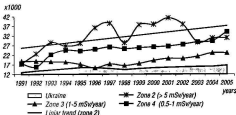


Fig. 10.13. Mortality of the population in 1991–2005 depending on the category of contamination zone and level of total irradiation dose, regulated by the legislation, per 1,000 population

During the entire postaccidental period the death rate in the four most contaminated districts of study (combined from zones 2, 3, 4) increased more distinctly than in Ukraine as a whole (Fig. 10.14).

These data indicate that radioactive contamination and prolonged exposure formed elevated levels of mortality. Analysis of correlations between the relative risk of mortality from all causes of death, tumors and non-tumor somatic pathology and accumulated collective radiation dose found in most cases weak relationship (0.07–0.34) between studied indices.

The vitality of the RCA population was 1.5–2.4 times less than in the control (zone 2 — 0.38 zone 3 — 0.42, zone 4 — 0.29, Ukraine — 0.60, Zhytomyr region — 0.56, Kyiv region — 0.55) and demographic crisis was more expressive (Fig. 10.15). Thus, if in the early 90s reproduction of RCA population was low negative (zone 2 — -5.6‰, zone 3 — -6.0‰, zone 4 — -9.1‰), in 2006 it became negative catastrophic (zone 2 — -20.6‰, zone 3 — -14.0‰, zone 4 — -21.5‰).



Fig. 10.14. Mortality of the population of Ukraine in 1986–2008 and in four investigated most radioactive contaminated districts (zone 2, 3, 4), per 1,000 population. The direct line — a line of trend on four areas

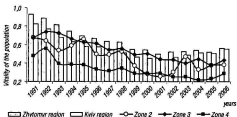


Fig. 10.15. Vitality of the population in Zhytomyr and Kyiv regions and in investigated districts (zone 2, 3, 4) in 1991–2006, per 1,000 population.

Demographic changes on RCA generated and made growing population losses. According to the balance method of estimation, for 18 years (1986–2003) complete demographic losses in the contaminated areas amounted to 48.8 thousand people. Net losses of population were formed by 25.2 thousand unborn and surplus (6.9 thousand) of deceased persons. Ivankiv and Ovruch districts are the leaders in absolute size of net losses. The loss of rural residents played a central role in the formation of net losses. Radioactively contaminated areas on average "received less" eight newborns in towns and 11 in rural areas per every thousand of average population (in the control respectively 4 and 2 infants).

The level of unborn losses per 1,000 women of child-bearing age from 8 in 1986 rose to 76 in 2001, i.e. 9.5 times and make 41.1% within the whole post-accidental period. The intensity of unborn losses in the control area was similar to the national level and its value for the 1986–2003 amounted to 14.2%, which is 3 times lower than in RCA.

Demographic losses of the population were formed through the "delay" of birth by women of most active generative age. The most significant in the total amount of losses was unborn contribution of women aged 20–29 years. At their expenses for every 1,000 women in post-accident period from 39 (Poltava region) to 215 (Narodychi district) births received less. In Ukraine in 1986–2003 comparing with 1979 intensity of unborn losses per women aged 15–49 years was 15.5%.

The hypothetical population losses because of high mortality for the period from 1986 to 2003 (compared to 1979) were formed in all investigated areas. By the absolute number the urban population had the greatest losses, except for districts of RCA, where their level was higher in a countryside, which were more suffered as a result of catastrophe.

The biggest losses through excess of the mortality in the postaccident period undergone the population at the age of 60 years and elder both on RCA and in the control (more than 96% from the total number of losses). In the children's age groups, by contrast, there was little increase saved lives by reducing their mortality. The largest decrease in the absolute number of deaths occurred in children under 1 year (about 3.0% of total losses).

During 1986–2003 losses of the working age population (15–59 years) ranged from 7.5% (Zhytomyr region) to 12.3% (Kiev region) in total losses were lower than in the control area (16.8%) and Ukraine as a whole (18.7%).

The number of saved lives of working age (in contaminated areas — 1123, in control Lohvytsky district — 223 people), was associated with the arrival in contaminated areas of the new population that had not undergone irradiation.

In 1986–2003 compared with 1979 figures of population loss through supermortality increased in all major classes of causes of death everywhere. Biggest population losses were formed by non-tumor somatic pathology (about 40.0% in female and almost 24.0% in male).

Tumors were the next significant class of death. Their contribution to total losses of the radioactively polluted areas in the 1986–2003 was 35.1%, while at the national level — only 15.9%. Much of the losses from tumors was formed by age group of 45–75 years,

Threatening in the post-accident period were the scales of mortality from external causes of death. Their share of population losses from all causes of death was about 20%. Now compared to 1979 due to external causes supermortality cause 17% of male and female 4% losses.

Demographic situation was also worsened through ecological migration [19]. During 1979–2003 migration losses of population attained in the Polisske district 28.3 thousand persons, in Narodychi — 15.6 thousand persons, in Luhyny — 10.4 thousand persons, in Ovruch — 23.9 thousand people. In the control Lohvytsky district it was less than 2.4 thousand persons. Only Ivankiv district grew due to migration and administrative-territorial reform on 0.8 million people.

Intensive outflow of youth, mainly women drastically undermined the basis of demographic reproduction of rural residents. Established in the most suffered regions and districts the peculiarity of demographic losses testify to intensification of depopulation and losses of labor potential. That is, RCA lose their social, economic and commercial purposes.

For post-accident period both regular and standardized mortality rates were increased. Their level increased during 1991–1995 mainly due to mortality from non-tumor somatic diseases and external causes (in radiation contaminated districts — by 36% in polluted regions — by 24% in the control — by 20%).

Age-related relative risks for causes of death suggest that the widespread increase in mortality was mainly caused by the working age population mortality from somatic diseases and external causes. In general upward trend of risk of death from tumors in patients over 45 years both on contaminated and in control areas there are groups of high-risk in relation to this pathology. In the Zhytomyr region these are children under 1 year and women aged 20–29 years in Kyiv and Chernihiv region — people aged 15–29 years.

The negative demographic situation in the RCA became the logical deepening of catastrophic changes of the most important demographic indices that have occurred since the early years of irradiation. According to our data (Table 10.7), in 1986–1987 due to the effects of acute exposure levels of growth spontaneous miscarriages, stillbirths, congenital anomalies at birth, reduced fertility, disturbance in sex ratio of newborns, infant mortality were increased. These data are confirmed in the studies of Franco-German Initiative for Chernobyl and demonstrate the negative impact of radiation on fertility and infant mortality after accidental exposure in 1986 [20].

Table 10.7. Consequences of exposure of a sharp emergency irradiation in 1986 on organism of the population of regions of the strong radiating control

Parameter	The level of changes in 1986–1987	The cause of changes
Spontaneous abortion	1.5 times increase	teratogenic action on fetus
Stillbirth rate	increase by 25–30%	teratogenic action on fetus
Congenital anomalies in birth	1.7 times increase	mutagenic action
Birth rate	decrease by 15–20%	sexual function decrease, fetus damage
Gender ratio in the structure of the newborn infants	increased number of boys	sexual chromosome damage
Infant mortality	increase by 20–30%	fetus damage

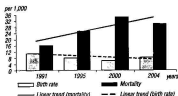


Fig. 10.16. Birth rate and mortality in the zone of unconditional (mandatory) resettlement, per 1,000 population.

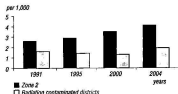


Fig. 10.17. Mortality of children in the age of 0-14 years, dwellers of zone 2 and the radiation contaminated districts, per 1,000 children.

higher than in the contaminated areas. This is evident in mortality among children aged 0-14 years (Fig. 10.17).

In contrast to the nationwide indices in contaminated districts high levels of infant mortality remained and in its structure increased mortality of infants aged 0-6 days (Table 10.8).

Table 10.8. The nature of changes in the structure of exposed infants mortality

Parameter	Character of changes
Mortality in the age of 0-6 days	Increase against a background of decrease of stillbirth
Mortality in the age of 0-27 days	The relative risk is authentically higher, than in the control
Early neonatal, perinatal, neonatal, postneonatal mortality	The weak correlation of parameters changes with the levels of soil contamination by ^{137}Cs , individual average and collective (by administrative districts) irradiation doses of thyroid gland and whole body

In view of known radiobiological data revealed changes we associate with radiogenic consequence of exposure to different systems of the body of parents, their sexual sphere, sex chromosome, embryo, fetus in the womb.

Accumulated in previous years violation of the organs and systems were realized since 1991 in the most contaminated areas in a long time reducing fertility, increasing total and infant mortality.

As it is visible from Fig. 10.16, in 2004 the birth rate in the zone of unconditional (mandatory) resettlement, in which radiation doses for a long time reached 5 mSv per year had decreased by almost 30%. At the same time mortality increased from 16.3% to 30.4%. These levels were considerably above national and regional.

Unlike other areas, mortality in all age groups in this zone was

In the neonatal mortality increased proportion of endogenous causes of death. The relative risk of infant mortality in the contaminated areas since 1991 probably exceeded 1.0 and was due to increased mortality in the neonatal period. In control areas the level of relative risk of infant mortality in this age was less than in polluted and did not affect

the increased relative risk of mortality. Epidemiological analysis found a weak correlation between changes in stillbirths, early neonatal, perinatal, neonatal, postneonatal mortality and radiation factors such as levels of ^{137}Cs soil contamination, the average individual and collective (in administrative districts) of the thyroid and total body radiation doses of population living in the most affected districts of Kyiv and Zhytomyr regions.

Simultaneously with the beginning of infant mortality growth since 1991 started increasing of general mortality.

According to the data set it should be recognized that due to the acute and long-term for 25 years chronic exposure to small doses of radiation from the time of conception and childhood negative systemic changes were formed in the body. The changes coincide with those obtained by other researchers.

General conclusion

Taking into account knowledge accumulated over 25 years the main consequences of the Chernobyl catastrophe in Ukraine should be recognized as:

1) large-scale radioactive contamination areas. In Ukraine, in a radius of 100 km around Chernobyl NPP was formed radioactively dangerous land (zones 1 and 2), which cannot be the residence of people, receipt of agricultural and other products, foods that meet national permissible levels. Involuntary resettlement of people in 1986–2008 led to desolation of area of more than 4.2 thousand km² (about 7.7% of the total radioactively contaminated area). A so-called environmental migration emerged in connection with the catastrophe and its consequences;

2) exposure of large numbers of people, increased acute human exposure in the first year after the catastrophe and long-term chronic exposure to low levels of radiation, simultaneous effects on the body of several types of external exposure, entry into organism of radionuclides from the set of high biological risk and types of radiation (gamma, beta, alpha), a coupling effect of external and internal radiation, which contributes in its totality enhanced effects of radiation on the body;

3) long duration (hundreds of years) of radioactive contamination of the environment, the formation of areas, entirely not suitable for living and production activities, loss of large areas of recreational, residential, historical, cultural and ethnographic purposes;

4) total depopulation of the two cities and over a hundred villages, irreversible degradation of at least 67 villages, one city and three settlements of urban type, accelerated degradation of about a thousand villages located in the northern part of Zhytomyr and Kyiv regions and in the western part of the Chernihiv region. In any of the 2,293 settlements referred in 1991 as contaminated in 25 years were not resumed pre-accidental conditions of residence by radiation factor;

5) limited conditions of life, work, nutrition, physical and mental suffering of millions of people and material damages from loss of property, work and earnings shortfall. Together with the effect of accidental exposure they adversely affected human health;

6) the incapacity of the affected countries and the international community within 25 years to overcome its consequences;

7) in the aggregate of surveyed medical and demographic indices the health of RCA inhabitants, submitted to chronic exposure and poor conditions of life and labor,

is worse than the population of Ukraine as a whole. The most negative conditions of life, work, high levels of exposure and mortality are for residents of zone 2;

8) epidemiological analysis of connections fertility, infant mortality, overall and cause-specific mortality with levels of radioactive contamination, radiation doses of thyroid gland and the average accumulated doses of radiation testify to their certain dependence on exposure. Effects of acute exposure in the first post-accidental year led to a drop in fertility in 1986, rising infant mortality in 1987, violation of the sexual proportion of newborns mainly by increasing the proportion of boys. In the following years as a result of chronic exposure in the most affected areas characteristic feature was the increased mortality mainly from somatic pathology. Its certain dependence from levels of contamination and irradiation was marked. This pattern was characteristic both at a level of regions and districts. In the totality, they have led to a more expressive demographic losses, decreased vitality and longevity of RCA inhabitants than residents in the country as a whole;

9) to improve the demographic situation on RCA it is necessary to create conditions by the radiation factor, which would meet national safety standards for levels of radioactive contamination and exposure of the population. These factors will eliminate existing socio-psychological stress in the affected as the basis of violated demographic guidelines to have children, stop chronic irradiation with small doses of radiation as a basis for the formation of destructive changes in humans and excess mortality rates and create economic conditions that would ensure decent standards of modern living conditions and labor;

10) taking into account the peculiarities of demographic losses formation depending on the contribution of certain classes of causes of death, measures to prevent them at RCA should be differentiated. Major efforts should be aimed for the prevention of mortality of able-bodied and elder than able-bodied ages, and among them — males. When formulating migration policy the existing radiation situation and the level of expected exposure doses must be taken into consideration.

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(M. Omelyanets, N. Ganko, N. Dubova)

CHAPTER 11 EPIDEMIOLOGY OF NON-TUMOR DISEASES IN THE REMOTE POSTRADIATION PERIOD

11.1 EFFECTS IN THE CHORNOBYL CLEAN-UP WORKERS OF 1986–1987

Non-tumor morbidity

Non-tumor morbidity in clean-up workers of 1986–1987 was studied on the data of the SRU, for 1988–2007. As on January 1, 2009 314,192 clean-up workers were registered in the SRU [1], 207,486 of them were clean-up workers of 1986–1987. A specific feature of age structure of clean-up workers of 1986–1987 is that these were mostly (93%) young and middle-aged males, 87% of them were under 40 at the time of their participation in recovery operations, average age of this cohort was 30.2 years.

On January 1, 2010, there were 91,253 people having data on dose in the SRU, of them 68,145 were clean-up workers of 1986–1987. Average individual doses from external irradiation for the majority of them were estimated to be 120–180 mSv, 15% having doses 250 mSv and more, 7.5% — 500 mSv and over. Average individual effective dose of whole body external radiation for the cohort was 163.7 mSv.

Distribution of clean-up workers of 1986–1987 with regard to age at the time of participation in recovery operations and whole body external dose is given in Table 11.1. In age group <40 years, 66.1% of persons had doses within interval 0.2–0.7 Gy, 23% — 0.05–0.199 Gy. In the age group ≥40 years, 9.6% of men had doses 0.2–0.7 Gy, 7.8% — 0.05–0.199 Gy.

Table 11.1. Distribution of clean-up workers of 1986–1987 with regard to the age at the time of participation in recovery operations and whole body external dose (persons)

Age groups	External doses, Gy					The total number of persons
	<0.05	0.05–0.099	0.1–0.199	0.2–0.249	0.25–0.7	
18–39	6754	18675	15121	14913	6609	59692
40–60	1046	1777	1414	1077	739	8453
Total	7800	20400	16400	16145	7400	68145

A retrospective-prospective epidemiological monitoring was conducted to investigate non-tumor morbidity (within 1988–2007) in clean-up workers of 1986–1987. Now, the health state of the Chernobyl clean-up workers 1986–1987 is estimated to be poor [2, 3].

Within observation period of 1988–2007 percentage of healthy individuals decreased from 67.6% to 5.4%, that of persons with chronic non-tumor diseases increased from 12.8% to 82.3% (Fig. 11.1), [4].

Fig. 11.2 presents dynamics of the overall non-tumor morbidity rates in cohort of clean-up workers of 1986–1987 as a whole and with dependence upon age at the time of participation in recovery operations, by 5-year observation periods. Over 1988–1997, no significant differences in overall non-tumor morbidity rates were noted in the cohort as a whole (regardless of age) and in the above mentioned age groups (under 40 and older). Since 1997, an increase in the overall morbidity rates has been observed. This growth was due to higher rates in clean-up workers aged ≥ 40 .

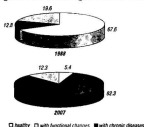


Fig. 11.1. Dynamics of integral health indices for the Chernobyl clean-up workers of 1986–1987 within 1988–2008

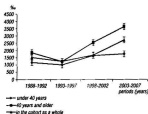


Fig. 11.2. Dynamics of cumulative non-tumor morbidity rates among the Chernobyl clean-up workers of 1986–1987 depending on the age at the time of participation in recovery operations, by 5-year observation periods

Structure of non-tumor morbidity in the cohort of clean-up workers as a whole and by age groups for 2007 is presented in Fig. 11.3. The following (digestive and circulatory systems diseases, diseases of nervous system and

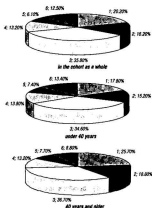


Fig. 11.3. The structure of non-tumor morbidity among clean-up workers of 1986–1987 for 2007. 1 — circulatory system diseases; 2 — diseases of nervous system and organs of sense; 3 — digestive system diseases; 4 — musculoskeletal system diseases; 5 — endocrine system diseases; 6 — other diseases

Table 11.2. Dynamics of non-tumor mortality rates among the Chernobyl clean-up workers of 1986–1987 in the cohort as a whole (regardless of age) by 5-year postradiation periods (%)

Diseases and nosological forms of diseases	Case ICD-9	Rates for observation years (periods)			
		1986–1992 (1)	1993–1997 (2)	1998–2002 (3)	2003–2007 (4)
Endocrine and metabolic diseases:					
- diabetes	240–249	148.2±7.15	107.9±5.4	176.4±8.82	166.6±8.33
- thyroid diseases	250	18.1±0.91	17.1±0.86	31.1±1.6	36.9±1.9 ^{ns}
- non-toxic nodular goiter	240–246	91.2±4.6	72.4±3.62	96.4±4.82	96.2±4.8
- thyrotoxicosis with or without goiter	241	4.0±0.19	7.0±0.3	13.5±0.68	21.4±1.1 ^{ns}
- acquired hypothyreoidism	242	2.1±0.1	2.8±0.12	2.6±0.11	1.5±0.08
- thyrotoxic	244	1.8±0.07	1.8±0.1	5.7±0.3	7.7±0.4 ^{ns}
- thyrotoxic	245	4.8±0.2	14.1±0.71	22.1±1.1	18.0±0.88 ^{ns}
Mental disorders	290–319	0.23±0.01	0.31±0.01	4.2±0.21	3.2±0.14 ^{ns}
Diseases of nervous system and organs of sense:	320–389	348.5±17.4	236.9±11.9	456.2±22.8	439.3±22.0
- vegetovascular dystonia	337	63.7±3.2	85.8±4.29	58.8±2.94	33.2±1.66
- cataracts	366	8.5±0.37	22.5±1.1	91.1±4.6	89.1±4.5 ^{ns}
Circulatory diseases:	390–459	350.8±17.5	281.3±14.1	411.4±20.6	440.0±22.0
- hypertension	401–406	185.7±9.3	86.2±4.31	102.8±5.14	85.2±4.26
- ischemic heart disease	410–414	129.6±6.45	117.9±5.9	153.6±7.68	150.3±7.5
- cardiovascular diseases	430–438	20.9±1.05	55.0±2.7	128.5±6.3	169.5±8.5 ^{ns}
Respiratory diseases:	460–519	119.8±6.0	114.0±5.74	229.2±11.5	246.7±12.3 ^{ns}
- chronic bronchitis, emphysema	491, 492	105.8±5.29	96.2±4.81	189.4±10.0	215.9±10.8 ^{ns}
- bronchial asthma	493	1.1±0.06	0.73±0.04	0.41±0.02	—
Digestive diseases:	520–579	343.3±17.2	302.7±15.1	737.4±36.9	971.2±48.6 ^{ns}
- gastric and duodenal ulcers	531, 532	55.2±2.8	41.9±2.1	50.4±2.52	53.2±1.06 ^{ns}
- gastritis, duodenitis	535	38.9±2.0	35.0±1.8	81.9±4.1	96.1±4.8 ^{ns}
- liver diseases	571	107.2±5.36	136.1±6.8	326.1±16.4	426.5±21.3 ^{ns}
- biliary diseases	574–576	81.8±4.1	88.0±4.3	235.4±11.8	322.5±16.1 ^{ns}
Genitourinary diseases:	580–589	2.0±0.11	1.9±0.11	4.4±0.2	4.1±0.17 ^{ns}
- urinary diseases	590–599	24.0±1.1	44.8±2.2	75.1±3.8	74.3±3.4 ^{ns}
Diseases of the skin and subcutaneous tissue	680–709	11.4±0.57	10.6±0.53	13.5±0.68	9.5±0.5
Musculoskeletal diseases:	710–739	163.0±6.2	154.9±7.8	302.3±15.1	359.4±18.0 ^{ns}
- dermopathy	720–724	59.0±2.95	36.8±1.78	51.3±2.6	54.2±1.1 ^{ns}
- osteopathy, chondropathy	730–739	66.1±0.31	74.8±1.74	184.9±9.1	240.4±12.0 ^{ns}
Total	240–739	1512.2±75.6	1245.2±62.3	1672.7±83.8	2714.2±135.7

* significant increase in rates relative to the first period; ^{ns} relative to the second period; ^{ns} relative to the third period

organs of sense, musculoskeletal and endocrine systems diseases) contributed mostly to health deterioration of contingent under study (regardless of age at the time of their participation in recovery operations). In fact, percentage of non-tumor diseases was 86.4% among persons aged 18–39 and 91.2% — in those aged 40–60 at the time of exposure.

The remote postChornobyl period was characteristic by growth of a wide range of non-tumor diseases in clean-up workers of 1986–1987 [5, 6], involving practically all body systems except skin and subcutaneous tissue (no significant changes were found) [7, 8]. The dynamics analysis of non-tumor diseases (Tables 11.2–11.4) in contingent under study revealed the existence of certain differences depending on age at the time of exposure. These differences were related both to morbidity growth rate and postradiation terms of disease development.

The substantial growth in mental disorders in both age groups was noted 11–15 years after exposure (Fig. 11.4). Higher rate was in younger age group.

It is not easy to explain this fact. It can be assumed in this case more expressed psychological stress caused by the youths' fear of the possible ionizing radiation risk to their health. It is not also improbable that mental disorders are the result of changes in brain structures and other systems [9]. During post-accident period, the incidence rates for the diseases of nervous system and organs of sense grew in both age groups

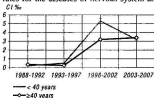


Fig. 11.4. Dynamics of mental disorders (code ICD-9, 290-319) in clean-up workers of 1986-1987 depending on age at the time of exposure

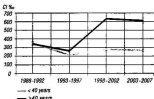


Fig. 11.5. Dynamics of nervous system diseases and organs of sense (code ICD-9, 320-395) in clean-up workers of 1986-1987 depending on age at the time of exposure

(Fig. 11.5). The highest incidence being observed within 11–15 postradiation years. The maximum increase in diseases was recorded among people aged 40–60 at the time of exposure.

Dynamics of vegeto-vascular dystonia is shown in Fig. 11.6.

The highest incidence rates for this pathology were established among clean-up workers (younger age group) within the first 5 years and 6–10 years after exposure. In subsequent years, incidence rates for the disease decreased in both age groups.

The dynamics observed in the development of cataracts was quite different (Fig. 11.7). The highest growth rate of the disease was recorded in people aged 40–60 at the time of exposure. However, a significant increase in morbidity in the older age group was found earlier — in 6–10 years after exposure while in younger one — in 11–15 years after exposure. Role of «Age» in this case is obvious. But it is worthy of notice that cataract incidence rates in the age group 29–54

Table 11.3. Dynamics of non-tumor morbidity rates among the Chernobyl clean-up workers 1986-1987 aged <40 at the time of their participation in recovery operations, by five-year postirradiation periods (%)

Classes and nosological forms of diseases	Code ICD-9	Rates for observation periods (years)			
		1988-1992 (1)	1993-1997 (2)	1998-2002 (3)	2003-2007 (4)
Endocrine and metabolic diseases:	240-289	158.1±7.9	103.0±5.2	137.2±6.3 ^{ss}	130.1±6.5
- diabetes	250	5.7±0.3	8.3±0.35	12.8±0.64	15.1±0.76 ^{ss,ss}
- thyroid diseases	240-246	121.6±6.1	80.4±4.0	93.5±4.7	89.8±4.5
- toxic nodular goiter	241	4.4±0.2	4.4±0.16	10.0±0.47	14.6±0.73 ^{ss,ss,ss}
- thyrotoxicosis with or without goiter	242	3.0±0.11	2.6±0.1	2.1±0.11	1.0 ± 0.04
- acquired hypothyroidism	244	1.5±0.08	1.6±0.07	5.4±0.3	7.8±0.39 ^{ss,ss,ss}
- thyroiditis	245	5.5±0.28	16.1±0.71	21.5±1.1	13.4±0.67 ^{ss}
Mental disorders	290-319	0.2±0.01	0.5±0.03	5.3±0.27	3.2±0.14 ^{ss}
Diseases of nervous system and organs of sense:	320-369	354.4±17.6	212.7±10.6	278.5±14.0	266.4±13.3
- vegetovascular dystonia	337	76.2±3.9	104.3±4.2	66.8±3.3	40.8±2.0
- cataracts	366	4.4±0.17	6.3±0.32	24.9±1.2	26.4±1.34 ^{ss,ss}
Circulatory diseases:	390-459	174.6±8.7	158.1±7.9	263.3±13.2	275.4±13.8
- hypertension	401-406	133.3±6.7	74.6±3.7	92.3±4.6	83.1±4.2
- ischemic heart disease	410-414	22.2±1.1	49.0±2.51	91.0±4.62	98.3±4.9 ^{ss,ss}
- cerebrovascular diseases	430-438	6.6±0.26	25.5±1.3	59.5±3.0	72.6±3.4 ^{ss,ss}
Respiratory diseases:	460-519	84.2±4.2	101.0±5.1	171.9±8.6	180.4±9.0 ^{ss,ss}
- chronic bronchitis, emphysema	491, 492	74.5±3.7	81.8±4.1	150.2±7.51	159.3±8.0 ^{ss,ss}
- bronchial asthma	493	0.7±0.04	0.7±0.04	0.3±0.02	-
Digestive diseases:	520-579	273.2±13.7	271.9±13.4	522.8±26.1	607.8±30.4
- gastric and duodenal ulcers	531, 532	45.7±2.3	35.7±1.8	41.8±2.1	38.3±1.9
- gastritis, duodenitis	535	37.4±1.9	35.6±1.78	67.4±3.4	68.2±3.41 ^{ss,ss}
- liver diseases	571	77.9±4.0	113.3±5.7	218.7±10.9	261.8±13.1 ^{ss,ss}
- biliary diseases	574-576	55.6±2.78	72.0±3.4	151.8±7.6	187.8±9.4 ^{ss,ss}
Genitourinary diseases:	580-589	2.0±0.1	1.9±0.09	3.5±0.18	3.4±0.14 ^{ss,ss}
- urinary diseases	590-599	16.9±0.8	31.6±1.58	51.5±2.6	48.8±2.4 ^{ss,ss}
Diseases of the skin and subcutaneous tissue	680-709	12.3±0.6	10.8±0.51	20.6±0.92	9.4±0.47
Musculoskeletal diseases:	710-739	105.2±5.26	122.0±6.1	208.3±10.4	240.0±11.7 ^{ss,ss}
- dorsopathy	720-724	38.3±1.9	30.0±1.42	40.9±2.1	43.0±2.21 ^{ss}
- osteopathy, chondropathy	730-739	43.1±2.2	61.6±3.1	123.8±6.19	155.7±7.8 ^{ss,ss}
Total	240-739	1169.8±58.4	1002.7±50.1	1643.3±82.2	1753.6±87.8

* significant increase in rates relative to the first period; ^{ss} relative to the second period; ^{ss,ss} relative to the third period

Table 11.4. Dynamics of non-tumor morbidity rates among the Chernobyl clean-up workers 1946–1987 aged ≥40 at the time of their participation in recovery operations, by five-year postirradiation periods (%)

Classes and nosological forms of diseases	Code ICD-9	Rates for observation periods (years)			
		1988–1992 (1)	1993–1997 (2)	1998–2002 (3)	2003–2007 (4)
Endocrine and metabolic diseases:	240–249	140.3±4.1	113.1±5.7	215.6±10.6	203.2±10.2 ^a
– diabetes	250	30.9±1.6	40.0±1.3	40.0±1.9	69.0±3.0 ^a
– thyroid diseases	240–246	60.6±3.0	64.6±3.21	90.2±5.0	100.3±5.02 ^a
– nontoxic nodular goiter	241	3.7±0.2	9.8±0.5	17.2±0.86	28.7±1.4 ^a
– thyroiditis with or without goiter	242	1.7±0.09	3.3±0.17	3.2±0.14	2.0±0.09 ^a
– acquired hypothyroidism	244	1.1±0.06	2.1±0.1	6.1±0.3	7.5±0.38 ^a
– thyroiditis	245	4.2±0.2	15.4±0.77	22.6±1.1	22.6±1.1 ^a
Mental disorders	290–319	0.35±0.02	0.24±0.01	3.2±0.14	3.4±0.15 ^a
Diseases of nervous system and organs of sense:	320–389	342.5±17.1	261.3±13.1	633.0±31.7	612.3±30.6
– vegetovascular dystonia	337	48.1±2.5	67.2±3.4	50.8±2.51	25.3±1.28
– cataracts	366	13.0±0.7	38.8±1.9	163.0±8.2	152.0±7.6 ^a
Circulatory diseases:	390–459	527.1±26.3	404.5±20.2	558.6±28.0	604.7±30.2
– hypertension	401–405	238.3±11.9	98.0±4.9	110.3±5.5	87.6±4.4
– ischemic heart disease	410–414	235.9±11.85	186.0±9.3	216.1±10.8	202.1±10.1
– cerebrovascular diseases	430–438	35.4±1.8	84.4±4.2	193.5±9.7	266.4±13.3 ^a
Respiratory diseases:	460–519	195.2±7.8	120.8±5.0	282.7±14.6	279.0±14.0 ^a
– chronic bronchitis, emphysema	481, 482	137.2±6.9	110.9±5.6	248.7±12.4	272.7±13.6 ^a
– bronchial asthma	493	1.4±0.05	0.75±0.04	0.5±0.02	—
Digestive diseases:	520–579	413.4±20.7	333.5±16.7	952.0±47.6	1333.7±66.9 ^a
– gastric and duodenal ulcers	531, 532	74.9±3.2	34.2±1.7	50.3±3.0	78.2±3.9 ^a
– gastritis, duodenitis	535	40.5±2.0	34.3±1.7	98.8±4.8	134.1±6.2 ^a
– liver diseases	571	156.7±6.8	197.0±7.9	437.9±21.9	591.6±29.6 ^a
– biliary diseases	574–576	107.3±5.4	103.8±5.2	319.0±16.0	457.5±22.9 ^a
Genitourinary diseases:	580–589	2.0±0.08	2.2±0.1	5.4±0.3	5.1±0.3 ^a
– urinary diseases	590–599	31.2±1.6	57.6±2.9	108.4±5.4	100.0±4.9 ^a
Diseases of the skin and subcutaneous tissue	680–709	10.8±0.5	13.0±0.5	16.6±0.81	9.9±0.5
Musculoskeletal diseases:	710–739	220.4±11.0	187.7±9.4	386.3±19.3	478.9±2.4 ^a
– dorsopathy	720–724	81.9±4.1	41.4±2.1	61.7±3.1	65.5±3.1 ^a
– osteopathy, chondroopathy	730–739	89.4±4.1	88.1±3.8	246.0±12.7	325.4±14.2 ^a
Total	240–739	1643.3±164.5	1232.6±99.1	2539.8±172.3	3630.0±181.0

^a Significant increase in rates relative to the first period; relative to the second period; relative to the third period

years (18–29 at the time of exposure) increased almost 6 times — from $4.4 \pm 0.2\%$ (for the period 0–5 years) to $24.5 \pm 1.2\%$ (for 11–15 years after exposure). Thus, it is here unlikely that age was the main impact factor.

Fig. 11.8–11.10 show the dynamics of non-tumor thyroid diseases. In the remote postaccident period, increased morbidity rates of acquired hypothyroidism and chronic thyroiditis were found.

A significant increase in thyroiditis incidence rates was observed in 6–10 years after exposure, that of acquired hypothyroidism — in 11 years. No age features in the development of these diseases were established. However, lower incidence rates for this pathology in younger clean-up workers within 16–20 postradiation years (with consistently higher rates in older age group) require thorough investigation. Perhaps the earlier terms of the development (regardless of the age of exposed people) are pathophysiological prerequisite for growth rate of thyroid diseases (nontoxic nodular goiter and acquired hypothyroidism).

Among endocrine diseases (Fig. 11.11), increased diabetes incidence rates deserve special attention. In clean-up workers aged 18–39 at the time of exposure, increased rates for the pathology were seen within 6–10 postradiation years, in older age group — in 11–15 years.

At present specialists in radiobiology and radiation medicine focus on radiation impact on cardiovascular diseases (in particu-

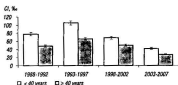


Fig. 11.6. Dynamics of vegeto-vascular dystonia (code ICD-9, 337) in clean-up workers of 1986–1987 depending on age at the time of exposure

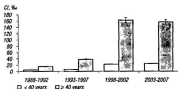


Fig. 11.7. Dynamics of cataract (code ICD-9, 366) in clean-up workers of 1986–1987 depending on age at the time of exposure

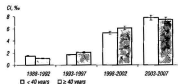


Fig. 11.8. Dynamics of acquired hypothyroidism (code ICD-9, 244) in clean-up workers of 1986–1987 depending on age at the time of exposure

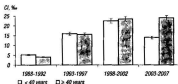


Fig. 11.9. Dynamics of thyroiditis (code ICD-9, 245) in clean-up workers of 1986–1987 depending on age at the time of exposure

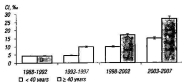


Fig. 11.10. Dynamics of nontoxic nodular goiter (code ICD-9, 241) in clean-up workers of 1986-1987 depending on age at the time of exposure

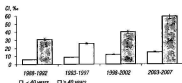


Fig. 11.11. Dynamics of diabetes (code ICD-9, 250) in clean-up workers of 1986-1987 depending on age at the time of exposure

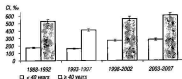


Fig. 11.12. Dynamics of circulatory diseases (code ICD-9, 390-459) in clean-up workers of 1986-1987 depending on age at the time of exposure

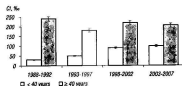


Fig. 11.13. Dynamics of ischemic heart disease (code ICD-9, 410-414) in clean-up workers of 1986-1987 depending on age at the time of exposure

lar low-dose effects of ionizing radiation). This is due to the fact that circulatory diseases occupy a leading place in the structure of death causes among adults [10, 11].

Our results showed that a remote period of the Chernobyl accident consequences was characterized by higher rates of non-tumor pathology of circulatory system among clean-up workers (Fig. 11.12).

Overall morbidity rates of these diseases increased, particularly of those with higher health risks — ischemic heart disease (Fig. 11.13) and cerebrovascular diseases (Fig. 11.14).

With higher incidence rates of these diseases among clean-up workers (older age group), a significant increase in incidence rates for cerebrovascular diseases was determined for 6–10 postradiation years; that in ischemic heart disease — within 6-year period (younger age group) and in 11 years (older age group). In the dynamics of hypertension no distinct regularities were observed.

The above features of post-accident dynamics of non-tumor diseases of nervous and endocrine systems, primarily thyroid and circulatory diseases, are also characteristic of respiratory (Fig. 11.15–11.16), digestive (Fig. 11.17–11.19), urinary (Fig. 11.20) and musculoskeletal diseases, in particular osteopathy and chondro-pathy (Fig. 11.21).

Considering the dynamics of some diseases — namely, mental disorders, vegetovascular dystonia, ischemic heart disease, biliary,

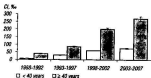


Fig. 11.14. Dynamics of cerebrovascular diseases (code ICD-9, 430-438) in clean-up workers of 1986-1987 depending on age at the time of exposure

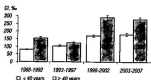


Fig. 11.15. Dynamics of respiratory diseases (code ICD-9, 480-519) in clean-up workers of 1986-1987 depending on age at the time of exposure

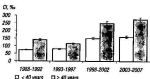


Fig. 11.16. Dynamics of chronic bronchitis, emphysema (code ICD-9, 491, 492) in clean-up workers of 1986-1987 depending on age at the time of exposure

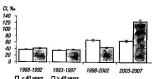


Fig. 11.17. Dynamics of gastritis, duodenitis (code ICD-9, 535) in clean-up workers of 1986-1987 depending on age at the time of exposure

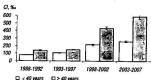


Fig. 11.18. Dynamics of the liver diseases (code ICD-9, 571) in clean-up workers of 1986-1987 depending on age at the time of exposure

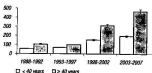


Fig. 11.19. Dynamics of biliary diseases (code ICD-9, 574-576) in clean-up workers of 1986-1987 depending on age at the time of exposure

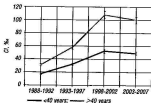


Fig. 11.20. Dynamics of urinary diseases (code ICD-9, 590-593) in clean-up workers of 1986-1987 depending on age at the time of exposure

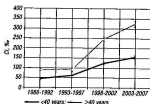


Fig. 11.21. Dynamics of osteopathy, chondropathy (code ICD-9, 730-735) in clean-up workers of 1986-1987 depending on age at the time of exposure

respiratory (chronic bronchitis, emphysema) and musculoskeletal diseases, young people as compared to those of middle age, are more sensitive to radiation factor.

As evidenced by the earlier period of morbidity growth in the above forms and groups of the diseases started in 6–10 years in younger clean-up workers and 11–15 — in middle-aged persons (40–60 at the time of exposure). Higher rates of non-tumor diseases in older people should be considered as a possible result of synergetic effect of such factors as «Aging» and «Radiation». Other negative endogenous factors are not excluded.

Dose-dependent effects

Risk assessment was used to determine dose-dependent effects of non-tumor morbidity in clean-up workers of 1986–1987 [12]. To establish correlation between impact of possible risk factor and a disease, relative and excess risks among exposed and unexposed persons were calculated [11]. The cohort of clean-up workers of 1986–1987 was taken as a control (unexposed), whole body external dose was <0.1 Gy. Thus we used an internal control.

It was very important to identify possible risk factors for non-tumor diseases (whole body external dose, age of exposed persons and time at risk). Particular attention was paid to those non-tumor diseases that have contributed and make main contribution to victims' health deterioration.

External doses of contingent under consideration range from 0.0 to 0.33 Gy (according to the Russian National Medical-Dosimetry Registry), from 0.0 to 0.7 Gy (SRU), from 0.0 to 1.4 Gy (CER).

Risk analysis of dose-dependent effects was carried out according to the SRU in cohort as a whole, depending on radiation dose for five-year periods at risk (Table 11.5–Table 11.12), with regard to the age at the time of exposure (Table 11.13–Table 11.15).

Table 11.5 presents data on RR values for circulatory diseases. Significant relative risks were established for 11–15 and 16–20 postradiation years. An ambiguity in dose intervals 0.05–0.099 and 0.1–0.199 Gy, stable values at doses 0.25–0.7 Gy were observed. Excess morbidity in this class of diseases was largely due to ischemic heart disease and cerebrovascular diseases.

Table 11.5. Relative risks for circulatory diseases (code ICD-9, 390–459) in clean-up workers 1986–1987 depending on whole body external dose and time after exposure

Dose groups (Gy)	Observation periods (years)			
	5 (1988–1992)	6–10 (1993–1997)	11–15 (1998–2002)	16–20 (2003–2007)
0.05–0.099	0.98 (0.86; 1.13)	0.92 (0.8; 1.06)	1.17 (1.03; 1.33)	0.9 (0.74; 1.09)
0.1–0.199	1.15 (1.0; 1.32)	0.95 (0.82; 1.1)	0.96 (0.84; 1.11)	1.38 (1.14; 1.66)
0.2–0.249	0.9 (0.77; 1.05)	0.93 (0.82; 1.05)	1.27 (1.14; 1.41)	0.96 (0.83; 1.12)
0.25–0.7	1.06 (0.89; 1.26)	0.9 (0.78; 1.05)	1.24 (1.1; 1.41)	1.12 (0.94; 1.33)

A similar trend was observed in the analysis of respiratory diseases (Table 11.8). Significant RR for respiratory diseases was revealed in dose group 0.05–0.099 Gy (the first 5 post-accident years) and at doses 0.1–0.199 Gy (the second five-year period). Over

observation period 1998–2002, a significant relative risk was found in all dose groups, in 16–20 years after exposure — in dose group 0.25–0.7 Gy. Additional morbidity is formed mainly by chronic bronchitis and emphysema.

Table 11.6. Relative risks for respiratory diseases (code ICD-9, 460–519) in clean-up workers 1986–1987 depending on whole body external dose and time after exposure

Dose groups (Gy)	Observation periods (years)			
	5 (1988–1992)	6–10 (1993–1997)	11–15 (1998–2002)	16–20 (2003–2007)
0.05–0.099	1.52 (1.18; 1.94)	0.92 (0.91; 1.12)	1.16 (0.92; 1.44)	0.69 (0.51; 0.94)
0.1–0.199	0.96 (0.73; 1.26)	1.14 (0.97; 1.48)	1.18 (0.94; 1.48)	0.99 (0.73; 1.34)
0.2–0.249	1.04 (0.79; 1.38)	1.02 (0.81; 1.29)	1.37 (1.14; 1.65)	1.09 (0.86; 1.43)
0.25–0.7	0.86 (0.61; 1.21)	1.08 (0.84; 1.41)	1.4 (1.12; 1.74)	1.23 (0.96; 1.63)

Based on risk-analysis, a significant RR for digestive diseases (Table 11.7) was found actually in all dose groups (6–10 years after exposure), in dose groups >0.2 Gy (11–15 years after exposure), and >0.1 Gy (16–20 years after exposure). In the morbidity structure, liver (Table 11.8) and biliary diseases are predominant.

Table 11.7. Relative risks for digestive diseases (code ICD-9, 520–579) in clean-up workers 1986–1987 depending on whole body external dose and time after exposure

Dose groups (Gy)	Observation periods (years)			
	5 (1988–1992)	6–10 (1993–1997)	11–15 (1998–2002)	16–20 (2003–2007)
0.05–0.099	0.72 (0.62; 0.84)	1.28 (1.09; 1.49)	0.94 (0.83; 1.06)	0.87 (0.73; 1.04)
0.1–0.199	0.74 (0.63; 0.87)	1.14 (0.97; 1.34)	0.89 (0.78; 1.01)	1.18 (0.99; 1.41)
0.2–0.249	0.78 (0.66; 0.92)	1.09 (0.96; 1.24)	1.31 (1.17; 1.46)	1.13 (0.98; 1.3)
0.25–0.7	1.11 (0.92; 1.32)	1.32 (1.15; 1.53)	1.66 (1.47; 1.86)	1.35 (1.16; 1.58)

Table 11.8. Relative risks for liver diseases (code ICD-9, 571) in clean-up workers 1986–1987 depending on whole body external dose and time after exposure

Dose groups (Gy)	Observation periods (years)			
	5 (1988–1992)	6–10 (1993–1997)	11–15 (1998–2002)	16–20 (2003–2007)
0.05–0.099	0.85 (0.63; 1.15)	1.09 (0.87; 1.37)	5.44 (3.83; 7.71)	0.53 (0.42; 0.67)
0.1–0.199	0.7 (0.51; 0.97)	1.12 (0.89; 1.42)	5.69 (4.01; 8.08)	0.73 (0.58; 0.92)
0.2–0.249	0.81 (0.59; 1.13)	1.28 (1.04; 1.58)	2.29 (1.9; 2.77)	1.38 (1.09; 1.73)
0.25–0.7	1.46 (1.05; 2.04)	1.71 (1.37; 2.13)	3.2 (2.65; 3.87)	1.97 (1.56; 2.5)

Significant RR for urinary diseases (Table 11.9) were found 5 years after the exposure (dose group 0.25–0.7 Gy), 10–20 years after the exposure (dose groups 0.2–0.249 Gy and 0.25–0.7 Gy).

True RR for the diseases of nervous system and sense organs (Table 11.10) was observed in all dose groups within the first five years after exposure. Morbidity is formed mainly by the diseases of vegetative nervous system, particularly vegeto-vascular dystonia, psychoneurological pathology, cerebrovascular diseases, including discirculatory encephalopathy, cataracts and others.

Table 11.9. Relative risks for urinary diseases (code ICD-9, 590-599) in clean-up workers 1986-1987 depending on whole body external dose and time after exposure

Dose groups (Gy)	Observation periods (years)			
	5 (1988-1992)	6-10 (1993-1997)	11-15 (1998-2002)	16-20 (2003-2007)
0.05-0.099	0.79 (0.45; 1.37)	0.97 (0.66; 1.42)	0.53 (0.37; 0.76)	1.04 (0.63; 1.73)
0.1-0.199	0.78 (0.44; 1.39)	1.03 (0.69; 1.54)	0.75 (0.53; 1.08)	1.27 (0.77; 2.11)
0.2-0.249	0.55 (0.29; 1.05)	1.23 (0.86; 1.78)	1.71 (1.3; 2.24)	1.48 (1.05; 2.09)
0.25-0.7	1.21 (0.64; 2.28)	1.87 (1.3; 2.7)	1.8 (1.32; 2.45)	2.12 (1.48; 3.03)

Table 11.10. Relative risks for the diseases of nervous system and organs of sense (code ICD-9, 320-389) in clean-up workers 1986-1987 depending on whole body external dose and time after exposure

Dose groups (Gy)	Observation periods (years)			
	5 (1988-1992)	6-10 (1993-1997)	11-15 (1998-2002)	16-20 (2003-2007)
0.05-0.099	1.15 (1.01; 1.33)	1.08 (0.93; 1.26)	1.17 (1.02; 1.34)	0.83 (0.67; 1.03)
0.1-0.199	1.09 (0.93; 1.24)	0.96 (0.81; 1.13)	0.81 (0.69; 0.94)	1.33 (1.08; 1.65)
0.2-0.249	1.11 (0.96; 1.28)	0.91 (0.78; 1.04)	1.01 (0.89; 1.14)	0.8 (0.67; 0.95)
0.25-0.7	1.62 (1.39; 1.89)	0.76 (0.64; 0.89)	1.45 (1.26; 1.68)	0.91 (0.74; 1.11)

In clean-up workers of 1986-1987, non-tumor thyroid diseases make up the major portion (75%) among endocrine diseases. Ten years after exposure, the significant RRs were found in dose group 0.25-0.7 Gy (Table 11.11). The following (acquired hypothyroidism, chronic thyroiditis and nontoxic nodular goiter) predominate in the structure.

Table 11.11. Relative risks for thyroid diseases (code ICD-9, 240-246) in clean-up workers 1986-1987 depending on whole body external dose and time after exposure

Dose groups (Gy)	Observation periods (years)			
	5 (1988-1992)	6-10 (1993-1997)	11-15 (1998-2002)	16-20 (2003-2007)
0.05-0.099	0.94 (0.7; 1.28)	1.15 (0.88; 1.53)	1.07 (0.81; 1.41)	0.7 (0.48; 1.03)
0.1-0.199	0.57 (0.41; 0.81)	0.73 (0.53; 1.02)	1.0 (0.75; 1.34)	0.8 (0.54; 1.19)
0.2-0.249	0.91 (0.66; 1.27)	1.02 (0.79; 1.33)	1.0 (0.78; 1.28)	0.6 (0.42; 0.88)
0.25-0.7	1.17 (0.81; 1.68)	1.16 (0.86; 1.55)	1.46 (1.13; 1.88)	1.7 (1.24; 1.96)

The analysis of dose-dependent risk for musculoskeletal diseases gave no reliable data (Table 11.12).

Table 11.12. Relative risks for musculoskeletal diseases (code ICD-9, 730-739) in clean-up workers 1986-1987 depending on whole body external dose and time after exposure

Dose groups (Gy)	Observation periods (years)			
	5 (1988-1992)	6-10 (1993-1997)	11-15 (1998-2002)	16-20 (2003-2007)
0.05-0.099	1.11 (0.9; 1.35)	0.9 (0.75; 1.09)	0.63 (0.53; 0.74)	0.63 (0.49; 0.81)
0.1-0.199	1.12 (0.91; 1.38)	0.84 (0.68; 1.02)	0.63 (0.53; 0.76)	0.88 (0.68; 1.13)
0.2-0.249	0.92 (0.74; 1.15)	1.02 (0.86; 1.21)	0.9 (0.77; 1.05)	0.9 (0.74; 1.1)
0.25-0.7	0.99 (0.77; 1.28)	0.93 (0.76; 1.14)	1.03 (0.86; 1.22)	0.83 (0.65; 1.07)

Risk analysis of non-tumor morbidity was also conducted in clean-up workers of 1986–1987. It was done with regard to external dose, age at the time of exposure and time at risk on the data for 5-, 10-, 15- and 20-year periods after exposure. Below are the results of the analysis of morbidity in subcohort (external radiation dose 0.25–0.7 Gy) as for this dose group more stable and reliable data were obtained.

Table 11.13 presents reliable RR values of non-tumor diseases in the cohort of clean-up workers as a whole (regardless of age).

Table 11.13. The relative risks (RR) for non-tumor morbidity among the Chernobyl clean-up workers 1986–1987 in the cohort as a whole, at doses 0.25–0.7 Gy, 5, 10, 15, 20 years after exposure

Classes and nosological forms of diseases	Code ICD-9	Postradiation periods (years)			
		5 1986–1992	10 1989–1997	15 1988–2002	20 1986–2007
Endocrine and metabolic diseases:	240–289			1.21 (1.11;1.4)	1.24 (1.17;1.3)
– diabetes	250				1.29 (1.08;1.5)
– acquired hypothyroidism	244	3.44 (1.38;8.6)	2.79 (1.41;5.5)		
– thyroiditis	245			1.25 (1.1;1.47)	1.27 (1.09;1.5)
Mental disorders	290–319			3.85 (2.58;5.8)	3.57 (2.48;5.1)
Diseases of nervous system and sense organs:	320–389	1.39 (1.3;1.48)	1.25 (1.19;1.3)	1.23 (1.18;1.3)	1.24 (1.2;1.3)
– vegeto-vascular dystonia	337	1.52 (1.4;1.65)	1.32 (1.23;1.4)	1.24 (1.17;1.3)	1.24 (1.17;1.3)
– cataracts	366			1.27 (1.06;1.5)	1.39 (1.18;1.6)
Circulatory diseases:	390–459			1.21 (1.16;1.3)	1.25 (1.21;1.3)
– ischemic heart disease	410–414			1.2 (1.09;1.3)	1.21 (1.12;1.3)
– cerebrovascular diseases	430–438	1.71 (1.14;2.6)	1.64 (1.38;2.0)	1.61 (1.44;1.8)	1.61 (1.5;1.77)
Respiratory diseases:	460–519	1.29 (1.12;1.5)	1.35 (1.2;1.47)	1.32 (1.24;1.4)	1.29 (1.2;1.37)
– chronic bronchitis, emphysema	491, 492	1.28 (1.1;1.47)	1.38 (1.26;1.5)	1.35 (1.2;1.45)	1.32 (1.2;1.41)
Digestive diseases:	520–579				
– gastritis, duodenitis	535	1.51 (1.22;1.9)	1.49 (1.3;1.71)	1.53 (1.37;1.7)	1.54 (1.4;1.69)
– liver diseases	571		1.27 (1.17;1.4)	1.45 (1.37;1.5)	1.4 (1.3;1.48)
Urinary diseases	590–599	1.37 (1.04;1.8)	1.4 (1.2;1.64)	1.46 (1.3;1.63)	1.43 (1.29;1.6)
Musculoskeletal diseases: osteopathy, chondroopathy	730–739		1.23 (1.1;1.38)	1.23 (1.14;1.3)	1.25 (1.16;1.4)

Data analysis shows that for most non-tumor diseases of various body systems dose-dependent effect was realized within 15 and 20 years at risk. The significant relative risks for some diseases (acquired hypothyroidism, vegeto-vascular dystonia, cerebrovascular diseases, chronic bronchitis, emphysema, gastritis, duodenitis and urinary diseases) were obtained for five postirradiation years. For ten years at risk — for liver diseases, osteopathy and hondroopathy.

Table 11.14 gives significant RR of non-tumor morbidity in clean-up workers under 40 years at the time of their participation in recovery operations depending on dose and time after the Chernobyl accident.

Table 11.14. Relative risks (RR) for non-tumor diseases among the Chernobyl clean-up workers of 1986-1987 aged <40 at the time of their participation in recovery operations, whole body external doses 0.25-0.7 Gy, 5, 10, 15, 20 years after exposure

Classes and nosological forms of diseases	Code ICD-9	Postirradiation periods (years)			
		5 (1988-1992)	10 (1988-1997)	15 (1988-2002)	20 (1988-2007)
Endocrine and metabolic diseases:	240-289			1.25 (1.1-1.32)	
– diabetes	244	2.95 (1.2-7.5)	2.7 (1.26-5.8)		
– acquired hypothyroidism	245			1.23 (1.03-1.5)	1.25 (1.07-1.5)
– thyroiditis	250			1.32 (1.04-1.7)	1.47 (1.19-1.8)
Mental disorders	290-319			3.4 (2.26-5.1)	3.2 (2.21-4.6)
Diseases of nervous system and sense organs:	320-389	1.38 (1.3-1.5)	1.26 (1.2-1.32)	1.26 (1.2-1.31)	1.27 (1.22-1.3)
– vegeto-vascular dystonia	337	1.49 (1.4-1.6)	1.3 (1.21-1.4)	1.24 (1.16-1.3)	
Circulatory diseases:	390-459		1.3 (1.1-2.4)	1.6 (1.3-2.9)	1.6 (1.2-2.8)
– ischemic heart disease	410-414		1.24 (1.06-1.4)	1.39 (1.26-1.5)	
– cerebrovascular diseases	430-438	2.0 (1.2-3.3)	1.91 (1.6-2.35)	1.81 (1.6-2.07)	
Respiratory diseases:	460-519	1.41 (1.2-1.6)	1.41 (1.3-1.55)	1.37 (1.28-1.5)	
– chronic bronchitis, emphysema	491, 492	1.42 (1.2-1.7)	1.45 (1.31-1.6)	1.41 (1.3-1.52)	1.55 (1.29-1.8)
Digestive diseases:	520-579		1.27 (1.2-1.34)	1.33 (1.2-1.39)	1.35 (1.3-1.4)
– gastritis, duodenitis	535	1.59 (1.3-1.99)	1.5 (1.3-1.74)	1.53 (1.37-1.7)	1.58 (1.41-1.7)
– biliary diseases	574-576	1.34 (1.11-1.6)	1.45 (1.3-1.63)	1.46 (1.3-1.59)	1.45 (1.3-1.56)
– liver diseases	571		1.24 (1.1-1.35)	1.34 (1.3-1.43)	1.36 (1.28-1.4)
Genitourinary diseases: urinary diseases	590-599	1.46 (1.07-2.0)	1.42 (1.2-1.69)	1.51 (1.3-1.71)	1.44 (1.29-1.6)
Musculoskeletal diseases:	710-739		1.24 (1.13-1.4)	1.26 (1.18-1.3)	1.29 (1.2-1.36)
– osteopathy, chondroopathy	730-739		1.33 (1.2-1.51)	1.33 (1.2-1.46)	1.35 (1.25-1.5)

In people under 40 with radiation doses 0.25–0.7 Gy, true RRs for non-tumor diseases were found: acquired hypothyroidism (5, 10 years after exposure); ischemic heart disease (10, 15 years after exposure); endocrine and metabolic diseases, particularly thyroiditis, diabetes, mental disorders (15, 20 years after exposure); vegeto-vascular dystonia, cerebrovascular and respiratory diseases (5, 10, 15 years after exposure); circulatory, digestive diseases (particularly liver diseases), musculoskeletal diseases, particularly osteopathy, hondropathy (10, 15, 20 years after exposure); diseases of nervous system and sense organs, chronic bronchitis, emphysema, biliary diseases, gastritis, duodenitis, urinary diseases (5, 10, 15, 20 years after exposure).

As for clean-up workers of 1986–1987 aged over 40, at doses 0.25–0.7 Gy (Table 11.15) significant RRs were found for some diseases: diseases of nervous system and sense organs, vegeto-vascular dystonia and liver diseases (all postradiation periods); endocrine and metabolic, digestive, biliary and urinary diseases (10, 15, 20 postradiation years); cerebrovascular diseases, gastritis and duodenitis (15, 20 postradiation years); cataract (10, 20 postradiation years); dorsopathy (10 postradiation years) and thyroid pathology (15 postradiation years).

Table 11.15. Relative risks (RR) for non-tumor diseases among the Chernobyl clean-up workers 1986–1987 aged ≥ 40 at the time of participation in recovery operations, whole body external doses 0.25–0.7 Gy, 5, 10, 15, 20 years after exposure

Classes and aetiological forms of diseases	Code ICD-9	Postradiation periods (years)			
		5 1986–1992	10 1993–1997	15 1998–2002	20 1993–2007
Endocrine and metabolic diseases:	240–289		1.23 (1.01;1.5)	1.26 (1.1;1.47)	1.22 (1.06;1.4)
– thyroid diseases	240–246			1.28 (1.1;1.56)	
Diseases of nervous system and sense organs:	320–389	1.62 (1.39;1.9)	1.24 (1.1;1.39)	1.2 (1.09;1.3)	1.17 (1.1;1.29)
– vegeto-vascular dystonia	337	1.74 (1.38;2.2)	1.37 (1.13;1.7)	1.24 (1.03;1.5)	1.22 (1.07;1.5)
– cataracts	366		1.78 (1.06;3.1)		1.51 (1.1;2.02)
Circulatory diseases:				1.41 (1.15;1.7)	1.42 (1.2;1.71)
– cerebrovascular diseases	430–438				
Digestive diseases:	520–579		1.37 (1.21;1.6)	1.33 (1.2;1.46)	1.3 (1.2;1.42)
– gastritis, duodenitis	535			1.47 (1.09;2.0)	1.34 (1.01;1.8)
– liver diseases	571	1.46 (1.1;2.04)	1.58 (1.29;1.9)	2.45 (2.1;2.89)	1.79 (1.6;2.06)
– biliary diseases	574–576		1.65 (1.3;2.1)	1.48 (1.23;1.8)	1.44 (1.22;1.7)
Urinary diseases	590–599		1.52 (1.08;2.2)	1.41 (1.09;1.8)	1.5 (1.2;1.88)
Musculoskeletal diseases:			1.21 (1.13;1.3)		
– dorsopathy	720–724				

Analytical epidemiological analysis of dose-dependent non-tumor morbidity proved for indicative of strong correlation of some classes and forms with dose in different periods after participation in recovery operations. Excess relative risks for certain classes and nosological forms of non-tumor diseases for 5, 10, 15, 20 years after exposure are presented in Table 11.16–11.18.

Table 11.16. Relative risks (RR) for non-tumor diseases among the Chernobyl clean-up workers 1986–1987 aged <40 at the time of their participation in recovery operations, whole body external doses 0.25–0.7 Gy, 5, 10, 15, 20 years after exposure

Classes and nosological forms of diseases	Code ICD-9	Postirradiation periods (years)			
		5 (1988–1992)	10 (1988–1997)	15 (1988–2002)	20 (1988–2007)
Endocrine and metabolic diseases:	240–289			1.25 (1.1–1.32)	
– diabetes	244	2.95 (1.2–7.5)	2.7 (1.26–5.8)		
– acquired hypothyroidism	245			1.23 (1.03–1.5)	1.25 (1.07–1.5)
– thyroiditis	250			1.32 (1.04–1.7)	1.47 (1.19–1.8)
Mental disorders	290–319			3.4 (2.26–5.1)	3.2 (2.21–4.6)
Diseases of nervous system and sense organs:	320–389	1.38 (1.3–1.5)	1.26 (1.2–1.32)	1.26 (1.2–1.31)	1.27 (1.22–1.3)
– vegeto-vascular dystonia	337	1.49 (1.4–1.6)	1.3 (1.21–1.4)	1.24 (1.16–1.3)	
Circulatory diseases:	390–459		1.3 (1.1–2.4)	1.6 (1.3–2.0)	1.6 (1.2–2.8)
– ischemic heart disease	410–414		1.24 (1.06–1.4)	1.39 (1.26–1.5)	
– cerebrovascular diseases	430–438	2.0 (1.2–3.3)	1.91 (1.6–2.35)	1.81 (1.6–2.07)	
Respiratory diseases:	460–519	1.41 (1.2–1.6)	1.41 (1.3–1.55)	1.37 (1.28–1.5)	
– chronic bronchitis, emphysema	491, 492	1.42 (1.2–1.7)	1.45 (1.31–1.6)	1.41 (1.3–1.52)	1.39 (1.29–1.5)
Digestive diseases:	520–579		1.27 (1.2–1.34)	1.33 (1.2–1.39)	1.35 (1.3–1.4)
– gastritis, duodenitis	535	1.58 (1.3–1.99)	1.5 (1.3–1.74)	1.53 (1.37–1.7)	1.56 (1.41–1.7)
– biliary diseases	574–576	1.34 (1.11–1.6)	1.45 (1.3–1.63)	1.46 (1.3–1.59)	1.45 (1.3–1.56)
– liver diseases	571		1.24 (1.1–1.35)	1.34 (1.3–1.43)	1.38 (1.28–1.4)
Genitourinary diseases:					
– urinary diseases	590–599	1.46 (1.07–2.0)	1.42 (1.2–1.69)	1.51 (1.3–1.71)	1.44 (1.29–1.6)
Musculoskeletal diseases:	710–739		1.24 (1.13–1.4)	1.26 (1.18–1.3)	1.29 (1.2–1.35)
– osteopathy, chondropathy	730–739		1.33 (1.2–1.51)	1.33 (1.2–1.46)	1.35 (1.25–1.5)

Data analysis (Table 11.16) shows that at doses 0.25–0.7 Gy in the cohort of clean-up workers as a whole significant ERRs were obtained for: acquired hypothyroidism (5, 10 postradiation years); endocrine and metabolic diseases, including thyroiditis; cataracts, circulatory diseases, particularly ischemic heart disease (15, 20 postradiation years); liver diseases, osteopathy, hondropathy (5, 10, 15 postradiation years); diabetes (20 years after exposure); diseases of nervous system and sense organs, particularly vegeto-vascular dystonia, cerebrovascular diseases, respiratory diseases, particularly chronic bronchitis, emphysema, gastritis, duodenitis, urinary diseases (5, 10, 15, 20 years after exposure).

Significant excess relative risks for non-tumor morbidity among those aged <40 (Table 11.17) were obtained for: acquired hypothyroidism (5, 10 postradiation years);

Table 11.17. Excess relative risk for non-tumor diseases in the Chernobyl clean-up workers 1986–1987 aged <40 at the time of their participation in recovery operations, whole body external doses 0.25–0.7 Gy (D average = 0.3 Gy), 5-, 10-, 15-, 20-year postradiation periods

Classes and nosological forms of diseases	Code ICD-9	Postradiation periods (years)			
		5 1988–1992	10 1988–1997	15 1988–2002	20 1988–2007
Endocrine and metabolic diseases:	240–289			0.71 (0.5;0.97)	1.0 (0.74;1.3)
– thyroid diseases	240–246			0.33 (0.12;0.6)	0.6 (0.34;0.8)
– acquired hypothyroidism	244	7.4 (1.1;13.7)	6.5 (1.5;11.5)		
– thyroiditis	245			0.89 (0.14;1.6)	0.89 (0.3;1.53)
– diabetes	250			1.2 (0.14;2.3)	1.8 (0.81;2.8)
Mental disorders	290–319			8.5 (4.9;8.9)	9.0 (6.1;11.9)
Diseases of nervous system and sense organs:	320–389	1.5 (1.2;1.8)	1.07 (0.8;1.32)	1.0 (0.81;1.2)	0.82 (0.8;1.09)
– vegeto-vascular dystonia	337	1.9 (1.5;2.3)	1.1 (0.8;1.4)	0.83 (0.57;1.1)	
– cataracts	366			1.17 (0.25;2.1)	
Circulatory diseases:	390–459		0.99 (0.66;1.3)	1.0 (0.8;1.2)	1.3 (1.1;1.5)
– hypertension	401–405	0.57 (0.1;1.1)	0.8 (0.38;1.2)	0.6 (0.28;0.9)	
– ischemic heart disease	410–414		0.56 (0.2;0.97)	1.3 (0.9;1.7)	
– cerebrovascular diseases	430–438	3.8 (1.03;6.6)	3.57 (2.3;4.5)	3.08 (2.4;3.8)	
Respiratory diseases:	460–519	1.83 (0.93;2.3)	1.4 (1.02;1.8)	1.35 (1.04;1.7)	
– chronic bronchitis, emphysema	491,492	1.5 (0.83;2.2)	1.75 (1.26;2.2)	1.59 (1.2;1.9)	1.59 (1.26;1.9)

circulatory diseases, particularly ischemic heart disease; musculoskeletal diseases, particularly osteopathy, hondropathy; liver diseases (10, 15, 20 years after exposure); endocrine and metabolic diseases, particularly thyroid diseases, thyroiditis, diabetes; cataracts, stomach ulcers (15, 20 years after exposure); mental disorders (20 years after exposure); diseases of nervous system and sense organs, particularly vegeto-vascular dystonia; hypertension, cerebrovascular pathology; respiratory diseases, particularly chronic bronchitis, emphysema; digestive diseases, particularly biliary diseases, gastritis, duodenitis; urinary diseases; dorsopathy (5, 10, 15, 20 years after exposure).

At doses 0.25–0.7 Gy, age ≥ 40 , significant ERR (Table 11.18) of non-tumor morbidity was obtained for: diseases of nervous system and sense organs (5 postradiation years); vegeto-vascular dystonia (5, 10 postradiation years); gastritis, duodenitis (15 postradiation years); cataracts (20 postradiation years); cerebrovascular diseases (15, 20 postradiation years); digestive diseases, particularly biliary and urinary diseases (10, 15, 20 postradiation years); liver diseases (10, 15, 20 postradiation years).

Table 11.18. Excess relative risk for non-tumor morbidity in the Chernobyl clean-up workers 1986–1987 aged ≥ 40 at the time of their participation in recovery operations, whole body external doses 0.25–0.7 Gy (Dose-rate = 0.3 Gy)

Classes and nosological forms of diseases	Code ICD-9	Postradiation periods (years)			
		5 1986–1987	10 1988–1997	15 1988–2002	20 1988–2007
Endocrine and metabolic diseases:	240–289		0.77 (0.67; 0.9)	0.87 (0.8; 0.97)	0.73 (0.66; 0.8)
– diabetes	250			0.93 (0.8; 1.07)	
Diseases of nervous system and sense organs:	320–389	2.1 (1.8; 2.4)	0.8 (0.73; 0.9)	0.87 (0.5; 0.78)	0.57 (0.5; 0.61)
– vegeto-vascular dystonia	337	2.5 (2.07; 3.0)	1.2 (1.03; 1.4)	0.8 (0.7; 0.91)	0.73 (0.64; 0.8)
– cataracts	366		2.6 (1.7; 4.08)		1.7 (1.36; 2.1)

Thus, summarizing the results of epidemiological studies of non-tumor morbidity among clean-up workers of 1986–1987 in post-accident period we can state the following:

- manifestation of dose-dependent effect in the form of non-tumor disorders in various systems and organs is variable due to radiosensitivity;
- the effect stabile depends on external radiation dose. In these studies, the most stable dose-dependent effect was observed at doses ≥ 0.2 Gy, and the most significant result was obtained in dose range 0.25–0.7 Gy;
- the risk of radiation-induced non-tumor effect rises with increasing radiation dose. The uncertainty was observed at radiation doses < 0.2 Gy that requires special study;
- manifestation of dose-dependent effects of non-tumor diseases among clean-up workers of 1986–1987 mainly took place in 11–15 years after exposure, some effects occurred in 16–20 years.

Non-tumor mortality in the Chernobyl clean-up workers of 1986–1987

During 1988–2007 non-tumor mortality in the cohort of clean-up workers of 1986–1987 as a whole increased from 2.12‰ (1988) to 10.78‰ (2007) ranging in age

groups <40 and ≥ 40 within 1.4–7.21‰ and 2.34–15.9‰ respectively (Fig. 11.22). Since 1997, mortality rate in the age group ≥ 40 was significantly higher than that in the age group <40.

Actual data on non-tumor mortality rates in the cohort of clean-up workers as a whole depending on age and time after exposure are given in Tables 11.19–11.21. Based on the data analysis, mortality increase in study contingent for 1988–2007 was due to the rise in circulatory diseases. Quite a significant contribution is observed due to respiratory and digestive diseases. Increased mortality rate for diabetes has been noted since 1997. Significant rise in mortality from diseases of nervous system and sense organs was established in 2007.

In data analysis mortality increase in people aged 40–60 at the time of participation in recovery operations and, consequently, exposure for the period of observation was proved. In subcohort of clean-up workers under 40, circulatory mortality increased from 0.9 to 5.6‰, in the older age group — from 2.0 to 13.4‰; respiratory diseases (chronic bronchitis, emphysema) — from 0.03 to 0.24‰ and from 0.01 to 0.4‰; digestive diseases — from 0.14 to 0.86‰ and from 0.24 to 1.22‰ respectively.

Mortality dynamics in clean-up workers for major non-tumor diseases with regard to age at the time of exposure is given in Fig. 11.23–11.25. The remote postChornobyl period was noted for a significant rise in circulatory mortality among clean-up workers [13] but the highest levels and

growth rates were established in people aged 40–60 at the time of exposure. Age differences were observed in 5 and especially 15–20 years after the accident. Here age range was 55–75 and 60–80 years.

Age had undoubtedly a major impact on the mortality of the contingent, significant increase in mortality growth rate observed in the older age group suggests the influence of other factors, particularly radiation one.

In the structure of causes of death from non-tumor diseases among clean-up workers, circulatory and digestive diseases contribute about 90%, while circulatory diseases — from 77 to 84% (Fig. 11.23). That is why considerable study has been given to risk factors for non-tumor mortality in clean-up workers, namely – risk of death from circulatory diseases.

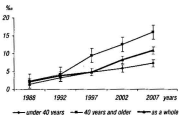


Fig. 11.22. Dynamics of non-tumor death rate among the Chornobyl clean-up workers of 1986–1987 depending on age at the time of participation in recovery operations, 1988–2007 (‰)

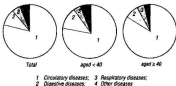


Fig. 11.23. Structure of non-tumor mortality among the Chornobyl clean-up workers of 1986–1987 depending on age at the time of participation in recovery operations, 2007 (%)

Table 11.19. Dynamics of non-tumor death rate among the Chernobyl clean-up workers of 1986–1987 in the cohort as a whole (regardless of age), 1988–2007 (%)

Classes and nosological forms of diseases	Code ICD-9	Retrospective periods				
		1988	1992	1997	2002	2007
Endocrine and metabolic diseases:						
– diabetes	240–249	0.03±0.002	0.07±0.04	0.11±0.006	0.1±0.004	0.13±0.007
– thyroid diseases	250	—	—	0.05±0.003	0.08±0.004	0.1±0.015
– toxic nodular goiter	240–246	0.002±0.0001	0.04±0.0017	0.1±0.003	0.02±0.001	0.006±0.0002
– thyroiditis	241	—	—	—	—	—
– thyrotoxicosis with or without goiter	242	—	—	—	—	—
– acquired hypothyroidism	244	—	—	—	0.02±0.001	0.004±0.0002
– thyroiditis	245	—	—	—	—	—
Mental disorders	290–319	0.008±0.002	0.02±0.001	0.05±0.003	0.06±0.0025	0.09±0.005
Diseases of nervous system and sense organs:	320–389	0.07±0.007	0.09±0.005	0.1±0.003	0.15±0.002	0.26±0.01
– vegeto–vascular dystonia	337	0.05±0.03	0.07±0.004	0.1±0.003	0.02±0.001	0.02±0.001
– cataracts	366	—	—	—	—	—
Circulatory diseases:	390–459	1.76±0.09	3.24±0.16	4.47±0.2	6.53±0.33	8.59±0.43
– hypertension	401–405	0.16±0.007	0.24±0.01	0.28±0.01	0.22±0.011	0.18±0.009
– ischemic heart disease	410–414	0.42±0.02	0.93±0.05	1.5±0.08	2.1±0.11	2.2±0.11
– cerebrovascular diseases	430–438	0.17±0.009	0.29±0.02	0.5±0.03	0.9±0.05	1.3±0.07
Respiratory diseases:	460–519	0.03±0.002	0.11±0.006	0.3±0.002	0.5±0.03	0.5±0.03
– chronic bronchitis, emphysema	491, 492	0.006±0.0002	0.15±0.008	0.24±0.01	0.3±0.02	0.38±0.017
– bronchial asthma	493	—	—	0.008±0.0004	0.002±0.0001	—
Digestive diseases:	520–579	0.19±0.01	0.28±0.011	0.44±0.02	0.7±0.04	1.09±0.06
– gastric and duodenal ulcers	531, 532	0.06±0.003	0.09±0.005	0.08±0.003	0.08±0.003	0.1±0.003
– gastritis, duodenitis	535	—	—	—	0.004±0.0002	0.01±0.0003
– liver diseases	571	0.11±0.006	0.14±0.008	0.28±0.01	0.38±0.02	0.52±0.03
– biliary diseases	574–576	—	—	0.04±0.0018	0.04±0.0018	0.06±0.0025
Genitourinary diseases:	580–589	0.008±0.0004	0.12±0.006	0.1±0.005	0.06±0.003	0.04±0.0018
– urinary diseases	590–599	0.01±0.0005	0.03±0.002	0.08±0.003	0.06±0.003	0.06±0.003
Diseases of the skin and subcutaneous tissue	680–709	—	—	0.004±0.0017	0.02±0.001	0.04±0.0017
Musculoskeletal diseases:	710–739	—	0.04±0.0017	0.02±0.001	0.06±0.0002	0.006±0.0003
– osteopathy	720–724	—	0.02±0.001	0.004±0.0002	0.006±0.0003	0.006±0.0003
– osteopathy	730–739	—	0.04±0.0017	0.008±0.0004	0.02±0.001	0.004±0.0007
– osteopathy, chondropathy	740–749	2.12±0.11	3.95±0.19	5.59±0.28	8.15±0.41	10.78±0.54
Total	240–739	—	—	—	—	—

*Reliable increase in the rates relative to 1988; *relative to 1997; *relative to 2002

Table 11.26. Dynamics of non-tumor death rate among the Chernobyl clean-up workers of 1986-1987 aged <40 at the time of participation in recovery operations, 1988-2007 (%)

Classes and nosological forms of diseases	Code ICD-9	Postirradiation periods				
		1988	1992	1997	2002	2007
Endocrine and metabolic diseases:	240-259	0.06±0.0027	0.11±0.006	0.2±0.01	0.05±0.003	0.09±0.005 ^{1,2}
- diabetes	250	—	—	0.02±0.001	0.04±0.0017	0.05±0.003 ^{1,2,3,4,5,6,7}
- thyroid diseases	240-246	0.024±0.002	0.09±0.005	0.18±0.008	0.02±0.001	0.01±0.0006 ⁸
- nontoxic nodular goiter	241	—	—	—	—	—
- thyroiditis with or without goiter	242	—	—	—	—	—
- acquired hypothyroidism	244	—	—	0.02±0.001	—	—
- thyrotoxis	245	—	—	—	—	—
Mental disorders	290-319	0.02±0.001	0.03±0.002	0.02±0.001	0.04±0.0017	0.1±0.005 ^{1,2,3,4,5,6,7}
Diseases of nervous system and sense organs:	320-389	0.1±0.005	0.12±0.006	0.18±0.008	0.19±0.009	0.24±0.01 ^{1,2,3,4,5,6,7}
- vegeto-vascular dystonia	337	0.04±0.0017	0.03±0.002	0.05±0.003	0.02±0.001	0.01±0.0005
- cataracts	366	—	—	—	—	—
Circulatory diseases:	390-459	0.9±0.05	2.69±0.14	4.09±0.2	4.86±0.23	5.58±0.28 ^{1,2,3,4,5,6,7}
- hypertension	401-405	0.05±0.003	0.07±0.004	0.06±0.005	0.14±0.006	0.12±0.005 ^{1,2,3,4,5,6,7}
- ischemic heart disease	410-414	0.24±0.01	0.93±0.05	1.08±0.054	1.24±0.05	1.38±0.07 ^{1,2,3,4,5,6,7}
- cerebrovascular diseases	430-438	0.07±0.004	0.1±0.005	0.14±0.005	0.24±0.01	0.34±0.017 ^{1,2,3,4,5,6,7}
Respiratory diseases:	460-519	0.3±0.02	0.2±0.01	0.18±0.007	0.26±0.01	0.3±0.02 ^{1,2,3,4,5,6,7}
- chronic bronchitis, emphysema	491, 492	0.026±0.001	0.11±	0.14±0.007	0.18±0.007	0.24±0.01 ^{1,2,3,4,5,6,7}
- bronchial asthma	493	—	—	—	—	—
Digestive diseases:	520-579	0.14±0.006	0.19±0.01	0.28±0.016	0.54±0.03	0.86±0.04 ^{1,2,3,4,5,6,7}
- gastric and duodenal ulcers	531, 532	0.1±0.005	0.12±0.006	0.06±0.003	0.09±0.003	0.03±0.002
- gastritis, duodenitis	535	—	—	—	0.02±0.001	0.02±0.001
- liver diseases	571	0.04±0.0017	0.07±0.004	0.12±0.005	0.26±0.01	0.52±0.03 ^{1,2,3,4,5,6,7}
- biliary diseases	574-576	—	0.01±0.001	0.09±0.001	0.02±0.001	0.03±0.002 ^{1,2,3,4,5,6,7}
Genitourinary diseases:	580-589	0.006±0.0003	0.009±0.001	0.06±0.003	0.03±0.002	0.03±0.002 ^{1,2,3,4,5,6,7}
- urinary diseases	590-599	0.03±0.002	0.03±0.002	0.006±0.0003	0.02±0.001	0.03±0.002 ^{1,2,3,4,5,6,7}
Diseases of the skin and subcutaneous tissue	680-709	—	—	0.006±0.0003	0.02±0.001	0.04±0.0017 ^{1,2,3,4,5,6,7}
Musculoskeletal diseases:	710-739	—	0.004±0.0002	0.008±0.0004	0.02±0.001	0.02±0.001 ^{1,2,3,4,5,6,7}
- dorsopathy	720-724	—	—	—	0.002±0.001	0.004±0.0002 ⁸
- osteopathy, chondroopathy	730-739	—	0.002±0.0001	0.004±0.0002	0.014±0.0007	0.006±0.0003 ^{1,2,3,4,5,6,7}
Total	240-739	1.4±0.08	3.27±0.15	4.79±0.24	5.8±0.3	7.21±0.38 ^{1,2,3,4,5,6,7}

1) reliable increase in the rates relative to 1988; 2) relative to 1992; 3) relative to 1997; 4) relative to 2002

Table 11.21. Dynamics of non-tumor death rate among the Chernobyl clean-up workers of 1986–1987 aged >40 at the time of participation in recovery operations, 1988–2007 (%)

Classes and nosological forms of diseases	Code ICD-9	Postaccident periods				
		1988	1992	1997	2002	2007
Endocrine and metabolic diseases:						
- diabetes	240–249	—	0.16±0.007	0.34±0.013	0.16±0.007	0.18±0.007 ¹⁹⁸⁸
- thyroid diseases	250	—	—	0.04±0.0017	0.12±0.005	0.16±0.007 ¹⁹⁸⁸
- thyroid diseases	240–246	—	0.1±0.005	0.17±0.009	0.006±0.0003	—
- nontoxic nodular goiter	241	—	—	—	—	—
- thyrotoxicosis with or without goiter	242	—	—	—	—	—
- acquired hypothyroidism	244	—	—	—	0.006±0.0003	0.01±0.0005 ¹⁹
- thyroiditis	245	—	—	—	—	—
Mental disorders	290–319	—	—	0.01±0.0005	0.063±0.003	0.08±0.002 ¹⁹⁸⁸
Diseases of nervous system and sense organs:	320–359	0.03±0.002	0.17±0.009	0.2±0.01	0.1±0.005	0.3±0.02 ¹⁹⁸⁸
- vegeto-vascular dystonia	337	0.03±0.002	0.2±0.01	0.14±0.006	0.016±0.0008	0.04±0.0017 ¹⁹
- catarracts	368	—	—	—	—	—
Circulatory diseases:	360–459	2.0±0.1	3.21±0.16	4.56±0.23	9.14±0.46	13.36±0.57 ¹⁹⁸⁸
- hypertension	401–405	0.08±0.004	0.26±0.01	0.42±0.02	0.3±0.015	0.26±0.014 ¹⁹
- ischemic heart disease	410–414	1.28±0.06	2.03±0.1	2.76±0.14	6.03±0.3	6.08±0.31 ¹⁹⁸⁸
- cardiovascular diseases	430–436	0.46±0.02	0.72±0.04	0.86±0.05	1.68±0.08	2.47±0.12 ¹⁹⁸⁸
Respiratory diseases:	460–519	0.65±0.003	0.21±0.01	0.34±0.02	0.63±0.03	0.63±0.03 ¹⁹⁸⁸
- chronic bronchitis, emphysema	491, 492	0.01±0.0005	0.15±0.008	0.3±0.02	0.52±0.03	0.4±0.017 ¹⁹⁸⁸
- bronchial asthma	493	—	—	0.01±0.0005	—	—
Digestive diseases:	520–579	0.24±0.01	0.38±0.02	0.78±0.04	0.52±0.05	1.22±0.06 ¹⁹⁸⁸
- gastric and duodenal ulcers	531, 532	0.04±0.0017	0.08±0.004	0.11±0.006	0.1±0.0005	0.16±0.01 ¹⁹⁸⁸
- gastritis, duodenitis	535	—	—	—	—	—
- liver diseases	571	0.18±0.01	0.28±0.12	0.44±0.02	0.56±0.03	0.86±0.04 ¹⁹⁸⁸
- biliary diseases	574–576	—	—	0.06±0.003	0.07±0.004	0.1±0.005 ¹⁹⁸⁸
Genitourinary diseases:	580–589	0.01±0.0005	0.015±0.0008	0.13±0.007	0.08±0.004	0.05±0.003 ¹⁹⁸⁸
- urinary diseases	590–599	—	0.06±0.003	0.12±0.006	0.06±0.003	0.07±0.004 ¹⁹⁸⁸
Diseases of the skin and subcutaneous tissue	680–709	—	—	—	0.02±0.001	0.04±0.0017 ¹⁹
Musculoskeletal diseases:	710–739	—	0.02±0.001	0.03±0.001	0.04±0.0017	0.04±0.0017 ¹⁹⁸⁸
- dermopathy	720–724	—	—	0.008±0.0003	0.014±0.0007	0.01±0.0005 ¹⁹⁸⁸
- osteopathy, chondriopathy	730–739	—	0.01±0.0005	0.008±0.0008	—	—
Total	240–739	2.34±0.12	4.17±0.21	9.46±0.47	12.5±0.63	15.9±0.8 ¹⁹⁸⁸

Available increase in the rates relative to 1988; relative to 1992; relative to 1997; relative to 2002

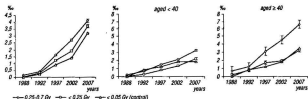


Fig. 11.24. Dynamics of non-tumor mortality among the Chernobyl clean-up workers of 1986–1987 depending on age at the time of their participation in recovery operations and whole body external dose, 1988–2007 (%)

When analyzing the dynamics of non-tumor mortality in clean-up workers, the dependence on whole body external dose and age at the time of exposure was found.

In the cohort as a whole (Fig. 11.24), the highest non-tumor mortality rates were established at radiation doses 0.05–0.249 and 0.25–0.7 Gy. The above pattern, depending on age at the time of exposure was found only in dose group 0.25–0.7 Gy; there was no age dependence at doses <0.25 Gy. Among clean-up workers aged 40–60 at the time of exposure, there were no differences in mortality rates for dose groups <0.05 and 0.05–0.249 Gy. In younger age group, mortality among people with radiation doses <0.05 appeared to be higher in comparison with dose groups 0.05–0.249 Gy, except for the last observation year (2007).

Regardless of age at the time of exposure the highest death rates from circulatory diseases were observed in dose groups 0.05–0.249 and 0.25–0.7 Gy (Fig. 11.25). However since 1997, death rate for clean-up workers (older age group) at doses 0.25–0.7 Gy was about twice as high as that of people under 40. In this case it may be the result of two potential impacts — “Aging” and “Ionizing radiation”.

A similar pattern is observed when analyzing the dynamics of mortality from respiratory (Fig. 11.26) and digestive diseases (Fig. 11.27).

Thus, analysis of relationship between circulatory, respiratory and digestive mortality and whole body external dose in the Chernobyl clean-up workers of 1986–1987 suggests a possible association between growth of mortality from these classes of non-tumor diseases and whole body external dose.

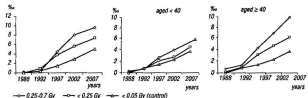


Fig. 11.25. Dynamics of death rates from circulatory diseases among the Chernobyl clean-up workers of 1986–1987 depending on whole body external dose and age at the time of participation in recovery operations, 1988–2007 (%)

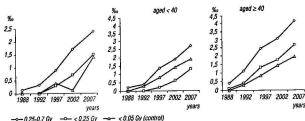


Fig. 11.26. Dynamics of mortality from respiratory diseases in the Chernobyl clean-up workers of 1986-1987 depending on whole body external dose and age at the time of their participation in recovery operations, 1988-2007 (%).

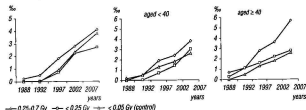


Fig. 11.27. Dynamics of mortality from digestive diseases among the Chernobyl clean-up workers of 1986-1987 depending on whole body external dose and age at the time of their participation in recovery operations, 1988-2007 (%).

To prove this hypothesis, a risk analysis of circulatory mortality among clean-up workers was conducted (Table 11.22, 11.23). In the study cohort (regardless of age) as a whole and also in subcohort of people aged over 40 at the time of exposure, significant RR of mortality was found in those with radiation dose 0.25-0.7 Gy for circulatory diseases, particularly hypertension, ischemic heart disease and cerebrovascular diseases (10, 15, 20 years after exposure).

Table 11.22. Relative risks (RR) for mortality from non-tumor circulatory diseases among the Chernobyl clean-up workers of 1986-1987 in the cohort as a whole (regardless of age), doses 0.25-0.7 Gy, 5-, 10-, 15-, 20-year postradiation periods

Classes and nosological forms of diseases	Code ICD-9	Postradiation periods (years)			
		5 1988-1992	10 1988-1997	15 1988-2002	20 1988-2007
Circulatory diseases:	390-459	—	2.6 (1.17;3.4)	2.9 (1.31;4.3)	2.4 (1.21;3.8)
– ischemic heart disease	410-414	—	3.1 (2.2;5.2)	2.78 (1.75;4.1)	2.81 (1.9;3.72)
– cerebrovascular diseases	430-438	—	1.97 (1.4;2.5)	2.34 (1.9;4.23)	2.41 (1.3; 3.7)

Table 11.23. Relative risks (RR) for mortality from non-tumor circulatory diseases among the Chernobyl clean-up workers of 1986–1987 aged ≥ 40 at the time of their participation in recovery operations, whole body external doses 0.25–0.7 Gy, 5-, 10-, 15-, 20-year postradiation periods

Classes and nosological forms of diseases	Code ICD-9	Postradiation periods (years)			
		5 1986–1992	10 1986–1997	15 1988–2002	20 1988–2007
Circulatory diseases:	390–459	—	1.27 (1.1;1.54)	1.35 (1.2;1.74)	1.64 (1.32;2.9)
– ischemic heart disease	410–414	—	1.21 (1.13;2.5)	1.25 (1.15;2.3)	1.32 (1.2;3.1)
– cerebrovascular diseases	430–438	—	1.31 (1.19;2.3)	1.42 (1.1;1.93)	1.67 (1.4;3.74)

Table 11.24 shows excess relative risk (ERR) for non-tumor mortality in the Chernobyl clean-up workers of 1986–1987 aged ≥ 40 at the time of participation in recovery operations at doses of whole body external radiation 0.25–0.7 Gy.

The analysis of mortality rates in subcohorts with doses < 0.25 Gy and in the age group under 40 at the time of exposure will be presented in the subsequent publications.

According to these data, a significant mortality ERR was obtained in this age group at doses 0.25–0.7 Gy for circulatory diseases, particularly hypertension, ischemic heart disease, cerebrovascular diseases for 10-, 15-, 20-year postradiation periods.

Table 11.24. Excess relative risks for mortality from non-tumor circulatory diseases among the Chernobyl clean-up workers of 1986–1987 aged ≥ 40 at the time of participation in recovery operations, external dose 0.25–0.7 Gy (Daverage = 0.3 Gy)

Classes and nosological forms of diseases	Code ICD-9	Postradiation periods (years)			
		5 1986–1992	10 1986–1997	15 1988–2002	20 2003–2007
Circulatory diseases:	390–459	—	0.9 (0.6;1.4)	1.2 (0.9;1.8)	0.67 (0.32;0.8)
– ischemic heart disease	410–414	—	0.56 (0.31;0.7)	0.83 (0.44;0.9)	0.73 (0.25;0.9)
– cerebrovascular diseases	430–438	—	1.03 (0.87;2.1)	1.4 (1.1;2.12)	0.97 (0.45;1.2)

Thus, considering the results of our study we can conclude the following:

- non-tumor mortality in clean-up workers of 1986–1987 grew within 1988–2007;
- circulatory, digestive and respiratory diseases predominate in structure of mortality;
- whole body external doses ranging from 0.05 to 0.7 Gy may be a factor of excess risk for death from these diseases;
- significant relative risks (RR) for circulatory mortality in the cohort of clean-up workers as a whole and in subcohort aged ≥ 40 at the time of exposure were found in dose group 0.25–0.7 Gy, 10, 15, 20 years after exposure; excess relative risks (ERR) were calculated for persons aged 40 and older at the time of exposure with doses of whole body external radiation 0.25–0.7 Gy, for 10, 15, 20 postradiation years;
- in our study of stochastic effects of small doses from short-term external radiation (< 1 Gy), here non-tumor mortality, duration of follow-up is crucial; in accordance with the results obtained, duration of medical follow-up can not be less than 10 years

after exposure. It is quite probable that for those exposed at a younger age this term should be extended;

- our values of excess RRs for cardiovascular mortality are similar to the results of the study of atomic bomb victims in Hiroshima and Nagasaki [14, 15] (Life Span Study), and data from the Russian National Medical and Dosimetric Registry [11].

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11.2 EPIDEMIOLOGICAL STUDIES OF NONTUMOR MORBIDITY AND MORTALITY IN ADULT EVACUEES: DYNAMICS AND EFFECTS OF LOW-DOSE IONIZING RADIATION

Non tumor morbidity

Evacuees are referred to the category of persons affected by the Chernobyl catastrophe at higher risks for non-tumor morbidity and mortality [1, 2]. The evidence derived from our 25-year study suggests a significant growth of somatic and psychosomatic diseases among evacuees in postevacuation period [3, 4]. Currently, non-tumor morbidity is a causative factor of disability and mortality [5, 6].

Epidemiological studies of non-tumor morbidity and mortality in adult at the time of the accident evacuees (15 years and older) were conducted during 1988–2007 using the data of the SRU. Analysis and evaluation were performed for five-year observation periods (1988–1992; 1993–1997; 1998–2002; 2003–2007) with proper regard for sex and age (<40 years and ≥40 years). Morbidity and mortality rates were calculated per 10³ person/years. Number of examined persons by observation periods (see Table 11.25).

Table 11.25. Number of examined persons in adult evacuees' cohort by sex and age groups (<40 and ≥40 years), five-year observation periods

Observation periods	Follow-up cohort, person/years, abs. (%)			Total number of persons aged, years, abs. (%)		Men aged, years, abs. (%)		Women aged, years, abs. (%)	
	total	men	women	<40	≥40	<40	≥40	<40	≥40
1988–1992	145532 (100)	57505 (39.5)	88027 (60.5)	65593 (45.1)	79939 (54.9)	25911 (45.1)	31595 (54.9)	39682 (45.1)	48344 (54.9)
1993–1997	171926 (100)	68752 (40.0)	103174 (60.0)	79577 (46.3)	92349 (53.7)	31872 (46.3)	36937 (53.7)	47725 (46.2)	55462 (53.8)
1998–2002	212350 (100)	88470 (41.7)	123880 (58.3)	98449 (46.4)	113901 (53.6)	41040 (46.3)	47523 (53.7)	57489 (46.3)	66558 (53.7)
2003–2007	235835 (100)	99742 (42.3)	136093 (57.7)	99277 (42.1)	136558 (57.9)	45880 (46.0)	53862 (54.0)	53397 (39.2)	82696 (60.8)

The results showed ambiguous rates of non-tumor diseases for different observation periods (Fig. 11.28).

For all periods significantly higher incidence rates against initial ones (1988–1992) were observed, with the highest rates being recorded in 1998–2002. In 2003–2007, a slight decline in rates was noted but still they were statistically higher than those of the first period. In 2003–2007, some reduction in incidence rates was apparently associated with manifestation of the majority of newly diagnosed diseases in the earlier stages of research and with mortality of the part of the study cohort.

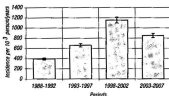


Fig. 11.28. Dynamics of non-tumor morbidity among adult evacuees for five-year observation periods

Over the period of study, specific changes in the structure of morbidity were recorded (Fig. 11.29).

Essential changes were observed in studies of the dynamics of non-tumor diseases by specific classes and nosological forms (Table 11.26–11.28).



Fig. 11.29. Structure of non-tumor diseases (%) among adult evacuees for 1988 and 2007

Table 11.26. Non-tumor morbidity rates for some classes and nosological forms in the adult evacuees' cohort in dynamics for five-year observation periods, per 10³ person/years

Classes and nosological forms of diseases	ICD-9	Observation periods			
		1988–1992	1993–1997	1998–2002	2003–2007
All diseases:	240–629; 680–739	392.5±1.3	661.5±1.1*	1154.6±3.4**	850.4±0.73***
Endocrine diseases:	240–279	29.9±0.45	32.7±0.43	38.4±0.42***	18.8±0.28
Thyroid diseases:	240–246	19.7±0.36	23.9±0.37*	24.4±0.33*	12.9±0.23
– nontoxic nodular goiter	241	1.7±0.11	2.0±0.11	2.4±0.11	3.5±0.12***
– acquired hypothyroidism	244	0.45±0.06	0.95±0.07	3.7±0.13***	1.3±0.07***
– thyroiditis	245	0.67±0.07	2.3±0.12*	3.5±0.13***	2.2±0.10*
– diabetes	250	3.0±0.14	3.1±0.13	3.3±0.13	2.0±0.09
Mental disorders	290–319	12.9±0.3	7.9±0.2*	6.6±0.18	2.0±0.09
Diseases of nervous system and sense organs:	320–389	40.7±0.5	85.1±0.7*	141.5±0.76***	56.5±0.48*
– vegeto-vascular dystonia	337	15.2±1.0	38.0±0.5*	50.4±0.8***	17.8±0.27*
Circulatory diseases:	390–459	84.4±0.7	103.0±0.73*	129.9±0.73***	72.1±0.53
– hypertension	401–405	17.6±1.1	15.1±0.3	19.2±0.3**	8.9±0.19
– ischemic heart disease	412–414	45.2±0.54	45.4±0.5	54.8±0.49***	30.7±0.35
– cerebrovascular diseases	437–438	7.9±0.23	21.4±0.35*	25.6±0.34***	13.1±0.23*
Respiratory diseases:	460–519	105.8±0.8	214.0±1.0*	298.9±1.0***	134.8±0.7*
– chronic bronchitis, emphysema	491–492	8.0±0.23	11.7±0.26*	21.5±0.3***	8.9±0.19
Digestive diseases:	520–579	54.9±0.6	97.2±0.7*	225.7±0.9***	125.9±0.68***
– gastric and duodenal ulcers	531–532	4.8±0.18	6.6±0.2*	9.2±0.2***	4.9±0.14*
– diseases of the liver, biliary tract and pancreas	571–577	24.9±0.41	51.2±0.53*	125.8±0.72***	74.8±0.54***
Genitourinary diseases	580–629	10.1±0.26	25.7±0.38*	41.1±0.43***	23.6±0.31*
Musculoskeletal diseases	710–739	30.6±0.45	65.2±0.6*	100.1±0.65***	47.6±0.44*

*significant rise in rates relative to those in 1988–1992; **as related to those in 1993–1997, +relative to those in 1998–2002

Table 11.27. Non-tumor morbidity rates in adult evacuees by some classes and nosological forms for five-year observation periods depending on sex, per 10³ person/years

Classes and nosological forms of the diseases	ICD-9	Observation periods			
		1986-1992	1993-1997	1998-2002	2003-2007
Men					
All diseases:	240-629; 680-739	348.1±2.0	574.3±1.9*	971.8±0.6**	528.4±1.5*
All diseases:	240-279	18.8±0.57	24.9±0.59*	26.2±0.54*	13.5±0.37
Endocrine diseases:	240-246	11.6±0.45	17.6±0.5*	17.0±0.43*	9.1±0.30
Thyroid diseases:	241	0.5±0.09	1.5±0.13*	1.05±0.11	2.5±0.16**
- nontoxic nodular goiter	244	0.24±0.06	0.36±0.07	2.4±0.16**	0.55±0.07**
- acquired hypothyroidism	245	0.38±0.08	0.86±0.11	2.0±0.15**	0.93±0.10*
- thyroiditis	290-319	11.5±0.45	8.1±0.34*	5.6±0.25**	2.2±0.15
Mental disorders	320-389	35.9±0.78	64.9±0.94*	110.2±1.05**	49.8±0.69*
Diseases of nervous system and sense organs:	337	12.4±0.46	23.8±0.56*	39.0±0.65**	15.6±0.39*
- vegetovascular dystonia	390-459	40.1±1.1	88.5±1.1*	104.6±1.02**	68.0±0.8*
Circulatory diseases:	401-405	16.7±0.53	13.1±0.43*	14.4±0.4*	7.3±0.27
- hypertension	412-414	31.4±0.73	40.7±0.75*	43.3±0.68**	24.8±0.49
- ischemic heart disease	437-438	5.7±0.3	17.3±0.5*	19.4±0.46**	12.1±0.35*
- cerebrovascular disease	460-519	103.0±1.3	1.49±1.5*	279.8±1.5**	133.4±1.1*
Respiratory diseases:	491-492	10.2±0.42	13.1±0.43*	19.5±0.46**	7.5±0.27
- chronic bronchitis, emphysema	520-579	51.2±0.92	86.1±1.1*	182.9±1.3**	102.6±0.96**
Digestive diseases:	531-532	7.5±0.36	9.3±0.37*	10.7±0.35*	6.7±0.26
- gastric and duodenal ulcers	571-577	19.2±0.57	41.5±0.76*	93.8±0.98**	55.8±0.73**
- diseases of the liver, biliary tract and pancreas	580-629	5.8±0.32	13.7±0.44*	23.2±0.51**	14.4±0.38**
Genitourinary diseases	710-739	29.5±0.71	54.2±0.86*	81.7±0.92**	38.9±0.61*
Women					
All diseases:	240-629; 680-739	421.4±1.7	719.6±1.4*	1284.4±4.7**	646.4±1.3*
All diseases:	240-279	37.2±0.64	37.9±0.59	47.1±0.6**	22.7±0.4
Endocrine diseases:	240-246	24.9±0.53	28.0±0.51*	29.6±0.48*	15.8±0.34
Thyroid diseases:	241	2.5±0.17	2.6±0.16	3.4±0.17	4.2±0.18**
- nontoxic nodular goiter	244	0.58±0.08	1.3±0.11	4.7±0.19**	1.8±0.11*
- acquired hypothyroidism	245	0.68±0.09	3.0±0.17*	4.5±0.19**	3.1±0.15*
- thyroiditis	290-319	13.9±0.39	7.8±0.27*	7.3±0.24*	1.9±0.12
Mental disorders	320-389	43.7±0.69	98.5±0.93*	163.8±1.05**	61.3±0.65*
Diseases of nervous system and sense organs:	337	17.0±0.44	47.4±0.66*	58.6±0.67**	18.7±0.37*
- vegetovascular dystonia	390-459	93.7±0.98	112.6±0.96*	147.9±1.0**	75.2±0.71
Circulatory diseases:	401-405	18.3±0.45	16.4±0.4	22.8±0.42**	10.1±0.27
- hypertension	412-414	50.3±0.74	48.5±0.67	63.4±0.69**	34.2±0.49
- ischemic heart disease	437-438	8.2±0.3	24.1±0.48*	30.0±0.48**	13.7±0.32*
- cerebrovascular diseases	460-519	107.6±1.0	220.1±1.3*	312.4±1.3**	135.8±0.93*
Respiratory diseases:	491-492	6.6±0.27	10.9±0.32*	22.9±0.42**	10.0±0.27*
- chronic bronchitis, emphysema	520-579	57.2±0.78	104.5±0.96*	256.2±1.2**	143.0±0.95**
Digestive diseases:	531-532	3.0±0.16	4.8±0.22*	8.0±0.25**	3.6±0.16
- gastric and duodenal ulcers	571-577	28.7±0.56	57.6±0.73*	148.6±1.0**	88.6±0.77**
- diseases of the liver, biliary tract and pancreas	580-629	16.4±0.43	33.7±0.56*	53.8±0.64**	30.2±0.46*
Genitourinary diseases	710-739	31.3±0.59	72.6±1.81*	113.3±0.9**	54.1±0.61*

*significant rise in rates relative to those in 1986-1992; **as related to those in 1993-1997, *relative to those in 1998-2002

Table 11.28. Non-tumor incidence rate among adult evacuees by some classes and nosological forms for five-year observation periods depending on age, per 10³ person-years

Classes and nosological forms of diseases	ICD-9	Observation periods			
		1988–1992	1993–1997	1998–2002	2003–2007
<40 years					
Total:	240–629; 680–739	388.7±3.1	578.8±2.3*	1043.6±3.0***	524.8±1.6*
Endocrine diseases:	240–279	38.4±0.75	32.3±0.63*	30.4±0.55***	12.2±0.35
Thyroid diseases:	240–246	29.7±0.66	26.3±0.57*	22.3±0.47***	9.2±0.30
– nontoxic nodular goiter	241	2.1±0.18	1.4±0.13	1.4±0.12	2.0±0.14
– acquired hypothyroidism	244	0.37±0.08	0.54±0.08	3.5±0.19***	0.9±0.10***
– thyroiditis	245	0.8±0.11	2.2±0.178	2.3±0.15*	1.2±0.11*
Mental disorders	290–319	15.9±0.49	8.8±0.33*	4.7±0.22***	1.2±0.11
Diseases of nervous system and sense organs:	320–389	50.3±0.85	77.3±0.95*	104.9±0.98***	40.7±0.63
– vegeto-vascular dystonia	337	21.4±0.57	42.3±0.71*	62.2±0.77***	23.4±0.48*
Circulatory diseases:	390–459	21.2±0.56	23.0±0.53	23.8±0.49*	10.0±0.32
– hypertension	401–405	9.5±0.38	6.8±0.29*	7.3±0.27	2.7±0.16
– ischemic heart disease	412–414	2.7±0.19	2.4±0.17	2.9±0.17	1.8±0.13
– cerebrovascular diseases	437–438	0.85±0.11	4.3±0.23*	3.1±0.18*	1.0±0.10
Respiratory diseases:	460–519	139.7±1.4	263.7±1.5*	372.1±1.5***	187.7±1.2*
– chronic bronchitis, emphysema	491–492	3.4±0.05	5.8±0.27*	16.3±0.4***	4.0±0.2
Digestive diseases:	520–579	57.6±0.91	83.3±0.98*	192.8±1.3***	101.4±0.96
– gastric and duodenal ulcers	531–532	4.3±0.26	5.9±0.27*	5.3±0.24*	2.0±0.14
– diseases of the liver, biliary tract and pancreas	571–577	25.5±0.62	40.1±2.2*	98.4±0.95***	51.6±0.7***
Genitourinary diseases	580–629	15.7±0.49	25.3±0.56*	35.3±0.59***	19.1±0.43*
Musculoskeletal diseases:	710–739	22.2±0.58	34.1±0.84*	51.4±0.7***	21.9±0.46
≥40 years					
Total:	240–629; 680–739	397.2±1.7	732.7±1.5*	1250.6±3.1***	648.7±1.3*
Endocrine diseases:	240–279	23.0±0.53	33.0±0.59*	45.3±0.62***	23.4±0.41
Thyroid diseases:	240–246	11.4±0.38	21.7±0.48*	26.0±0.47***	6.4±0.22
– nontoxic nodular goiter	241	1.4±0.13	2.4±0.16	3.3±0.17*	4.4±0.18***
– acquired hypothyroidism	244	0.5±0.08	1.3±0.12	3.9±0.18***	1.8±0.11*
– thyroiditis	245	0.8±0.09	2.5±0.16*	4.3±0.19***	2.9±0.15*
Mental disorders	290–319	10.5±0.36	7.4±0.28	8.1±0.27*	2.5±0.14
Diseases of nervous system and sense organs:	320–389	32.8±0.63	91.8±0.95*	173.1±1.1***	67.2±0.68*
– vegeto-vascular dystonia	337	9.0±0.33	34.2±0.6*	40.3±0.58***	13.4±0.31*
Circulatory diseases:	390–459	133.7±1.2	172.0±1.2*	221.5±1.2***	117.6±0.87
– hypertension	401–405	24.4±0.55	22.2±0.48*	29.5±0.5***	13.4±0.31
– ischemic heart disease	412–414	80.3±0.96	82.4±0.9	99.7±0.89***	51.1±0.59
– cerebrovascular diseases	437–438	13.7±0.41	36.1±0.61*	45.1±0.61***	21.8±0.4*
Respiratory diseases:	460–519	76.5±0.94	171.3±1.2*	235.5±1.25***	95.2±0.79*
– chronic bronchitis, emphysema	491–492	11.8±0.38	16.8±0.42*	26.0±0.47***	12.5±0.30*
Digestive diseases:	520–579	52.8±0.79	108.7±1.0*	254.1±1.3***	143.1±0.95***
– gastric and duodenal ulcers	531–532	5.2±0.25	8.3±0.3*	12.1±0.32***	7.0±0.23*
– diseases of the liver, biliary tract and pancreas	571–577	24.4±0.55	59.7±0.78*	151.3±1.1***	91.6±0.78***
Genitourinary diseases	580–629	9.4±3.4	26.0±0.52*	45.1±0.62***	26.8±0.44*
Musculoskeletal diseases	710–739	37.5±0.67	92.1±0.95*	142.3±1.0***	66.1±0.67*

*Significant rise in rates relative to those in 1988–1992; ***as related to those in 1993–1997, +relative to those in 1998–2002

In 1988 as well as in 2007, digestive, circulatory and respiratory diseases were predominant, however their ranking has changed significantly: 1988 — circulatory, respiratory and digestive diseases; 2007 — digestive, circulatory and respiratory diseases. Changes concerned other diseases as well.

In 2003–2007, as compared with all previous periods, significantly higher level of disease of non-toxic nodular goiter was recorded. Statistically higher, against the first and second periods, were rates for some diseases (acquired hypothyroidism, digestive diseases, particularly those of the liver, biliary tract and pancreas). Morbidity of thyroiditis, nervous system and sense organs, particularly vegeto-vascular dystonia, cerebrovascular diseases, respiratory diseases, stomach and duodenum ulcers, genitourinary and musculoskeletal diseases significantly exceeded appropriate rates of the first period only. For other diseases statistically lower rates were recorded.

It is notable that in the first period (1988–1992) level of some non-tumor diseases is sufficiently high, it was either equal to that of the second period (1993–1997) or exceeded that of the last one (2003–2007). It was relevant to endocrine, thyroid diseases, nontoxic nodular goiter, diabetes, vegeto-vascular dystonia, circulatory diseases (hypertension, ischemic heart disease), stomach and duodenum ulcer. Considering the highest rates of mental disorders in the initial period (compared with the next ones) a possible correlation of non-tumor diseases development in evacuees and stress experienced during evacuation time may be assumed.

Certain features of non-tumor morbidity among adult evacuees were identified depending on sex. In comparison with men, women had higher disease rates through all observation periods (Fig. 11.30).

In both men and women, the highest reliable rates were recorded within 1988–2002, while in 2003–2007 incidence rates significantly exceeded those of 1988–1992 and practically corresponded to those of 1993–1997.

Regarding changes in morbidity by some classes and nosological forms, the following is noteworthy (Table 11.27). In 2003–2007, rate of almost all diseases was higher in women (except for stomach and duodenum ulcers). Most of the rates statistically exceed those of the first period.

Men had significantly higher (against the first period) incidence of thyroiditis, diseases of nervous system and sense organs (particularly vegeto-vascular dystonia), circulatory diseases (including cerebrovascular pathology), respiratory, digestive (including diseases of the liver, biliary tract and pancreas), genitourinary and musculoskeletal diseases.

In women, statistical excess of the first period rates was caused by acquired hypothyroidism, thyroiditis, diseases of nervous system and sense organs (particu-

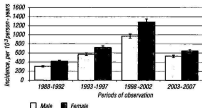


Fig. 11.30. The dynamics of non-tumor morbidity in adult evacuees with regard to sex for five-year observation periods.

laly vegetato-vascular dystonia), cerebrovascular diseases, respiratory diseases (including chronic bronchitis and emphysema), digestive diseases (including stomach and duodenum ulcers, diseases of the liver, biliary tract and pancreas), genitourinary and musculoskeletal diseases.

In 2003–2007, rates for some diseases (regardless of sex) significantly exceeded those of the second period. As for men, it concerns acquired hypothyroidism, digestive (particularly liver, biliary tract and pancreas) and genitourinary diseases; as for women — digestive diseases (including liver, biliary tract and pancreas).

Most of changes concerned incidence of nontoxic nodular goiter. In 2003–2007, the specified pathology rates in men were 5 times higher compared with those for the first period and almost 2.5 times — as compared to the third one. The highest rates were recorded in 2003–2007. In women, the excess was by a factor of 1.2–1.7 higher (in comparison with all previous periods). Steady increase in incidence rate of non-toxic nodular goiter among evacuees being resulted from higher radiation exposure of the thyroid gland in 'victims' group needs special attention from health authorities. It is necessary for early prevention of potential negative consequences (malignant transformation) among evacuees.

Age-dependent changes in non-tumor diseases were evident (Fig. 11.31).

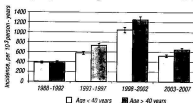


Fig. 11.31. The dynamics of non-tumor morbidity among adult evacuees depending on age for five-year observation periods

The tendency was proved by the dynamics of disease by some classes and nosological forms (Table 11.28).

According to our findings, most disease rates were higher in people over 40 years. It mostly concerned cardiovascular diseases, particularly ischemic heart disease and cerebrovascular pathology.

Incidence of endocrine disorders was almost 2 times higher in people over 40, yet in 2003–2007, rates of thyroid diseases in younger persons were significantly higher compared with the elder contingent. Higher rates for respiratory diseases and vegeto-vascular dystonia were also observed among persons under 40.

Incidence of non-toxic nodular goiter in evacuees under 40 was practically stable throughout the entire study period, whereas it increased gradually in those aged 40 and older, the highest rates, significantly different from those of the previous periods, were recorded in 2003–2007.

As shown in the figure, incidence rate in persons aged 40 and older exceeded that of younger contingent through all observation periods. During 1988–2007, the trend remained the same as in the cohort as a whole. In 2003–2007, incidence rate, regardless of age, was significantly higher than that of the first period and was practically consistent with that of the second one.

Non-tumor mortality

Results of epidemiological studies of non-tumor mortality among adult evacuees within 1988–2007 showed its gradual increase in the cohort as a whole and separately for men and women. The highest rates were found for the period of 2003–2007 (Fig. 11.32).

Mortality rates in males and females were almost equal.

Throughout all study periods, cardiovascular morbidity was leading in the structure of mortality. At different times, its contribution ranged from 45 to 83%. In 2007, the percentage of cardiovascular disease increased to 88.5% (Fig. 11.33), 84.9% in men and 90.7% in women. Diseases of nervous system and sense organs, respiratory and digestive diseases also make certain contribution to the structure of evacuees' death. In different years, contribution of respiratory diseases varied from 1.3 to 12.5%, digestive diseases — from 0.7 to 8.1%, diseases of nervous system and sense organs — from 0.2 to 4.0%.

Among circulatory diseases ischemic heart disease ranks first (39.5%). Thus in 2007, death from ischemic heart disease was observed in men (66% of cases) and in women (60.3%). Hypertension contributed 11%, cerebrovascular pathology — 3.4%.

An analysis of causes-of-death dynamics by five-year observation periods also revealed the dominant role of cardiovascular diseases, particularly ischemic heart disease (Table 11.29). As seen from the table, evacuees' death rate (observation period 2003–2007) from circulatory diseases in general was near twice as much as the initial rate (1988–1992), the same was true of ischemic heart disease.

Throughout first three periods, the hypertension death rate remained unchanged with notable increase in 2003–2007. Any significant excess of cerebrovascular mortality was observed during study (Fig. 11.34–11.37).

The analysis revealed significant increase in death rate from diseases of nervous system and sense organs (mainly vegeto-vascular dystonia diseases primarily due to chronic bronchitis, emphysema and digestive diseases mostly due to diseases of the liver, biliary tract and pancreas).

Mortality rates of genitourinary, musculoskeletal diseases, diseases of skin and subcutaneous tissue, mental disorders were minimal throughout the study period. Mortality from endocrine diseases was mainly attributed to diabetes.

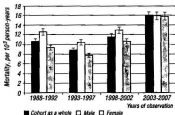


Fig. 11.32. Dynamics of non-tumor mortality in adult evacuees for five-year observation periods



Fig. 11.33. Structure of non-tumor mortality (%) among adult evacuees, 2007

Table 11.29. The dynamics of non-tumor death rate by some classes and nosological forms of diseases in adult evacuees' cohort for five-year observation periods, per 10³ person/years

Classes and nosological forms of diseases	ICD-9	Observation periods			
		1988–1992	1993–1997	1998–2002	2003–2007
All diseases:	240–829; 680–739	10.6±0.27	8.8±0.23	11.5±0.23	15.9±0.26 ^{*,***}
Endocrine diseases:	240–279	0.04±0.02	0.18±0.03*	0.07±0.02	0.09±0.02*
– diabetes	250	0.03±0.01	0.09±0.02*	0.06±0.02	0.08±0.02*
Diseases of nervous system and sense organs:	320–389	0.05±0.02	0.17±0.03*	0.16±0.03**	0.4±0.04 ^{*,***}
– vegeto-vascular dystonia	337	0.01±0.008	0.1±0.02*	0.04±0.01	0.2±0.03*
Circulatory diseases:	390–459	6.9±0.2	5.8±0.2	8.0±0.19 ^{*,***}	11.8±0.22 ^{*,***}
– hypertension	401–405	0.1±0.03	0.09±0.02	0.18±0.03	1.2±0.07 ^{*,***}
– ischemic heart disease	412–414	3.6±0.16	3.4±0.14	4.5±0.15 ^{*,***}	7.7±0.18 ^{*,***}
– cerebrovascular diseases	437–438	1.1±0.09	0.9±0.07	0.9±0.07	0.6±0.05
Respiratory diseases:	460–519	0.2±0.04	0.3±0.04	0.2±0.03	0.7±0.05 ^{*,***}
– chronic bronchitis, emphysema	491–492	0.1±0.03	0.1±0.02	0.1±0.02	0.2±0.03 ^{*,***}
Digestive diseases:	520–579	0.2±0.04	0.2±0.03	0.3±0.04	0.6±0.05 ^{*,***}
– gastric and duodenal ulcers	531–532	0.02±0.01	0.04±0.02	0.01±0.007	0.06±0.02**
– diseases of the liver, biliary tract and pancreas	571–577	0.1±0.03	0.1±0.02	0.2±0.03	0.4±0.04 ^{*,***}

*significant rise in rates relative to those in 1988–1992; **as related to those in 1993–1997; ***relative to those in 1998–2002

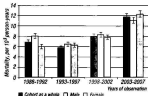


Fig. 11.34. Dynamics of mortality from circulatory diseases in adult evacuees for five-year observation periods

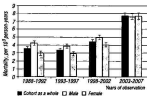


Fig. 11.35. Dynamics of mortality from ischemic heart disease in adult evacuees for five-year observation periods



Fig. 11.36. Dynamics of mortality from hypertension in adult evacuees for five-year observation periods

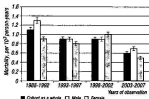


Fig. 11.37. Dynamics of mortality from cerebrovascular diseases in adult evacuees for five-year observation periods

Table 11.30. The dynamics of non-tumor mortality for some classes and nosological forms of diseases in adult evacuees' cohort for five-year observation periods depending on sex, per 10³ person/years

Classes and nosological forms of diseases	ICD-9	Observation periods			
		1988-1992	1993-1997	1998-2002	2003-2007
Men					
All diseases:	240-629; 680-739	12.6±0.47	10.4±0.39	12.9±0.38	15.9±0.40 ^{*,**,*}
Endocrine diseases:	240-279	0.07±0.03	0.19±0.05	0.05±0.02	0.02±0.01
– diabetes	250	0.03±0.02	0.1±0.04	0.03±0.02	0.04±0.02
Mental disorders	290-319	0.03±0.02	0.03±0.02	0.06±0.03	0.43±0.07 ^{*,**,*}
Diseases of nervous system and sense organs:	320-389	0.05±0.03	0.22±0.05 [*]	0.16±0.04 ^{*,**}	0.2±0.04 [*]
– vegeto-vascular dystonia	337	0	0.15±0.05	0.06±0.03	0.03±0.02
Circulatory diseases:	390-459	8.1±0.37	6.5±0.3	8.3±0.3	11.1±0.33 ^{*,**,*}
– hypertension	401-405	0.14±0.05	0.09±0.04	0.2±0.05	0.73±0.09 ^{*,**,*}
– ischemic heart disease	412-414	4.3±0.27	3.9±0.24	5.0±0.24	7.6±0.27 ^{*,**,*}
– cerebrovascular diseases	437-438	1.3±0.15	0.9±0.11	0.9±0.1	0.7±0.08
Respiratory diseases:	460-519	0.3±0.07	0.3±0.07	0.3±0.06	0.9±0.09 ^{*,**,*}
– chronic bronchitis, emphysema	491-492	0.21±0.05	0.19±0.05	0.16±0.04	0.24±0.1
– bronchial asthma	493	0	0.03±0.02	0.01±0.01	0.03±0.02 [*]
Digestive diseases:	520-579	0.3±0.07	0.35±0.07	0.44±0.07	0.73±0.09 ^{*,**,*}
– diseases of the liver, biliary tract and pancreas	571-577	0.23±0.06	0.26±0.06	0.28±0.06	0.54±0.07 ^{*,**,*}
Genitourinary diseases	580-629	0.03±0.02	0.04±0.02	0.09±0.03	0.03±0.02
Urinary diseases	581-599	0.03±0.02	0.06±0.03	0.07±0.03	0.03±0.02
Women					
All diseases:	240-629; 680-739	9.3±0.32	7.7±0.27	10.6±0.29	15.8±0.34 ^{*,**,*}
Endocrine diseases:	240-279	0.02±0.15	0.14±0.04	0.08±0.03	0.15±0.03 [*]
– diabetes	250	0.03±0.18	0.09±0.03	0.07±0.02	0.12±0.03 [*]
Mental disorders	290-319	0	0	0.02±0.01	0
Diseases of nervous system and sense organs:	320-389	0.05±0.02	0.14±0.04	0.15±0.03	0.44±0.06 ^{*,**,*}
– vegeto-vascular dystonia	337	0.02±0.02	0.09±0.03	0.03±0.02	0.24±0.04 ^{*,**,*}
Circulatory diseases:	390-459	6.1±0.26	5.3±0.23	7.8±0.25	12.3±0.3 ^{*,**,*}
– hypertension	401-405	0.09±0.03	0.1±0.03	0.17±0.04	1.5±0.1 ^{*,**,*}
– ischemic heart disease	412-414	3.1±0.19	3.0±0.17	4.1±0.03	7.7±0.24 ^{*,**,*}
– cerebrovascular diseases	437-438	0.9±0.1	0.8±0.09	1.0±0.09	0.5±0.06
Respiratory diseases:	460-519	0.2±0.05	0.19±0.04	0.18±0.04	0.63±0.07 ^{*,**,*}
– chronic bronchitis, emphysema	491-492	0.05±0.03	0.1±0.03	0.07±0.02	0.13±0.03
– bronchial asthma	493	0.02±0.02	0.02±0.01	0.008±0.008	0.04±0.02
Digestive diseases:	520-579	0.1±0.03	0.1±0.03	0.2±0.01	0.5±0.06 ^{*,**,*}
– diseases of the liver, biliary tract and pancreas	571-577	0.03±0.18	0.06±0.02	0.15±0.03	0.03±0.05
Genitourinary diseases	580-629	0.05±0.02	0.05±0.02	0.04±0.02	0.33±0.05 ^{*,**,*}
Urinary diseases	581-599	0.02±0.02	0.01±0.01	0.008±0.008	0.03±0.01

*significant rise in rates relative to those in 1988-1992; **ex related to those in 1993-1997; ***relative to those in 1998-2002

Analysis of non-tumor mortality by sex (Table 11.30) showed higher rates in men.

And yet in 2003–2007, total mortality of men and women became equal (see Fig. 11.32). As in the cohort as a whole, in men and women the highest mortality rates were established for circulatory diseases, particularly ischemic heart disease. The contribution of hypertension and cerebrovascular pathology did not exceed 12–15%. Much lower mortality rates for diseases of nervous system and sense organs, respiratory and digestive diseases were registered in both sexes.

In 2003–2007, however, they significantly exceeded the initial rates (1988–1992). In 2003–2007, among women as compared to men, higher death rates for endocrine diseases, especially diabetes were found. Mortality proved to be age-dependent (Table 11.31).

A significant dependence of mortality on age (Table 11.31).

Throughout all periods, much lower rates were observed in people under age 40 as compared to older persons. Yet, their gradual increase was recorded in both cohorts; the highest rate being registered in 2003–2007.

The greatest difference in mortality rates was found for circulatory diseases. In persons 40 years and older, mortality from this class of diseases, as well as hypertension and ischemic heart disease was significantly higher in 2003–2007 against all previous periods. Cerebrovascular mortality remains almost unchanged through all periods of study (Fig. 11.38).

In younger persons, a significant increase in hypertension mortality rates has been observed. The death rates from ischemic heart disease and cerebrovascular pathology were similar through all periods of study. In both age cohorts, a significant increase in death rates for diseases of nervous system and sense organs (Fig. 11.39), respiratory (Fig. 11.40) and digestive diseases (Fig. 11.41) was found at higher rates in persons of 40 and older.

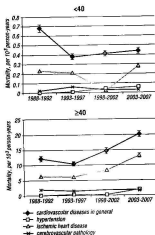


Fig. 11.38. Dynamics of mortality from circulatory diseases in adult evacuees depending on age for five-year observation periods

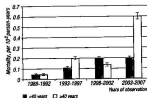


Fig. 11.39. Dynamics of mortality from diseases of nervous system and sense organs in adult evacuees depending on age for five-year observation periods

Table 11.31. The dynamics of non-tumor mortality by some classes and nosological forms of diseases in adult evacuees' cohort for five-year observation periods depending on age, per 10³ person-years

Classes and nosological forms of diseases	ICD-9	Observation periods			
		1988-1992	1993-1997	1998-2002	2003-2007
<40 years					
All diseases:	240-829; 680-739	1,3±0,14	1,6±0,14	1,5±0,12	1,9±0,14
Endocrine diseases:	240-279	0,02±0,02	0,08±0,03	0,04±0,02	0,02±0,01
- diabetes	250	0,02±0,02	0,03±0,02	0,04±0,02	0
Mental disorders	290-319	0	0,03±0,02	0,06±0,02	0
Diseases of nervous system and sense organs:	320-389	0,05±0,03	0,11±0,04*	0,18±0,04*	0,21±0,04***+
- vegeto-vascular dystonia	337	0	0,10±0,03*	0,06±0,02	0,12±0,03**+
Circulatory diseases:	390-459	0,68±0,1	0,38±0,07	0,41±0,06	0,44±0,07
- hypertension	401-405	0	0,01±0,01	0,04±0,02	0,06±0,02***
- ischemic heart disease	412-414	0,23±0,06	0,21±0,05	0,23±0,05	0,28±0,05
- cerebrovascular diseases	437-438	0,02±0,02	0,06±0,03	0,03±0,02	0,03±0,02
Respiratory diseases:	460-519	0,02±0,02	0,05±0,03	0,06±0,02	0,58±0,08***+
- chronic bronchitis, emphysema	491-492	0,02±0,02	0,01±0,01	0	0,07±0,03***+
- bronchial asthma	493	0	0,01±0,01	0	0,02±0,01*
Digestive diseases:	520-579	0,05±0,03	0,19±0,06	0,1±0,03	0,22±0,05*
- diseases of the liver, biliary tract and pancreas	571-577	0,03±0,02	0,1±0,03	0,03±0,02	0,17±0,04**+
Genitourinary diseases	580-629	0,05±0,03	0,01±0,01	0,04±0,02	0,01±0,01
Urinary diseases	581-599	0,03±0,02	0	0,03±0,02	0,01±0,01
≥40 years					
All diseases:	240-829; 680-739	18,2±0,47	14,9±0,4	20,0±0,4	24,2±0,42**
Endocrine diseases:	240-279	0,06±0,03	0,2±0,05	0,1±0,03	0,15±0,03*
- diabetes	250	0,05±0,02	0,1±0,03	0,06±0,02	0,14±0,03*
Mental disorders	290-319	0,03±0,02	0	0,04±0,02	0,04±0,02
Diseases of nervous system and sense organs:	320-389	0,05±0,02	0,2±0,05	0,14±0,04	0,6±0,07***+
- vegeto-vascular dystonia	337	0,03±0,02	0,1±0,03	0,02±0,01	0,2±0,04*
Circulatory diseases:	390-459	12,2±0,39	10,5±0,33	14,6±0,35	20,1±0,38***+
- hypertension	401-405	0,2±0,05	0,2±0,05	0,3±0,05	2,0±0,12***+
- ischemic heart disease	412-414	6,3±0,28	6,1±0,26	8,2±0,27	13,1±0,31***+
- cerebrovascular diseases	437-438	1,9±0,15	1,5±0,13	1,7±0,12	1,7±0,11
Respiratory diseases:	460-519	0,4±0,07	0,4±0,07	0,4±0,06	0,8±0,08***+
- chronic bronchitis, emphysema	491-492	0,2±0,003	0,2±0,05	0,2±0,04	0,3±0,05
- bronchial asthma	493	0,03±0,0004	0,03±0,02	0,02±0,01	0,05±0,02
Digestive diseases:	520-579	0,3±0,06	0,2±0,05	0,5±0,07	0,9±0,08***+
- diseases of the liver, biliary tract and pancreas	571-577	0,01±0,01	0,03±0,02	0,03±0,02	0,1±0,03***+
Genitourinary diseases	580-629	0,14±0,04	0,17±0,04	0,3±0,05	0,6±0,07***+
Urinary diseases	581-599	0,04±0,02	0,08±0,03	0,08±0,03	0,1±0,03

* — significant rise in rates relative to those in 1988-1992; ** — as related to those in 1993-1997, + — relative to those in 1998-2002

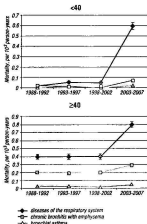


Fig. 11.40. Dynamics of mortality from respiratory diseases in adult evacuees depending on age for five-year observation periods

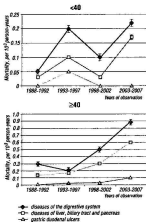


Fig. 11.41. Dynamics of mortality from digestive diseases in adult evacuees depending on age for five-year observation periods

In addition, a significant increase in mortality from stomach and duodenum ulcer was noted in older persons while that from bronchial asthma – among younger people.

In 1993–1997 and 2003–2007, thyroid mortality was observed in both age cohorts: with higher rates in older persons (0.11 ± 0.03 vs 0.04 ± 0.02) — 1993–1997 and among those under 40 (0.02 ± 0.01 vs 0.007 ± 0.005) — 2003–2007. Mortality was due to thyrotoxicosis with or without goiter.

Risks for non-tumor morbidity and mortality under the effect of low-dose ionizing radiation

Numerous post-Chernobyl studies in cohorts of clean-up workers, evacuees and residents of contaminated areas have revealed dose-related effects in the development of some non-tumor disease forms and non-tumor mortality in low-dose range [7, 8]. Today the radiation effects on the development of non-tumor diseases and mortality at low-dose radiation was found. It was done on the results of health monitoring of Hiroshima and Nagasaki A-bomb survivors [9, 10], radiologic investigations [11], under occupational exposure [12].

Taking into account the urgency of the problem and the lack of consensus so far in the assessment of the effects of low-dose ionizing radiation, a special research is very

important. Identification of radiation risks in a wide dose range may provide the most informative evidence of dose-dependent effects of non-tumor morbidity and mortality.

Ionizing radiation effects were investigated under the influence of whole body external and thyroid doses. The effect of low doses from whole-body external radiation was studied in dose subcohorts 0.05–0.099 Gy; 0.1–0.199 Gy; 0.2–0.249 Gy; 0.25–0.32 Gy (group of exposed persons) compared with dose subcohort <0.05 Gy (group of unexposed persons — control). The range of whole body external doses was 0.01–0.32 Gy. The average whole-body external doses in those subcohorts were 0.0236 Gy; 0.073 Gy; 0.157 Gy; 0.222 Gy; 0.301 Gy. Absolute (AR), relative (RR) risks [13] and their excess [14] were calculated in exposed groups. Risks were calculated with cumulative rates for 2003–2007 using a contingency table [15], confidence interval being calculated by the following formula:

$$CI = e^{-\ln(x) \pm 1.96 \cdot \sqrt{\text{Var}[\ln(x)]}},$$

where e — base of natural logarithm (approximately = 2.718); $\ln$ — natural logarithm; $\text{Var}[\ln(x)]$ — variance of $\ln(x)$; x — absolute or relative risks of morbidity (mortality), excess absolute (EAR per 10^3 person/years, Gy) or relative risk (ERR Gy $^{-1}$).

Distribution of the number of adult evacuees by whole body external doses is presented in Table 11.32.

Table 11.32. Distribution of adult evacuees depending on whole body external doses, 10^3 persons

Total number of examined persons	Doses, Gy				
	<0.05	0.05–0.099	0.1–0.199	0.2–0.249	0.25–0.32
25 708	2 421	1 993	7 867	8 670	4 757

Risk of non-tumor diseases depending on thyroid exposure was investigated in dose subcohorts 0.3–1.99 Gy and ≥ 2.0 Gy. Dose subcohort <0.3 Gy was used as control. Thyroid doses ranged from 0.001 to ≥ 2.0 Gy. Distribution of adult evacuees depending on thyroid doses is presented in Table 11.33.

Table 11.33. Distribution of adult evacuees depending on thyroid dose, 10^3 persons

Total number of examined persons	Doses, Gy		
	<0.3	0.3–1.99	≥ 2.0
1 593	436	894	238

Risks of non-tumor diseases depending on thyroid exposure

Study of non-tumor morbidity risk under thyroid exposure is of particular importance. Within the first days after the accident, thyroid exposure to radioactive iodine was a major factor for internal exposure. The accumulation of radioactive iodine in the thyroid was considered to be a factor of increased morbidity in exposed contingent. As is now established, post-accident period was characteristic of nonstochastic thyroid effects in victims and children born to them [16, 17]. Acquired hypothyroidism, autoimmune thyroiditis, nontoxic nodular goiter are the most prevalent nonstochastic disorders [18, 19]. Along with thyroid pathology due to functional and structural changes in thyroid caused by irradiation, immune and hormonal disorders in the body as a whole there is an increased risk for many other non-tumor diseases [20].

The results show that with thyroid exposure to doses $0.3 - <2.0$ Gy some diseases (circulatory diseases, particularly ischemic heart disease and cerebrovascular pathology, musculoskeletal diseases) are significantly dose-dependent. Increase in thyroid doses (2.0 Gy and more) results in higher risks for the above diseases. Significant risks for mental disorders and digestive diseases, including diseases of the liver, biliary tract and pancreas were also recorded (Table 11.34).

Table 11.34. Relative risks for some non-tumor diseases among adult evacuees depending on thyroid dose, 1989-2005

Classes and ecological forms of diseases	ICD-9	Dose, Gy	
		0.3 — <2.0	≥ 2.0
		RR (CI)	RR (CI)
Circulatory diseases:	390-459	1.89* (1.64-2.18)	2.91* (2.51-3.37)
– ischemic heart disease	410-414	3.22* (2.50-4.15)	4.38* (3.37-5.68)
– cerebrovascular diseases	430-438	2.00* (1.41-2.85)	4.83* (3.46-6.73)
Digestive diseases:	520-579	1.02 (0.86-1.22)	1.40* (1.15-1.71)
– diseases of the liver, biliary tract and pancreas	571-577	1.06 (0.82-1.35)	1.34* (1.01-1.79)
Musculoskeletal diseases	710-739	1.22* (1.01-1.49)	2.69* (2.21-3.26)
Mental disorders	290-319	1.10 (0.65-1.88)	1.82* (1.02-3.26)

* $p < 0.05$

Ambiguous risks of non-tumor morbidity in case of thyroid exposure were sex- and age- dependent. Statistically significant RR rates of exposed men and women ($0.3 - <2.0$ Gy), as compared to that of dose subgroup <0.3 Gy were registered for the whole class of circulatory diseases, in particular ischemic heart disease and cerebrovascular pathology, digestive diseases as a whole, musculoskeletal diseases. However, the highest risk of circulatory diseases in general and ischemic heart disease in particular was found in women. In contrast, a higher relative risk of cerebrovascular disease was

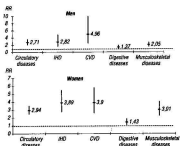


Fig. 11.42. Statistically significant relative risks for some non-tumor diseases among adult evacuees over the period 1989-2005 depending on sex and thyroid doses $0.3 - <2.0$ Gy. Comparison group – subcohort with radiation doses <0.3 Gy

recorded in men. The risk of digestive diseases was almost the same for men and women, while that for musculoskeletal diseases was higher in women (Fig. 11.42).

Another picture was observed at doses ≥ 2.0 Gy. Risk of developing circulatory diseases was higher in men. This is true for the entire class of cardiovascular pathology as well as separately for ischemic heart disease and cerebrovascular pathology (Fig. 11.43).

RR varied depending on thyroid doses and age of examined persons. It was found that with increasing exposure to ≥ 2.0 Gy significant relative risks grew, number of cases and age groups increased with true risk of certain non-tumor diseases (Table 11.35).

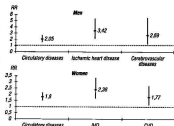


Fig. 11.43. Statistically significant relative risks for circulatory diseases among adult evacuees within 1989–2005 depending on sex and thyroid doses ≥ 2.0 Gy. Comparison group – subcohort with radiation doses <0.3 Gy

Table 11.35. Relative risks of some non-tumor diseases among adult evacuees depending on age (at the time of medical examination) and thyroid doses as compared with dose subgroup <0.3 Gy. Observation period 1989–2005

Classes and nosological forms of diseases (ICD-9)	Dose, Gy	Age, years				
		18–29	30–39	40–49	50–59	60 and >
		RR (CI)	RR (CI)	RR (CI)	RR (CI)	RR (CI)
Endocrine diseases (240–279):	0.3 – <2.0	1.11 (0.55–2.20)	1.54 (0.90–2.64)	0.58 (0.33–0.94)	0.79 (0.47–1.35)	3.07* (1.02–9.23)
	≥ 2.0	—	—	—	—	—
– diabetes (250)	0.3 – <2.0	—	—	—	0.75 (0.19–2.99)	3.21 (0.48–21.64)
	≥ 2.0	—	—	5.83* (3.51–8.33)	—	—
Mental disorders (290–319)	0.3 – <2.0	1.05 (0.33–3.33)	0.67 (0.28–1.60)	1.93 (0.56–6.65)	3.02 (0.42–21.65)	2.35 (0.32–17.67)
	≥ 2.0	—	—	4.8* (1.51–15.26)	8.93* (1.66–48.09)	17.67
Circulatory diseases (390–459):	0.3 – <2.0	0.60 (0.23–1.55)	1.45 (0.68–2.39)	0.76 (0.35–1.66)	1.09 (0.81–1.47)	2.15* (1.74–2.67)
	≥ 2.0	—	—	—	—	2.75* (2.22–3.41)
– ischemic heart disease (412–414)	0.3 – <2.0	—	3.83 (0.55–26.88)	0.97 (0.44–2.13)	1.53 (0.91–2.59)	2.72* (1.95–3.81)
	≥ 2.0	—	—	—	1.9* (1.08–3.34)	2.74* (1.93–3.90)
– cerebrovascular diseases (437–438)	0.3 – <2.0	0.42 (0.03–6.16)	2.73 (0.35–21.36)	1.53 (0.62–3.77)	2.55* (1.27–5.13)	2.50* (1.23–2.41)
	≥ 2.0	—	—	—	2.34* (1.56–3.23)	3.28* (2.10–5.11)

Classes and nosological forms of diseases (ICD-9)	Dose, Gy	Age, years				
		18-29	30-39	40-49	50-59	60 and >
		RR (CI)	RR (CI)	RR (CI)	RR (CI)	RR (CI)
Respiratory diseases (480-519)	0.3 - <2.0	1.33*	0.77	1.08	0.57	1.08
		(1.05-1.68)	(0.61-0.98)	(0.84-1.40)	(0.42-0.77)	(0.77-1.52)
	≥2.0	—	—	—	—	—
Digestive diseases (520-579)	0.3 - <2.0	2.29*	0.83	0.72	0.92	1.40
		(1.33-3.66)	(0.56-1.22)	(0.52-1.00)	(0.51-1.40)	(0.91-2.13)
	≥2.0	2.75*	—	—	—	2.02*
		(1.19-4.18)	—	—	—	(1.31-3.08)
- diseases of the liver, biliary tract and pancreas (571-577)	0.3 - <2.0	2.94*	0.75	0.74	0.89	1.65
		(1.31-6.57)	(0.42-1.34)	(0.47-1.18)	(0.51-1.55)	(0.87-3.13)
	≥2.0	3.01*	—	—	—	2.69*
		(1.12-8.23)	—	—	—	(1.08-4.05)
Musculoskeletal diseases (710-739)	0.3 - <2.0	0.92	1.45	0.65	1.01	1.35
		(0.32-2.65)	(0.84-2.56)	(0.43-0.97)	(0.57-1.53)	(0.96-1.90)
	≥2.0	—	—	—	2.26*	2.2*
		—	—	—	(1.52-3.37)	(1.58-3.07)

* $p < 0.05$

Analysis showed that at doses 0.3 - <2.0 Gy (compared with dose subcohort <0.3 Gy) significant relative risks were found only within age groups 18-29, 50-59, 60 and older. In those aged 18-29 significant risks were registered for respiratory, digestive diseases, including diseases of the liver, biliary tract and pancreas.

In persons aged 50-59, significant risk for cerebrovascular diseases was observed; for endocrine and circulatory diseases, particularly ischemic heart disease and cerebrovascular pathology — in those aged 60.

Thyroid exposure to doses ≥2.0 Gy among persons of 18-29 years results in higher risks for digestive diseases, including diseases of the liver, biliary tract and pancreas.

In 40-49-year old, significant risks of diabetes and mental disorders were observed; in those aged 50-59, unlike the previous interval risk for mental disorders became more evident, risks for ischemic heart disease and cerebrovascular pathology increased. In persons 60 years and older, statistically significant risks of some diseases (endocrine, digestive, including diseases of the liver, biliary tract and pancreas, musculoskeletal) were found. Risk estimates of cardiovascular diseases, particularly ischemic heart disease and cerebrovascular pathology were increasing.

Risks of non-tumor diseases depending on whole body external exposure.

Determination of relative, absolute and excess risks

A study on relative risks for non-tumor diseases in adult evacuees at various doses of whole body external irradiation has been conducted. Gradual increase in RR and number of cases was observed; increased level of risk with dose being proved. The maximum RR for most diseases was found in dose range 0.25-0.32 Gy (Table 11.36).

The smallest (in comparison with control) number of diseases with a statistically significant risk was registered in dose subcohort 0.05-0.099 Gy (class of diseases of nervous system and sense organs, particularly vegetovascular dystonia, circulatory and digestive diseases, including gastritis and duodenitis).

Table 11.35. Relative risks of non-tumor diseases by some classes and nosological forms among adult evacuees within 2003–2007 depending on whole body external dose (comparison dose subcohort <0.05 Gy)

Classes and nosological forms of diseases (ICD-9)	Radiation dose, Gy							
	0.05–0.099		0.1–0.199		0.2–0.249		0.25–0.32	
	RR	CI	RR	CI	RR	CI	RR	CI
Endocrine diseases (246–279):	1.01	0.78;1.32	1.20	0.83;1.25	0.89	0.71;1.08	1.45*	1.18;1.79
Thyroid diseases (240–246):	1.07	0.78;1.48	1.10	0.86;1.41	0.92	0.71;1.18	1.37*	1.06;1.77
– thyroiditis (245)	0.46	0.16;1.29	0.81	0.43;1.53	0.92	0.50;1.72	1.49*	1.09;2.79
Diseases of nervous system and sense organs (320–389):	1.34*	1.09;1.66	2.04*	1.73;2.40	1.25*	1.07;1.87	1.23*	1.03;1.47
– vegeto-vascular dystonia (337)	2.04*	1.12;3.71	4.44*	2.72;7.25	1.45	0.86;2.43	1.17	0.66;2.06
– cataracts (366)	1.73	0.74;4.04	2.74*	1.36;5.45	1.58	0.78;3.21	2.94*	1.45;5.96
Circulatory diseases (390–459):	1.32*	1.11;1.56	1.53*	1.34;1.75	1.32*	1.15;1.51	1.83*	1.42;1.87
– hypertension (401–405)	0.89	0.64;1.24	1.35*	1.07;1.72	1.01	0.79;1.29	1.40*	1.23;1.55
– ischemic heart disease (412–414)	1.30	0.97;1.74	1.24	0.96;1.57	1.19	0.94;1.51	1.27*	1.01;1.63
– cerebrovascular diseases (437–438)	1.45	0.97;2.16	1.75*	1.27;2.42	1.56*	1.13;2.16	1.94*	1.39;2.71
Respiratory diseases (460–519):	1.15	0.90;1.46	0.92	0.76;1.12	1.07	0.89;1.29	0.97	0.79;1.19
– chronic bronchitis, emphysema (491–492)	0.86	0.56;1.32	0.92	0.67;1.26	0.92	0.67;1.26	0.78	0.55;1.12
Digestive diseases (520–579):	1.21	1.06;1.38	0.94	0.84;1.05	1.04	0.93;1.16	1.25*	1.12;1.40
– gastritis, duodenitis (535)	1.52*	1.01;2.28	1.15	0.82;1.62	1.52*	1.09;2.12	1.66*	1.17;2.35
– diseases of the liver and biliary tract (571–576)	1.12	0.91;1.39	0.92	0.77;1.09	1.01	0.85;1.19	1.27*	1.06;1.51
– diseases of pancreas (577)	0.68	0.62;1.27	0.84	0.64;1.11	0.83	0.63;1.08	1.24*	1.06;1.50
Genitourinary diseases (580–629):	0.97	0.71;1.33	0.92	0.72;1.17	1.10	0.87;1.39	1.35*	1.06;1.73
– urinary diseases (581–599)	1.37	0.87;2.17	1.44*	1.00;2.08	1.69*	1.18;2.43	1.90*	1.30;2.77
– prostate diseases (600–608)	1.30	0.73;2.32	1.12	0.70;1.79	1.52	0.97;2.40	2.03*	1.28;3.24
Musculoskeletal diseases (710–739):	1.11	0.90;1.37	1.05	0.89;1.24	0.92	0.78;1.09	1.27*	1.08;1.39
– osteopathy, chondropathy (730–739)	1.10	0.82;1.76	1.17	0.93;1.47	1.09	0.86;1.37	1.39*	1.09;1.77

*p<0.05

With increasing doses to 0.1–0.249 Gy number of diseases with significant RR increased due to the following nosological forms: cataract, hypertension, cerebrovascular pathology, urinary diseases. The greatest number of non-tumor diseases with true dose-effect relationship was noted in dose subcohort 0.25–0.32 Gy.

Statistically significant RRs were estimated for endocrine diseases, diseases of nervous system and sense organs, circulatory, digestive, genitourinary and musculoskeletal diseases.

Among them significant risks were established for thyroid diseases, particularly thyroiditis, cataracts, hypertension, ischemic heart disease, cerebrovascular pathology, gastritis, duodenitis, diseases of the liver, biliary tract and pancreas, genitourinary

diseases, including urinary and prostate diseases, musculoskeletal diseases, including osteopathy and chondropathy. In most cases, with further gain in dose effect RR value increased or insignificant risk became significant.

Thus, RR of cataract increased from 1.73 to 2.94, of circulatory diseases — from 1.32 to 1.83, cerebrovascular pathology — from 1.45 to 1.94, digestive diseases — from 1.21 to 1.25, gastritis and duodenitis — from 1.52 to 1.66. Insignificant at lower doses risks for some diseases (endocrine diseases, particularly thyroid diseases and thyroiditis, diseases of the liver, biliary tract and pancreas, genitourinary, including prostate diseases, musculoskeletal diseases, particularly osteopathy and chondropathy) become significant.

Absolute risk calculations are also indicative of the fact that risk of the most non-tumor diseases is dose-dependent (rise in dose levels causes an increase in risk values) (Table 11.37).

Table 11.37. Absolute risks (AR) of non-tumor diseases by some classes and nosological forms among adult evacuees within 2003–2007 depending on whole body external dose, per 10³ person/years

Nosological forms of diseases (ICD-9)	Radiation dose, Gy				
	<0.05	0.05–0.099	0.1–0.199	0.2–0.249	0.25–0.32
acquired hypothyroidism (244)	1.2 (0.3; 3.8)	1.4 (0.5; 4.5)	1.4 (0.8; 2.5)	1.4 (1.0; 2.9)	2.1 (1.1; 3.9)
thyroiditis (245)	1.8 (0.4; 6.0)	1.0 (0.2; 3.9)	1.9 (1.1; 3.1)	2.8 (1.3; 3.3)	5.0 (3.3; 7.4)
diabetes (250)	2.0 (1.4; 8.1)	2.4 (1.0; 5.8)	1.9 (1.1; 3.1)	1.1* (1.0; 3.3)	6.0 (4.2; 8.7)
vegeto-vascular dystonia (337)	2.4 (1.1; 5.5)	9.6 (6.2; 14.9)	16.7 (14.1; 19.9)	5.8 (3.2; 6.0)	4.6 (3.0; 6.8)
cataracts (366)	1.2 (0.3; 1.4)	3.8 (1.9; 7.7)	4.4 (3.2; 6.1)	3.6 (1.6; 3.9)	7.5 (5.4; 10.4)
hypertension (401–405)	12.2 (8.5; 17.6)	17.8 (12.9; 24.6)	19.0 (18.2; 22.3)	17.2 (14.3; 19.8)	21.6 (17.8; 26.2)
ischemic heart disease (412–414)	16.3 (11.9; 22.3)	23.5 (17.7; 31.3)	20.6 (17.7; 24.1)	22.0 (16.3; 22.2)	33.3 (20.4; 37.0)
cerebrovascular diseases (437–438)	9.8 (6.8; 14.6)	13.9 (9.7; 20.1)	17.2 (14.6; 20.4)	15.5 (10.6; 17.7)	18.5 (15.0; 22.6)
gastritis, duodenitis (535)	8.9 (5.9; 13.7)	12.0 (8.1; 17.8)	9.4 (7.5; 11.9)	13.1 (10.3; 15.1)	14.3 (11.3; 18.2)
diseases of the liver and biliary tract (571–576)	32.6 (26.1; 40.8)	39.9 (32.0; 49.7)	32.2 (28.5; 36.5)	31.7 (28.5; 37.8)	45.2 (39.5; 51.8)
diseases of pancreas (577)	10.6 (7.2; 15.6)	12.0 (8.1; 17.8)	11.2 (9.1; 13.8)	10.8 (8.4; 13.2)	16.2 (12.9; 20.2)
urinary diseases (581–599)	10.2 (6.9; 15.1)	7.2 (4.3; 12.0)	9.4 (7.5; 11.9)	12.1 (8.5; 14.4)	15.4 (12.2; 19.3)
prostate diseases (600–608)	4.3 (2.8; 8.6)	6.7 (4.0; 11.4)	5.5 (4.1; 7.5)	7.2 (5.1; 8.5)	9.9 (7.4; 13.2)
osteopathy, chondropathy (730–739)	15.9 (11.6; 21.8)	22.1 (16.5; 29.6)	20.9 (17.8; 24.4)	18.9 (16.0; 21.2)	27.2 (22.9; 32.3)

As seen from the data obtained, the significant values of absolute risks for non-tumor morbidity by most classes and nosological forms were recorded at doses 0.25–0.32 Gy.

An increase in risk by a factor of 1.5 to 2.0 and more in exposed cohorts versus unexposed one was noted. At the same time, the absolute risk of thyroiditis increased 2.8 times, diabetes — three times, ischemic heart disease and prostate diseases — over two times. Risk of developing cataract was the highest (6.25 times).

It should be mentioned that significant risks for the development of some diseases were observed within the particular dose intervals: acquired hypothyroidism (0.25–0.32 Gy), thyroiditis (0.1–0.199 Gy), cataracts (0.05–0.099 Gy).

As for most diseases, the maximum reliably confirmed risk was observed within the dose range 0.25–0.32 Gy, excess absolute and relative risks were calculated at a specified dose level (Table 11.39). As seen from the data, the highest reliably confirmed excess absolute risks were found for ischemic heart disease, hypertension, diseases of the liver and biliary tract, musculoskeletal diseases.

Table 11.39. Excess absolute and relative risks of non-tumor diseases by some classes and nosological forms among adult evacuees within 2003–2007 at whole body external doses 0.25–0.32 Gy

Classes and nosological forms of diseases	ICD-9	Excess absolute risk, EAR per 10 ⁴ person-years, Gy	Excess relative risk, ERR Gy ⁻¹
Endocrine diseases, including:	240–279	28.0 (21.2; 37.1)	1.67 (1.35; 2.07)
– diabetes	250	5.1 (2.6; 10.3)	3.41 (2.03; 5.74)
– thyroid diseases:	240–246	19.1 (13.4; 27.1)	1.38 (1.06; 1.78)
– thyroiditis	245	7.1 (3.1; 16.6)	3.25 (1.76; 6.01)
Vegeto-vascular dystonia	337	7.1 (2.9; 17.5)	0.62 (0.35; 1.39)
Cataracts	366	21.0 (8.4; 68.1)	7.18 (3.54; 14.6)
Hypertension	401–405	31.3 (20.8; 47.1)	1.23 (0.96; 1.60)
Ischemic heart disease	412–414	59.5 (33.2; 94.6)	1.19 (0.92; 1.53)
Cerebrovascular diseases	437–438	29.1 (18.5; 45.8)	3.48 (2.48; 4.88)
Gastritis, duodenitis	535	17.9 (11.1; 29.0)	2.45 (1.73; 3.49)
Diseases of the liver and biliary tract	571–576	42.2 (32.5; 54.8)	1.0 (0.83; 1.20)
Diseases of pancreas	577	3.7 (2.4; 5.4)	1.24 (0.94; 1.64)
Genitourinary diseases:	580–629	21.9 (15.6; 30.9)	1.31 (1.02; 1.69)
– urinary diseases	581–599	17.4 (11.0; 27.3)	3.33 (2.28; 4.97)
– prostate diseases	600–608	2.4 (1.2; 4.7)	3.83 (2.40; 6.12)
Musculoskeletal diseases:	710–739	42.2 (17.3; 54.7)	0.75 (0.63; 0.90)
– osteopathy, chondropathy	730–739	37.8 (33.9; 54.2)	1.44 (1.13; 1.84)

There is also an excess absolute risks for endocrine diseases (including thyroid diseases), genitourinary diseases (including urinary), osteopathy and chondropathy.

Calculation results of excess relative risk per unit dose suggest that some non-tumor diseases in adult evacuees were dose-dependent. The highest was excess relative risk of cataracts. Excess relative risks for diabetes, thyroiditis, cerebrovascular, urinary and prostate diseases were also rather high.

Risk of death from non-tumor diseases depending on whole body external exposure. Estimation of absolute, relative and excess risks of mortality

The study analyzed relative mortality risks among evacuees for all non-tumor diseases, recorded as causes of death (circulatory diseases, including hypertension, ischemic heart disease, cerebrovascular pathology, respiratory, digestive diseases, including those of the liver, biliary tract and pancreas, genitourinary diseases, including urinary diseases, mental disorders, endocrine diseases, particularly thyroid diseases). The above dose subcohorts were compared with control (subcohort <0.05 Gy). The results showed highly significant relative risks of mortality due to all non-tumor diseases combined in all subcohorts with doses over 0.05 Gy (Fig. 11.44).

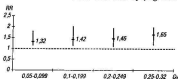


Fig. 11.44. Relative risks of adult evacuees' mortality from all non-tumor diseases depending on whole-body external doses

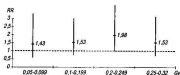


Fig. 11.45. Relative risks of adult evacuees' mortality from ischemic heart disease with regard to whole body external doses

In determining risks by causes of death, statistically significant RR of ischemic heart disease was recorded in dose subcohort 0.2-0.249 Gy (Fig. 11.45).

Analysis of causes of death on estimates of absolute risks, excess absolute and relative risks showed the highest rates of death from circulatory diseases, particularly ischemic heart disease, digestive and genitourinary diseases (prostate diseases in particular).

Thus, significant absolute risks of mortality at all dose intervals were established only for circulatory diseases, including ischemic heart disease and digestive diseases (Table 11.39).

Table 11.39. Absolute risks of mortality for some classes and nosological forms among adult evacuees, 2003-2007, depending on whole body external doses, per 10^3 person/years

Diseases (ICD-9)	Doses, Gy				
	<0.05	0.05-0.099	0.1-0.199	0.2-0.249	0.25-0.32
Circulatory diseases (390-459):	7.4 (4.6; 11.8)	9.5 (6.1; 15.0)	10.9 (8.6; 13.5)	11.1 (9.1; 13.5)	9.3 (6.9; 12.5)
- ischemic heart disease (412-414)	4.1 (2.2; 7.7)	6.0 (3.4; 10.6)	5.8 (4.4; 7.8)	7.6 (6.0; 9.7)	5.9 (4.1; 8.5)
- cerebrovascular diseases (437-438)	1.6 (0.6; 4.4)	1.5 (0.5; 4.7)	2.2 (1.3; 3.5)	1.3 (0.7; 3.5)	1.7 (0.8; 3.4)
Respiratory diseases (460-519)	1.0 (0.3; 3.8)	1.2 (0.4; 4.0)	1.7 (1.0; 2.9)	1.5 (0.9; 2.6)	1.1 (0.4; 2.5)
Digestive diseases (520-579)	1.6 (0.6; 4.4)	3.5 (1.7; 7.4)	3.1 (2.0; 4.6)	3.0 (2.0; 4.4)	2.5 (1.4; 4.5)

In addition, at doses 0.1–0.199 Gy, a significant absolute risk of death was recorded for cerebrovascular pathology and respiratory diseases. Maximum values of absolute risks of death from circulatory diseases, particularly ischemic heart disease were registered in dose range 0.2–0.249 Gy. Obtaining reliable absolute risks of death from particular non-tumor diseases within different dose intervals suggests a possible relation to dose. But to determine the final values further study is needed provided a sufficiently large number of examined persons with updated individual doses.

In determining excess absolute risks per unit dose (EAR per 10^3 person-years, Gy), a statistically significant excess was recorded for cardiovascular diseases, particularly ischemic heart disease and digestive diseases (Table 11.40).

Table 11.40. Excess absolute risks of mortality by some classes and nosological forms among adult evacuees, 2003–2007, whole body external doses 0.25–0.32 Gy

Disease	ICD-9	Excess absolute risk, EAR per 10^3 person-years, Gy
Circulatory diseases:	390–459	6.0 (3.5; 10.5)
– Ischemic heart disease	412–414	5.8 (2.8; 12.0)
Digestive diseases	520–579	2.9 (1.9; 9.0)

In dose range 0.25–0.32 Gy, the highest was excess absolute risk of circulatory mortality, with nearly the same EAR death rate (except ischemic heart disease). Excess absolute risk of digestive mortality was half as much.

We have calculated excess relative risk per unit dose (ERR Gy⁻¹). As a result, significant excess death rates for endocrine diseases, ischemic heart disease, gastritis and duodenitis, urinary and prostate diseases were established (Table 11.41).

Table 11.41. Excess relative risks of mortality for some classes and nosological forms among adult evacuees, 2003–2007, whole body external doses 0.25–0.32 Gy

Disease	ICD-9	Excess relative risk, ERR Gy ⁻¹
Endocrine diseases	240–279	1.50 (1.21; 1.85)
Ischemic heart disease	412–414	3.12 (2.23; 4.38)
Gastritis, duodenitis	535	2.19 (1.54; 3.12)
Urinary diseases	581–589	2.99 (2.04; 4.37)
Prostate diseases	600–608	3.42 (2.14; 5.47)

The highest excess relative risk per unit dose was established for ischemic heart disease, prostate and urinary diseases. Excess of deaths from endocrine diseases was almost half as much.

Conclusion

In summary, due to epidemiological research of the dynamics in non-tumor morbidity and mortality, postevacuation radiation effects among adult evacuees, the following results were found:

- ambiguous non-tumor disease rates in different observation periods. Through all periods, a significantly higher disease rates against initial ones (1988–1992) were

observed, yet the highest rates were recorded in 1998–2002. In 2003–2007, a slight decline was noted, nevertheless, the rates were statistically higher than those of the first period. Very high rates of certain non-tumor diseases (endocrine, thyroid and circulatory diseases, stomach and duodenum ulcer, etc.) in the early observation period, similar or higher rates in the second and final periods of the study may be associated with stress experienced during evacuation;

- the higher incidence rate in women as compared to men and in people over 40 in comparison with the younger ones;
- gradual growth of death rate with its peak in 2003–2007. Among causes of death circulatory diseases, primarily ischemic heart disease prevailed. Mortality in men exceeds appropriate rates for women; people over 40 contributed mostly to its formation;
- significant risks for the development of non-tumor diseases under thyroid exposure to doses 0.3 – <2.0 Gy and ≥ 2.0 Gy as compared with the control (<0.3 Gy). The increase of thyroid doses to 2.0 Gy and over caused risks higher rates, augmented diseases incidence and made bigger the number of age groups with significant risks of certain non-tumor diseases. Age and sex dependence of risk was revealed;
- increasing risk and non-tumor incidence with rise in whole body external doses. The maximum risk for most diseases was found in dose range 0.25–0.32 Gy;
- determination of the highest excess absolute risk for: ischemic heart disease (EAR = 59.5; CI = 33.2; 94.6), hypertension (EAR = 31.3; CI = 20.8; 47.1), diseases of the liver and biliary tract (EAR = 42.2; CI = 32.5; 54.8), musculoskeletal diseases (EAR = 42.2; CI = 17.3; 54.7); excess relative risk for: cataracts (ERR^{-1} = 7.18, CI = 3.54; 14.6), diabetes (ERR^{-1} = 3.41; CI = 2.03; 5.74), thyroiditis (ERR^{-1} = 3.25, CI = 1.76; 6.01), cerebrovascular diseases (ERR^{-1} = 3.48, CI = 2.48; 4.88), urinary (ERR^{-1} = 3.33, CI = 2.28; 4.87) and prostate diseases (ERR^{-1} = 3.83, CI = 2.40; 6.12);
- possible correlation between risk of non-tumor mortality and whole body external dose. Close relationship between death formation on the estimates of absolute risk, excess absolute and relative risks for circulatory diseases (ischemic heart disease and cerebrovascular diseases), digestive diseases (gastritis and duodenitis), genitourinary diseases (urinary and prostate diseases). Was recorded excess absolute risks (EAR per 10^3 person/years, Gy) for mortality from circulatory diseases matched the values of 6.0 (3.5, 10.5), ischemic heart disease — 5.8 (2.8, 12.0), digestive diseases — 2.9 (1.9, 9.0). Excess relative risks ERR^{-1} for mortality from ischemic heart disease were 3.12 (2.23, 4.38), urinary diseases — 2.99 (2.04, 4.37), that of prostate — 3.42 (2.14, 5.47), gastritis, duodenitis — 2.19 (1.54, 3.12);
- these data suggest a promising research direction. However, the deeper results are possible with extensive studies of specified individual doses from whole body external radiation.

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CHAPTER 12 CARDIOVASCULAR DISEASES IN CHORNOBYL ACCIDENT CLEAN-UP WORKERS 25 YEARS UPON

There are over 25 million residents in Ukraine suffering the cardiovascular diseases (CVD). The Chornobyl accident clean-up workers are attributed to population group of Ukraine where the CVD prevalence, incidence, disability rate, in-ward admittance rate and mortality are reliably higher than in both the entire population and other Chornobyl catastrophe survivors [1].

Yearly 7.5 million people die worldwide due to CVD. CVD prevail within mortality causes for all the years in Chornobyl catastrophe clean-up workers exposed to radiation doses up to 1 Sv. The CVD mortality in clean-up workers is dramatically higher than mortality from cancer and other diseases [2]. It had increased within five years upon the accident from 3.1% to 13.4%, reaching 46.2% during the third decade. The CVD proportion in the entire mortality is up to 70%. Eight from 91 Chornobyl catastrophe clean-up workers who had survived the acute radiation syndrome have died from the CVD. In most of them the sudden cardiac death occurred [1]. The mentioned above substantiate considering the CVD in Chornobyl catastrophe clean-up workers as not only a health issue but rather a socio-economic one.

Because of a decisive impact of CVD on population health, large financial expenditures due to the high costs of managing especially the in-ward, the CVD issue is focused every 2–3 years at the world, European and national levels. The approaches in prophylaxis and management of the most prevalent CVD have recently evolved dramatically. The new groups of medications have appeared requiring the clarification of their efficiency. It is especially relevant to arterial hypertension and coronary heart disease (CHD). The new proposals on modification of prophylaxis and management taking into account the high risk are put forward in the last Recommendations of World and National levels. However they are relevant to the patients' population in a whole where lifestyle features are different and therefore are not applicable directly to the Chornobyl catastrophe clean-up workers.

Excess of some CVD and cerebrovascular diseases in particular was revealed in survivors of Hiroshima and Nagasaki A-bombing [3], nuclear power plants and nuclear industry accidents [4], and after therapeutic radiation exposure (i.e. radiotherapy) [5]. However some data clarification is required here. In their compare to values and indices

from the Chernobyl catastrophe survivors the respective peculiarities of the wide-scaled accident are to be considered anyway.

Data on radiation exposure peculiarities presented from countries most affected by the Chernobyl catastrophe [1, 6, 7] indicate to the most dramatic impact of radiation and other accident factors on the Chernobyl catastrophe clean-up workers of 1986–1987 period. Therefore the mentioned group is considered as a critical by dose criterion.

Peculiarities of onset and course of CVD in the Chernobyl catastrophe clean-up workers described here are based on data from 9,423 patients exposed to up to 1 Sv of ionizing radiation (IR). The studies were launched in 1986 and go on nowadays providing real estimates of the onset and course of most prevalent CVD in Chernobyl catastrophe clean-up workers as a most critical group for dose criterion.

(I. Khomazuk)

12.1 ARTERIAL HYPERTENSION

The arterial hypertension (AH) remains one of the most often registered diseases since the Chernobyl catastrophe among all groups of survivors and especially in Chernobyl catastrophe clean-up workers [1]. AH incidence is still increasing being over the respective indices in other population groups. Damage to target organs, acute cardiovascular complications, and heart failure (HF) are the most frequent causes of death in survivors suffering the AH. Therefore focus of prophylactic and therapeutic arrangements on the adequate blood pressure (BP) control and prevention/regression of injuries to target organs appears of especial importance. Estimate and stratification of the general risk is a precondition here. The extra risk factors (RF) are identified now with link to AH genesis and progression being proved, that can be a target for AH pharmacotherapy. The oligosymptomatic/asymptomatic cases with high risk require a special concern. Even in case of a high normal BP against the background of a single target organ damage one can consider the cardiovascular risk.

Proportion of arterial hypertension within cardiovascular in-ward admissions

As of year 2009 the proportion of AH in the Chernobyl catastrophe clean-up

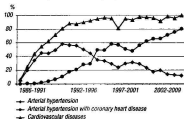


Fig. 12.1. Arterial hypertension incidence within cardiovascular diseases structures among Chernobyl accident clean-up workers in 1986–2009 ($n=9,423$)

workers admitted to hospitals because of CVD was 92.5%. In 80.4% it was associated with a CHD. There are 11.9 million AH patients registered in Ukraine making up 26% of the entire population.

According to our data analysis for the admission rate of Chernobyl catastrophe clean-up workers suffering AH there are meaningful Chernobyl catastrophes by years within 1986–2009 period (Fig. 12.1).

As shown the AH proportion had increased within first five years from 5.4% to 55.8%. It was represented mainly by the AH not associated with any other conditions. In five years its incidence had reached 48.9%. The incidence of AH associated with CHD was 6.8%. Further the AH proportion increased gradually reaching 69% in 1992. Its association with CHD had increased also reaching 10.6%. Ten years upon the accident within total amount of AH of 73.8% the proportion of AH associated with CHD had reached 29.4%. The proportions of AH associated and not associated with CHD had become equal. After that the AH associated with CHD prevailed with its proportion reaching 80.4% within an entire structure of CVD in Chernobyl catastrophe clean-up workers.

Effects of radiation exposure

External radiation doses (ERD) exceeding 5 cSv officially registered in Chernobyl catastrophe clean-up workers of 1986 were established in 89.9%, of 1987 — in 81.9%. In 1988 the number of clean-up workers with ERD >5 cSv had decreased to 13.5%, and in 1989 — to 3.5% respectively. Therefore clean-up workers of 1986–1987 period can be regarded as a most critical group.

AH incidence in Chernobyl catastrophe clean-up workers depending on ERD is shown at Fig. 12.2.

Within a dose range of 25–60 cSv the AH incidence had reached 38% reliably exceeding the respective values in doses range up to 5 cSv (20.1%), 5–9.9 cSv (22.5%) and 10–24.9 cSv (9.7%) respectively. Excess of an AH risk in ERD 0.5 Gy among the clean-up workers was found in other studies [8]. These data conform to results received from Chernobyl catastrophe clean-up workers living in Russian Federation. The comparably meaningful dose dependence with 95% confidence interval for the AH was found for them [9]. More rapid i.e. about for the 10 years onset of AH and high incidence of AH stage II, left ventricular hypertrophy (LVH) were found in clean-up workers compared to the general population within framework of a clinical study of IR impact on AH course and endothelial function [10].

Just vascular disorders lead to heart muscle injury as heart tissues are in especial conditions due to continuous heart function and intensity of myocardial biochemical metabolic processes.

Destabilizing effect of IR on cellular membranes initiates the peroxidation processes. Disorders of the balanced function of pro- and antioxidant systems lead to increase of the active oxygen species and origination of oxidative stress. High amount of mitochondria in myocardium is the source of higher intensity of a free-radical oxidation than in other tissues. Such a process leads to functional capacity exhaustion and produces conditions for myocardial damage. Summarization

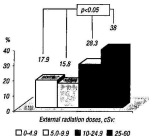


Fig. 12.2. Arterial hypertension incidences in Chernobyl accident clean-up workers depending on external radiation doses

of study results from the Chernobyl catastrophe clean-up workers in the nearest years upon the accident showed [1] the abnormal lipid peroxidation and pro/antioxidant balance exacerbating along with radiation dose increase.

Other factors related to Chernobyl accident in genesis of the arterial hypertension

Situation occurred around the Chernobyl NPP became the source both the IR and some other substances and compounds hazardous for a human. Several substances i.e. lead, boron, magnesian lime, trisodium phosphate and others were used for the decrease of radioactive releases. Burning of the plastic construction elements was also the source of hazardous impact. The environmental content of heavy metals was proven as 3–10-fold increased after the accident. Lead was considered the most hazardous for human among them.

The environmentally stipulated contact of clean-up workers with the hazardous substances was combined with their occupational and household incorporation. There was an intensive occupational exposure to the lead and some other hazardous substances in the clean-up workers of 1986 engaged in work at the most dangerous sites. The clean-up work was accompanied by severe psychoemotional strain.

Stress and blood pressure elevation

The excessive psychoemotional strain was one of the important factors influencing the AH genesis in a substantial part of Chernobyl catastrophe clean-up workers especially of 1986–1987 work period. Sympathetic nervous system activation under such circumstances induces the intensification of catecholamine production and the BP elevation respectively. Serum catecholamine content in the clean-up workers soon after the accident was dramatically elevated. Daily urine excretion of epinephrine, norepinephrine and dopamine was substantially increased. Their concentration in daily urine was directly related to the index of heart rate variability (HRV) reflecting the sympathetic and parasympathetic activity of the autonomous nervous system. Increase of epinephrine excretion over the 14 µg/day, of norepinephrine — over 40 µg/day, and of dopamine above 766 µg/day was accompanied by the elevation of power ratio within low to high frequency range (LF/HF) ≥ 3.5 units, and by the increase of a Stress Index (SI) > 100 units. The direct confidential link was revealed between epinephrine, norepinephrine and dopamine concentrations and SI.

Systolic BP in the clean-up workers who had experienced the serious psychoemotional burden was higher by 10–15% vs. cases of a mild psychoemotional load. The AH had occurred 3-fold more often during the high-stress work. The psychoemotional factor at a workspace correlated closely with target organs damage, left ventricular (LV) wall thickness, and increased CHD risk.

The epinephric effect of sympathicotonia occurs first of all through the direct impact on circulation. The myocardial contractility, stress index, and heart rate increase and as a result the increase of cardiac index occurred. Amplification of sympathetic activity led also to the excessive vascular tone and increase of total peripheral vascular resistance.

The indirect effects were of important role in the nature of sympathicotonia. The negative impact of catecholamines on vascular endothelium with nitric oxide synthesis depression and hyperproduction of endothelin-1 predisposed to the increase of total

peripheral vascular resistance. The increased sympathetic activity was shown being accompanied by the intensified sodium reabsorption due to the direct effect on kidney endothelium and blood flow decrease in medullary areas.

Stable activation of the sympathetic part of autonomous nervous system leads to activation of the renin-angiotensin-aldosterone system (RAAS). Increase of the angiotensin concentration in circulation potentiated the activation of the already activated sympathetic centers. Stimulation of aldosterone secretion stipulated by angiotensin II impact also predisposes to BP elevation. The increased secretion of arginine-vasopressin under stress conditions is an important component of AH development in the clean-up workers.

The peculiarities of interaction between the systemic regulation disorders occurring in a stress and the activation of stress-limiting and antihypertensive pathways of various levels should be taken into account. The insufficient activation of the latter becomes one among other regularities of the AH origination in clean-up workers.

Noteworthy is the combination of psychoemotional strain under the emergency with especial labor conditions of clean-up workers, rotational regimen of work with a prolonged working day, and high requirements towards personal job responsibilities. According to data available the workers at a far north under the rotation regimen were peculiar with a high variability of systolic and diastolic BP (SBP and DBP, respectively), both with daily BP profile abnormalities [11]. The overwhelming majority of clean-up workers were males in whom not only pronounced pressor response was typical in stress situations but also the slowed return of BP value to normal level. The reverse correlation was found between the work intensity and BP elevation after it.

There are therefore quite a bit of data testifying the role of psycho-emotional stress in the AH development in clean-up workers. Daily monitoring of a BP (BPDm) can be considered promising for diagnostics psycho-emotional stress. The daily average SBP increase over 135 mm Hg and DBP increase above 85 mm Hg with SBP and DBP difference in working days and days off 5% and more appeared valuable as diagnostic criteria for the stress-induced AH. The BP compare during working hours and a free-time of the same day indicated the difference of 10%.

The psychological strain related to negative experience and reminiscences of bygone events was not decreasing within post-accident period in majority of clean-up workers and especially in evacuated from Prypiat city. The integral score of negative reminiscences in clean-up workers of 1986–1987 remains about twofold higher. The emotional strain predisposes to appearing and fixation such RF as smoking, dyslipidemia, and alcohol consumption. It is associated with insufficient nighttime BP lowering and nighttime hypertension stipulating the high risk of danger in prognosis cardiovascular events.

Presented data of stress effect on the AH genesis substantiate the necessity of not only the antihypertensive therapy application in clean-up workers but also the tools for psycho-emotional strain decrease.

Non-radiation factors in arterial hypertension genesis among Chernobyl accident clean-up workers

According to our data at least one RF was registered in 97.7% of the clean-up workers, 1–2 RF are found in 40%, and 3 and more — in 57.7% of them (Fig. 12.3). Respective values in non-exposed persons were 89%, 55% and 34% respectively ($p < 0.05$).

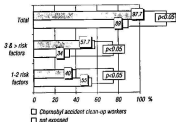


Fig. 12.3. Non-radiation risk factors incidence in Chornobyl accident clean-up workers suffering the arterial hypertension

the angiotensinogen, angiotensin converting enzyme (ACE), and angiotensin I receptor gene polymorphism. The elevated concentration of angiotensin I predispose to the BP increase through angiotensin II synthesis by means of systemic adrenergic stimulation and RAAS activation. Genes of aldosterone, nitric oxide, glucocorticoid, insulin receptors, β - and α -adrenoreceptors are also considered among those involved in AH genesis [12]. Possible role of other genes is not excluded here. Gene defects are far not identical in separate AH patients. Peculiarities of genetic defects involved in BP elevation and stabilization in the clean-up workers are subject to clarification.

Body mass excess had been found in 38% of the clean-up workers suffering AH. Its negative impact was due to the increase of circulating blood volume producing the extra load on LV.

Smoking habit was revealed in 55.2% of the clean-up workers. Despite the short-term BP elevation after smoking its magnitude can reach 11/5 mm Hg, and the prolonged smoking can predispose to stabilization of the high BP.

Age issues of arterial hypertension development. Aging of the clean-up workers is natural along with time since the Chornobyl NPP accident. Along with age the cardiac output and vascular elasticity decrease, the kidney circulation and humoral regulation of BP becomes different. Structural and functional disorders of vascular intima and media are of important role in arterial elasticity decrease. The amount of elastic fibers undergoes depletion against the collagen ones increase on the contrary stipulating vascular, valvar and cardiac cord sclerosis and fibrosis.

Adaptation capacity decrease and regulatory pathway disorders were found in clean-up workers aged up to 60 being typical for persons over 60 in the general population that can be regarded as premature onset and development of involutional processes.

Age-dependent analysis of BPFDM data in clean-up workers suffering the AH indicated the consistent increase of average diurnal, nighttime and daytime SBP along with an age. In the age group of 34–39 the patients suffering mild AH were prevalent, whereas in 40–49 years the moderate severe AH was the uppermost. BP deviations along with aging were different from data in non-exposed persons with quantitatively higher and earlier manifestations.

The body mass excess (BME), genetic predisposition, age over 55 years and smoking were predominant here.

Genetic predisposition has been identified in 31.7% of the clean-up workers. The AH was present also in their first-line relatives. Close correlation was found between the BP in parents and children.

The most number of studies has been focused on polymorphism of genes involved in synthesis control of the RAAS components. Data are available on the AH relation to

Thus combination of radiation, chemicals, and other accident factors with some non-radiation factors stipulates the mutual amplification of hazardous effect i.e. synergism in the AH development in clean-up workers.

Clinical peculiarities of arterial hypertension in Chernobyl accident clean-up workers

There are consistent differences between the clinical patterns of AH in clean-up workers and general population. The reliably higher incidence of subjective manifestations, BP profile disorders, early onset of the LVH, higher probability of diastolic dysfunction and LV systolic overload, myocardial ischemia, more pronounced decrease of physical load tolerability are of especial importance here.

Peculiarities of subjective manifestations. The thorough analysis of complaints and case history data indicated to the variability and higher incidence of AH subjective manifestations as against the patients in general population where scanty clinical presentation was typical. Headache was found in 80.4% of them vs. 60.2% in control. Irritability was present in 83.4% being 1.5-fold over the control value. Sleep disorders as frequent awakening, insomnia at a nighttime and somnolence during a day were presented by 27.4% of the clean-up workers (twice more often than in control). The 71.2% of clean-up workers experienced memory disorders (54.2% in control). Anxious feeling was widely prevalent (91.7% vs. 66.6% in control). Emotional instability in the clean-up workers suffering an AH (80.4%) twice exceeded the control values. Working ability was decreased in most of the clean-up workers. AH in clean-up workers were peculiar with a fixed onset and development in a younger age.

Hypertension peculiarities in Chernobyl accident clean-up workers. Classification of the clinical BP levels in clean-up workers according to the 2009 standards indicated that BP in 38.2% of them was within 140–159/90–99 mm Hg, in 36.4% was 160–179/100–109 mm Hg, and in 25.5% reached and exceeded 180/110 mm Hg. Time course by the AH stages is shown at Fig. 12.4.

According to these data the AH I was predominant in clean-up workers within first few years upon the accident. AH I and AH II incidence had become equal in 1993 and later the AH II incidence continued to increase. AH III cases appeared first in 1990 with respective incidence increase up to 3.3% in 2000 and to 30% in 2009. In total within 25 years the initial predominance of AH I evolved into the dramatic increase of AH II and slower one of AH III. Although the AH II is more prevalent among all years since the Chernobyl catastrophe.

Blood pressure diurnal monitoring is of substantial advantage over the office and clinical



Fig. 12.4. Arterial hypertension stages evolution in Chernobyl accident clean-up workers in 1986–2009 (n=3,423)

BP checking. As expected the SBP and DBP from BPDM in clean-up workers were lower than its clinical values. Average diurnal systolic BP level was 142.9 ± 1.9 mm Hg, diastolic — 91.3 ± 1.3 mm Hg, heart rate — 69.8 ± 0.6 per minute.

Under the comparable deviations of the average diurnal, daytime and nighttime SBP and DBP, BP time index (TI) in the clean-up workers and AH patients not exposed to IR the more pronounced variability, pulse BP and incidence of insufficient nighttime BP lowering was found. Just the high BP variability was considered responsible for the damage of target organs.

As distinct from normally typical diurnal BP rhythm with its decrease by 10–20% at a sleep then such a profile type of BP in clean-up workers suffering AH was preserved in 60.6%, in case of AH II — in 53.5%. Its incidence in the entire population of AH patients was 60–80%. Insufficient nighttime BP lowering within up to 10% occurred in 37.6% of the clean-up workers, up to 20% — in 6.7%, whereas a nighttime BP excess over the daytime (night-peaker) was found in 4.8%. Insufficient nighttime BP lowering in the clean-up workers was associated with an amplified sympathetic or decreased parasympathetic activity. Deviation from the permissible day/night variability of SBP $\geq 15/15$ mm Hg and DBP $\geq 14/12$ mm Hg was registered too.

More substantial abnormalities of the BPDM parameters were consistent to the exposure to ≥ 25 cSv in persons with long period of work in 1986 under the rotation shift regimen and having high integral score of negative reminiscences of the Chornobyl catastrophe events. Under the exposure within dose range of 25–50 cSv the weak correlation was revealed between the ERD and SBP diurnal variability ($r=0.35$; $p<0.05$), a nighttime ($r=0.31$; $p<0.05$), and DBP nighttime variability ($r=0.3$; $p<0.05$). There was a consistent dependence of the increased SBP, DBP, BP TI, BP variability in AH patients and duration of clean-up work.

According to our data the work under the rotation shift regimen can lead in 2–3 years to the SBP increase by 10–20 mm Hg, DBP — by 10–15 mm Hg. It corresponds to the data on the entire population patients working under the same schedule type [11]. Negative reminiscences on the Chornobyl catastrophe events are of especial concern in the remote period upon accident. High integral score of reminiscences was associated with diurnal BP profile abnormalities. There was an integral score of 18.9 ± 1.7 in case of insufficient nighttime BP lowering, 22.3 ± 3.4 in a nighttime hypertension, 20 ± 2.8 in a profound nighttime lowering, whereas 13.8 ± 1.2 in patients having normal diurnal BP profile ($p<0.05$).

The AH duration was of high importance. Increase of the diurnal SBP and DBP indices occurred mainly due to the daytime variations. Maximum SBP deviations were exceeding the average ones by 34.8 mm Hg, maximum DBP deviations — by 23 mm Hg. The BP TI was increasing from 40% in the very first years up to 50% further. The SBP and DBP variability increased within range of 3–5 mm Hg. The SBP diurnal variability abnormalities defined at most its deviations at a daytime, whereas the DBP — mainly at a nighttime. Extent of the morning time elevation of SBP and DBP increased by few mm Hg along with the AH duration. Incidence of the diurnal BP abnormalities had increased by more than 10% upon 5 years and by the 15% after 10 years. Rate of the morning time BP elevation was increasing substantially along with AH duration, and 10 years upon the difference for SBP had reached 31.7% and for DBP — 25.7%. Incidence

of its abnormalities was higher in DBP by 16.7%. Along with AH duration the number of patients having normal BP profile had decreased by 12.8% within a 10-year period. Simultaneously the incidence of an insufficient nighttime BP lowering had increased by 7.5% and of a deep nighttime lowering — by 5.5%.

Despite the robust advantage of the BPDm it cannot be considered as a complete substitution tool for the office of clinical BP checking.

Damage to target organs in Chernobyl accident clean-up workers suffering arterial hypertension

According to our data the LVH was of top priority within target organs injuries being diagnosed in 77.9% of clean-up workers suffering the AH. Microalbuminuria and/or serum creatinine elevation was found in 16% of cases.

Features of the left ventricular hypertrophy

LVH is recognized as an independent risk factor of myocardial infarction, stroke, CHD, HF and death independently from the BP level. The integral analysis of epidemiological data had shown that LVH predisposes to 2.3-fold increase of cardiovascular mortality [13]. There is a direct link between LVH grade and AH patients' survival. According to our data the LVH possess over 95% within target organ damage in the clean-up workers suffering the AH. Therefore early detection and Chernobyl catastrophe racterization of LVH in clean-up workers appear of more and more actual. Electrocardiography (ECG) and echocardiography are of a priority position among the contemporary tools of LVH diagnostics. According to international standards the ECG-markers of LVH and its regression are Sokolov-Lyon criterion and Cornell voltage criterion of a QRS ventricular complex duration.

Value of left ventricular hypertrophy electrocardiographic signs in Chernobyl accident clean-up workers suffering arterial hypertension. Electrocardiographic signs of LVH were fixed only in a part of such cases (Table 12.1), i.e. the Sokolov-Lyon criterion >38 mm — in 16.3% and Cornell voltage criterion in 15%. In severe LVH their incidence was 5- and 2-fold higher respectively.

Table 12.1. Incidence of electrocardiography signs of left ventricular hypertrophy in Chernobyl accident clean-up workers suffering from arterial hypertension

Criteria	Left ventricular hypertrophy		
	average	moderate MMI 125–170 g/m ²	severe MMI >170 g/m ²
Number of patients	257	126	131
Sokolov-Lyon criterion >38 mm	16.3%	4.8%	25% *
range	10–64 mm	10–45 mm	14–64 mm
average	28.6±1.4	24.4±0.9	30.7±1.1*
Cornell voltage criterion >2400 mm*ms	15.0%	9.2%	19.3%
range	540–8528	540–2940	600–8528
average	1714.5±77.8	1457.5±70.8	1904.3±98.1*

MMI — myocardial mass index; *significant difference between moderate and severe LVH, $p < 0.05$

Despite the insufficient sensitivity of LVH ECG-signs they convincingly indicate to the LVH presence due to the high specificity (95%). However the ECG reveals

LVH in a low amount of cases especially if there are mild or moderate presentations. According to the Framingham study data the ECG-signs of LVH are an independent prognostic factor. If they appear the mortality rate within 5 years reaches 35% in men and 20% in women 35–64 years and 50/35% older than 65 years respectively. According to population study data the ECG-criteria of LVH is associated with a 3-fold CHD risk excess, in case of possible LVH signs – with 1.5-fold excess. The probability of cardiovascular complications increases significantly 2–4 times if the voltage signs of LVH are associated with negative T-wave or ST segment depression. Related to them risk in absence of LVH voltage signs is 2-fold lower. The advisability of LVH voltage criteria as the markers of LVH and its regression under management is out of doubt. Abnormal ST segment and T-wave can be applied for the LV volume or pressure overload, or ischemia detection.

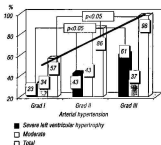


Fig. 12.5. Left ventricular hypertrophy incidence against arterial hypertension grade in Chernobyl accident clean-up workers.

Its basic index the myocardial mass index (MMI) was over 170 g/m^2 in 51.8% and within range of $125\text{--}170 \text{ g/m}^2$ — in 48.2% of cases. Relatively early wall thickening with no trend to rapid progression was peculiar here. The LV posterior wall thickness (LVPWTh) was over 11 mm in 76%, being 12–13 mm in 53.95% 14–15 mm — in 20%, and 16–17 mm — in 2.1%. The LVH incidence increased along with the BP level (Fig. 12.5) being the highest in BP >180/110 mm Hg. In the last case the LVH incidence had reached 98%, whereas in BP within range of 160–180 mm Hg it was 86%, and in BP <160/95 mm Hg — 57% ($p<0.05$). The severe LVH was present in 61%, 43%, and 23% respectively ($p<0.05$).

Compare of the average absolute values of principal echocardiographic indices of LVH with BP levels indicated (Table 12.2) that there was gradual increase of MMI depending on the BP level ($p<0.01$) exceeding at that the control values ($p<0.001$).

The LVPWTh and the interventricular septum thickness (IVSTh) were increased comparing to control ($p<0.001$).

Value of echocardiographic signs of left ventricular hypertrophy. ECG indicates only through an indirect evidence the cardiac dilatation, the echocardiography provides visual estimates of LV wall thickness and cavity size. It is considered a reference non-invasive diagnostic tool for LVH. Despite some technical limitations such as variability of results between the performers, impact of BME and chronic obstructive pulmonary disease on the imaging quality the echocardiography is anyway more sensitive method than ECG. The LVH is revealed then in 50–70% of cases.

The LVH was found through the echocardiography application in 77.9% of the clean-up workers suffering AH.

Table 12.2. Average echocardiographic indices ($M \pm m$) of left ventricular hypertrophy in Chernobyl accident clean-up workers suffering arterial hypertension

Indices	Control (no CSD), n=81	BP 140–159/90–99 mm Hg, n=126	BP 160–179/100–109 mm Hg, n=120	BP >180/110 mm Hg, n=84
MMI, g/m^2	100.6 $\pm$ 1.8	144.0 $\pm$ 2.9*	161.3 $\pm$ 1.6* ^a	181.0 $\pm$ 5.3* ^a □
LVPWth, mm	9.3 $\pm$ 0.1	11.4 $\pm$ 0.1*	11.9 $\pm$ 0.1* ^a	12.6 $\pm$ 0.2* ^a □
IVSTh, mm	9.1 $\pm$ 0.2	11.4 $\pm$ 0.2	12.2 $\pm$ 0.1* ^a	13.0 $\pm$ 0.2* ^a □

Significant differences: *vs. control; ^avs. data in AH I; □ vs. data in AH 2. MMI — myocardial mass index; LVPW — left ventricle apocardial mass; IVSTh — interventricular septum thickness.

According to the variability of body mass and myocardial wall thickness the LV geometry was different in 77.9% of patients. The concentric LVH was prevalent (diagnosed in 50.9%), the eccentric LVH was found in 27% of patients. The mean MMI values in concentric hypertrophy were higher and significantly exceeded those in eccentric one by 13.7 g/m^2 ($p < 0.001$). The LVPWth in concentric LVH was 12.9 $\pm$ 0.1 mm, in eccentric one — 11.7 $\pm$ 0.1 mm ($p < 0.01$), the IVSTh — 13.4 $\pm$ 0.1 mm and 11.7 $\pm$ 0.1 mm respectively ($p < 0.01$). There were direct correlations between the LV geometry type and myocardial thickness ($r = 0.79$; $p < 0.001$), MMI ($r = 0.74$; $p < 0.001$), AH grade ($r = 0.37$; $p < 0.001$), SBP ($r = 0.4$; $p < 0.01$) and DBP ($r = 0.31$; $p < 0.01$), and AH duration ($r = 0.34$; $p < 0.01$).

Enlargement of the left atrium (LA) adjacent to LV in clean-up workers was the consistent manifestation of heart structural remodeling in AH. Its average anteroposterior size in a concentric LVH exceeded one in normal geometry by 2.7 mm, LA index — by 1.3 mm/m^2 ($p < 0.001$), ratio of LA dimension to LV end-diastolic dimension (LA/EDD) — by 0.03 ($p < 0.01$) respectively. In eccentric LVH the LP dimension difference from normal geometry was 1.9 mm, LA index — 1 mm/m^2 ($p < 0.001$). In eccentric LVH the LA dimension difference to normal geometry was 1.9 mm, LA index — 1 mm/m^2 ($p < 0.01$). LA cavity increase over 40 mm in concentric LVH was registered in 22.6%, in eccentric one — in 12.3% of cases, and the LA/EDD index over 0.75 was found in 29.2% and 9% respectively. The LA dimension correlated with MMI ($r = 0.52$; $p < 0.001$). The pulmonary vein pressure increase can be expected under the LA functional activity deterioration and dilatation predisposing to congestion in pulmonary circuit. LA enlargement >42 mm was accompanied by the 1.8-fold risk increase of atrial fibrillation (AF).

Progression of the LVH was associated also with gradual increase of the average diurnal and nighttime SBP. The most pronounced abnormalities of BPDM indices were found in $MMI > 170 g/m^2$ and were characterized by the significant increase of BP TI making up 100% in one half of the examined persons, by the BP variability increase mainly at a nighttime, and by the diurnal BP profile abnormalities. Significant link was found between the LVH and pulse BP >53 mm Hg. Case incidence of pulse BP >53 mm Hg against the pronounced LVH was 49.2%, moderate LVH — 34.9%, and in LVH absence — 18.5% ($p < 0.05$). There was a correlation between the pulse BP and MMI ($r = 0.30$; $p < 0.01$).

The every third case of LVH was associated with heart rate at rest above 75 min^{-1} , that was considered the possible RF of sudden death. In majority of clean-up workers having heart rate >75 min^{-1} the MMI exceeded 170 g/m^2 .

Analysis of the LVH incidence against the increasing ERD up to 60 cSv (Fig. 12.6) indicated their interdependence. In exposure to 25–60 cSv the LVH incidence exceeded

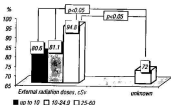


Fig. 12.6. Left ventricular hypertrophy incidence depending on external radiation doses

along with relative predominance of sympathetic one. In epinephrine excretion excess over $14 \mu\text{g/day}$ the MMI increased by 28 g/m^2 ($p<0.05$). There was a correlation between epinephrine excretion in daily urine and MMI ($r=0.32$; $p<0.05$).

Excessive production of angiotensin II having the direct proliferative effect on cardiomyocytes and vascular smooth muscle cell predispose to activation of hypertrophy and proliferation in the clean-up workers. Possible LVH regression under the ACE inhibitors administration convincingly proves the RAAS role in its genesis.

Other factors of non-radiation nature was revealed in 88.3% of the clean-up workers suffering LVH, two and more factors were present in 65.5% of LVH. The smoking habit was met with equal incidence in both groups, however the MMI value in smoking patients was by 9 g/m^2 higher than in non-smokers ($p<0.05$).

The LVH incidence increased along with age for every ten years reaching 95.7% in the age range of 60–69. The MMI every 10 years increased by $40\text{--}50 \text{ g/m}^2$. There were correlations between age and LVPWth ($r=0.35$; $p<0.01$), IVStH ($r=0.31$; $p<0.01$), and MMI ($r=0.28$; $p<0.05$).

The excessive body mass as it predisposed to increase of circulating blood volume made therefore an extra load on the LV. In LVH among clean-up workers suffering AH the number of persons having normal body mass was 5%, whereas with no LVH — 14.3%. The body mass index over 30 kg/m^2 was found under LVH in 55%.

Genetic predisposition was found in 31.7% of clean-up workers suffering LVH vs. 5% with no LVH. Taking into account possible manifestation of genetic predisposition to LVH the search of specific genes responsible for its genesis is going on. The ACE gene was intensively studied as a LVH marker.

In ERD 10–24.9 cSv combination with 2 and more RF the LVH incidence was 83%, with 1 RF — 75%. In ERD >25 cSv combination with 2 and more RF, occupational exposure to xenobiotics the LVH incidence reached 100%.

LVH genesis in AH among the clean-up workers depending on the peculiarities of long-term pressure load interference with radiation and non-radiation factors stipulates the need of combined therapy.

Cardiac dysfunction in left ventricular hypertrophy

Diastolic dysfunction (DDF) was an early sign of heart remodeling in clean-up workers suffering the AH. The DDF mainly the LV relaxation was found in 64.6% of

the values in exposed up to 10 cSv by 14.2%, to 10–14.9 cSv, and was higher than in patients having no registered ERD (for the sort term involved in the clean-up work).

The consistent for clean-up workers activation of the sympathoadrenal system and RAAS was important in LVH genesis. At the BP level of 140/95 mm Hg they experience the abnormalities of average-diurnal HRV characterizing the parasympathetic activity decrease

LVH patients. The average-diurnal BP elevation, high BP variability, insufficient night-time BP decrease, elevated MMI value, and larger duration of disease were the DDF predictors. There was a significant link between the ratio of maximal velocity early and lately diastolic filling (E/A), isovolumetric relaxation time (IVRT) and myocardial mass. DDF development in clean-up workers suffering AH was as a rule preceding the systolic dysfunction. In DDF the heart geometry remained normal in 5%, the concentric LVH was present in 83% and eccentric LVH with no dilatation — in 12%. More severe diastolic dysfunction in survivors suffering AH occurred in concentric LVH. LV peak early filling velocity decrease of 2.1 cm/sec and peak late filling velocity increase by 16.1 cm/sec ($p<0.05$), E/A ratio decrease by 0.34 units vs. normal LV geometry ($p<0.05$) were found. E/A ratio less than 1 unit were registered in 56% of patients suffering the concentric LVH. Average IVRT values in concentric hypertrophy were higher and exceeded the respective values in groups of normal geometry and eccentric LVH by 42.2% and 27.9% ($p<0.05$).

Diastolic dysfunction in clean-up workers suffering LVH stipulated by AH was both isolated and combined with a systolic one. In most of the clean-up workers suffering uncomplicated AH the ejection fraction (EF) was not less than 55%. More pronounced EF abnormalities occurred in case of HF, the EF<55% was registered in 6%.

Hypertensive angiopathy and its clinical appraisal in Chernobyl accident clean-up workers suffering arterial hypertension

Along with heart remodeling in clean-up workers suffering AH the vascular remodeling occurred with hypertensive angiopathy syndrome origination, consisting of arterial and venous components. Vascular deformations as angular and loop-shaped deflections were found in 60% of cases and thickening of intima-media complex (IMC) in 92% by about 1.28 ± 0.21 mm vs. control. IMC echogenicity was homogeneously increased with flattened layer differentiation. Arterial abnormalities were concomitant to internal jugular veins dilation with decreased velocity there. The IMC thickening over 1 mm reflected the atherosclerotic injury. Carotid atherosclerotic plaques found in 43% being homogenous, of moderate echogenicity, and localized mainly in bifurcation of common carotid artery, in some cases spreading to internal carotid artery ostium. Stenosis grade at that not exceeded 40% of vascular diameter.

Index of aortic remodeling correlated with LVH ($r=0.44$; $p<0.05$). Aortic wall thickness in clean-up workers suffering AH without LVH was on average not exceeding 4 mm. In LVH of 25% it was more than 5 mm, whereas in AH association with CHD it was 5.9–6.1 mm. Ratio of aortic wall thickness to its lumen reached 0.22 ± 0.0001 units indicating to hemodynamically significant blood flow obstruction.

Focal or generalized retinal arteriostenosis was not regarded as a target organ injury according to the European Society of Hypertension and European Society of Cardiology recommendations as arterial abnormalities grade 1-2 are frequently found with no significant evidence of their prognostic role. According to our experience the frequency of such disorders exceeded 80% in surveyed persons. Hypertensive angiopathy had led to deterioration of vascular wall elastic properties, increase of peripheral vascular resistance and was an important unfavorable factor of heart remodeling. It also stipulated the decrease of cerebral blood supply. Therefore such abnormalities can be considered as markers of an unfavorable course of AH.

Left ventricular hypertrophy and myocardial ischemia

Decrease of coronary flow reserve can be a serious consequence of LVH in clean-up workers suffering the AH. It occurs even in an absence of coronary stenosis as a result of coronary microcirculation disorders due to myocardial mass and myocardial oxygen demand increase. In decrease of density and number of functioning capillaries as a result of cardiomyocyte hypertrophy the hypoxia appears even under the absence of any other predisposition for ischemia. Possibility of heart rhythm disorders (HRD) especially of high-grade ventricular extrasystoles and ventricular tachycardia under the transient ischemia stipulates sudden cardiac death risk.

According to the results of electrocardiography daily monitoring (ECG DM) the mainly silent myocardial ischemia episodes were revealed in every fifth clean-up worker suffering the LVH even in the absence of CHD signs. Total average duration of ischemia per one patient a day was 52.3 ± 4.9 min. Depth of the ST-segment depression by 0.08 sec from the j point not exceeded 1.5 mm being mainly 1.23 ± 0.3 mm. Average number of ischemia episodes per one patient was 3.6 ± 0.3 , the average episode duration — 14.6 ± 1.75 min. It was consistently expressed under physical load in all patients having ischemia episodes. Number of ischemia episodes under physical load per one patient in LVH was 1.8-fold higher vs. its absence, and its duration daily increased 2.5 times making up 24.5 min.

Painless myocardial ischemia in clean-up workers reached 73.3% from its total duration. In its presence the MMI value was higher vs. in its absence by 9.2 g/m^2 ($p < 0.05$). In 70-90% it was associated with heart rate increase, LV diastolic dysfunction. The role of stenosis or lack of stimulus to coronary arteries dilation, heart rate increase, physical load, psychological factors cannot be excluded.

There was a correlation of number and duration of ischemia episodes with myocardial mass ($r = 0.33$; $p < 0.05$). Myocardial mass index in case of ischemia lasting over 20 minutes was higher by 20.9% ($p < 0.05$) vs. lower duration.

Left ventricular hypertrophy and heart rhythm disorders

According to ECG DM the heart rate in clean-up workers suffering the AH was up to $69.8 \pm 0.6 \text{ min}^{-1}$, the daytime exceeded the average diurnal by 6.9%, the nighttime rate was less than diurnal by 10.7% and less than daytime by 16.9%. According to the YALUE trial results the heart rate increase in AH leads to complications risk increase. In patients having the highest heart rate the primary endpoint risk was significantly 1.64-fold higher, the HF risk was higher 1.89 times, and the sudden cardiac death risk was 1.27 times higher. Our studies showed that an increased heart rate was a serious RF of LVH. The MMI value was up to 170 g/m^2 and more in 33.5% clean-up workers suffering AH if the heart rate at rest had been 75 min^{-1} and more.

Decrease of total HRV (SDNN-I, TP, triangular index, coefficient of variation), of parasympathetic activity (RMSSD, HFp) both with amplification of sympathetic one (LFp, LFp/HFp, IC) were consistent in the clean-up workers suffering AH. Significant exacerbation of autonomous regulation disorders characterized by further decrease of HRV statistical and spectral parameters, sympathetic and parasympathetic activity against preserved relative superiority of sympathetic nervous system activity was found in extremely high risk patients.

Heart rhythm disorders were found in 22% of clean-up workers suffering LVH stipulated by the AH. Ventricular HRD attributed to increased risk of sudden cardiac death were registered in 15.9% of LVH patients vs. 5.7% in absence of the latter ($p<0.05$). Quantity of isolated ventricular extrasystoles (VE) per one examined person was 115 ± 10.3 that exceeded 6.1 times ($p<0.05$) the respective value in no LVH. Paired and group VE were registered in 9.6% only in LVH.

The MMI value in VE exceeded the respective index in patients with no HRD by 17.2%. Myocardial mass gain was 3.1% in supraventricular extrasystoles (SVE).

Direct correlations between the quantity ($r=0.75$; $p<0.01$) and duration ($r=0.9$; $p<0.01$) of myocardial ischemia episodes per round the clock and the VE were found. In HRD the number of diurnal ischemia episodes was 2.2-fold higher, the average ischemic episode duration — 2-fold, integral duration of ischemia per patient — by 44.8 min round the clock ($p<0.05$). Total number of VE including the high-grade ones correlated with basal heart rate increase ($r=0.5$; $p<0.01$) that cannot be underestimated in risk assessment for complications and mortality.

Microalbuminuria and creatinine content elevation in Chernobyl accident clean-up workers suffering arterial hypertension

Glomerular ischemia in arterial hypertension stipulated by the progressing depletion of kidney circulation and vascular resistance increase can predispose to onset of microalbuminuria. It was found in 16% of the clean-up workers. Noteworthy that kidney circulation related to involuntary disorders can occur in the absence of cardiovascular or kidney disease. The glomerular filtration rate after 40 years of age decreases on average by 8 mL/min per 1.73 m^2 within each next decade. The physiological microalbuminuria can occur as a result of increased permeability of glomerular membrane, spreading of sclerosis and hyalinosis by 12–30%. In 68% of the clean-up workers suffering microalbuminuria the age was within 39–49 years and in 32% was over 50. The nighttime hypertension or insufficient BP lowering was peculiar for 58% of them, whereas the increased BP variability and pulse BP over 60 mm Hg were registered in 8.3% of patients. There was a significant correlation between microalbuminuria and office SBP value both with an average-diurnal pulse BP.

Mean level of creatininemia in microalbuminemia was $87\pm7.3\text{ }\mu\text{mol/L}$ vs. $67.4\pm2\text{ }\mu\text{mol/L}$ in its absence ($p<0.05$). In separate cases the serum creatinine elevation was within 115–133 $\mu\text{mol/L}$. The AH duration in all microalbuminemia and/or serum creatinine elevation cases exceeded 5 years, whereas in one-half of cases it was 10–16 years. At that low compliance was typical for most of patients.

The LVH was named an “enemy of the kidneys” at the American Society of Hypertension congress in 2010. It was found over the 6,000 male patients that each MMI elevation by 42 g/m^2 was accompanied by serum creatinine elevation risk by 45.7%. Therefore the MMI value should be taken into account for the prediction of kidney function decrease in the clean-up workers suffering AH.

Associated clinical conditions

Nowadays the AH association in clean-up workers with CHD and HF is becoming urgent. The $\text{ERD}\geq 25\text{ cSv}$, older age, disease duration, higher BP and LVH levels,

non-radiation RF presence mainly dominant in atherosclerosis progressing, and low compliance are consistent for such patients.

In most of them it was a stable angina mainly of FC II (77.5%). Average age of patients with angina was 54.9 ± 1.05 years, disease duration was 12.4 ± 1.3 years vs. 48.9 ± 0.5 and 9.1 ± 0.4 years at the absence of clinical conditions linked to AH ($p < 0.05$). The LV MMI was higher by 22.8 g/m^2 ($p < 0.05$) making up $180.5 \pm 6 \text{ g/m}^2$. In 88.5% of examined persons the RF were identified dominant in atherosclerosis progress, and in 57.5% of cases there were more than 2 RFs.

Stable BP elevation, LVH, irregular management, age over 60, AH duration over 10 years, physical and emotional load, metabolic disorders, BME were the leading factors predisposing to higher incidence and progressing of the HF. A direct link was established between the HF stage and DBP, SBP variability, load index. Diurnal BP profile with lowering or paradoxical elevation of a nighttime BP was most consistent. Such phenomena were the predictors of exacerbation of LV structure and geometry remodeling, its systolic overload, and myocardial ischemia. The HF development in AH was accompanied by further decrease of total HRV (SDNN-I, TP, TI, CV), parasympathetic activity (RMSSD, HFp) and amplification of sympathetic activity (LFp, LFp/HFp, IC).

Strokes related to AH had occurred in 3.6% of patients with mean age of 55.7 ± 1.8 years, disease duration 14 ± 1.1 years, $BP \geq 180/110 \text{ mm Hg}$ with/pulse $BP > 65 \text{ mm Hg}$. They had more frequent association with CHD and HE. They had the MMI value of $194.6 \pm 15.8 \text{ g/m}^2$, the concentric LVH was found in 82%.

Taking into account the impact of associated clinical conditions on the AH course in clean-up workers and related stable disability, life-span shortening, such patients therefore were attributed to the especially high risk category with the purpose of further management strategy choice directed not only to the target BP value maintenance but the effect on modifiable RFs and minimization of target organs damage.

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12.2 FEATURES OF CORONARY HEART DISEASE AFTER MYOCARDIAL INFARCTION IN CHORNOBYL ACCIDENT CLEAN-UP WORKERS

After two decades since Chornobyl catastrophe the coronary heart disease (CHD) in clean-up workers who have survived the myocardial infarction (MI) becomes one of key health issues in all groups of survivors. MI and its consequences are now ones among other leading causes of disability and mortality in the accident survivors. Chronic CHD in clean-up workers who had survived the MI is featured by the high rate of in-ward admissions. Sixteen from every 100 of clean-up workers have the chronic CHD that by 11.5% exceeds the respective value in population of Ukraine.

MI incidence and prevalence now vs. previous years in Chornobyl catastrophe survivors has increased nearly twice. Further increase here is expected due to natural aging of the survived population and combination impact of radiation and non-radiation Chornobyl catastrophe factors. Passed MI makes great influence on all the further life hampering the ability of previous activity and employment. Unfavorable prognosis after

the survived MI stipulates the elevated risk of repeated MI, sudden cardiac death, and chronic heart failure. If the sudden death risk in an entire population is under 1% then among the MI survivors it is over 30%.

From our survey of the 9,423 clean-up workers there were 215 patients with passed MI in whom the specific features were studied.

Radiation and non-radiation factors in Chernobyl accident clean-up workers suffering coronary heart disease with underwent myocardial infarction

Issue of MI in persons exposed to IR is not a new one. Review of data available for IR effects on cardiovascular system indicates to possibility of its severe involvement with MI that can be lethal in acute radiation syndrome. The fine-focal injuries with hemorrhages were found under pathological study in 21 person and MI — in 6 men from 27 clean-up workers deceased from the acute radiation syndrome [14]. Those findings occurred after the uniform gamma- and beta-irradiation within 4–15 Sv dose range.

Available data on MI and other cardiovascular and cerebrovascular disease in Hiroshima A-bombing survivors [3] and in survivors of nuclear power plants and nuclear industry accidents [4] require concern and clarification.

Evidences on coronary effects after low dose irradiation mainly under the occupational exposure are debatable at a time. According to [15] there is a significant excess of spontaneous lethality from cardiovascular disease and specifically from MI after occupational exposure in doses exceeding the permissible levels. And there are opinions that morbidity and mortality are lower than population level and are not correlated with ERD, maximum yearly dose and employment history in persons whose occupational experience was related to IR. According to [16] the CHD incidence was 5.2–28.8% and did not exceed the population level in persons exposed to gamma-radiation in doses over the permissible levels.

According to our data the MI incidence had increased significantly along with ERD value (Fig. 12.7).

In 23.3% of clean-up workers with passed MI the ERD was less than 10 cSv (3.9 cSv mean). ERD up to 10 cSv was in 31% of clean-up workers with no MI history. Exposure in dose range 10–24.9 cSv was established in 32.3% of clean-up workers who had survived MI, i.e. by 9% higher vs. irradiated up to 10 cSv. ERD exceeded 25 cSv (42.4 cSv mean) in 44.4% of subjects. Difference in MI incidence depending on the ERD up to 10 cSv was linearly increasing towards 21.1% ($p < 0.05$). Thus in combination of IR dose > 25 cSv with traditional risk factors the incidence of MI among the clean-up workers was nearly 2-fold higher vs. exposed to less than 10 cSv.

Situation that occurred after the wide-scale Chernobyl NPP accident was the source of both the IR, and other sub-

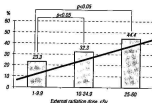


Fig. 12.7. Myocardial Infarction incidence and external radiation dose in Chernobyl accident clean-up workers

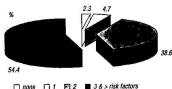


Fig. 12.8. Risk factors incidence in Chernobyl accident clean-up workers with myocardial infarction history

to cumulative effect of some or other xenobiotics. As of occupational affiliation the 49.3% of MI survivors have had a contact with cumulative impact of lead and other substances vs. 18.8% of patients with no MI history ($p < 0.05$). Mostly they were vehicle drivers, welders, fitters, and miners. Xenobiotics are considered as a principal stimulus of vascular damage in the respective occupational groups.

Genetic predisposition and lifestyle features along with IR and other factors related to Chernobyl catastrophe were preceding the MI in majority of surveyed persons. At least one of those was found in 97.7% of cases, two were present in 93%, and three and more — in 54.4% (Fig. 12.8)

Age range of 45–55 years was critical for MI in clean-up workers (70%). MI incidence at the age up to 45 years 2-fold exceeded the respective value in the entire population. Genetic predisposition, intensive tobacco smoking, occupational contact to xenobiotics, psychological strain due to negative reminiscences about the Chernobyl catastrophe prevailed among other factors predisposing to MI up to 45 years of age, whereas the AH, LVH, excess of body mass, and diabetes mellitus were more relevant after 45 years of age.

The share of those period factors has decreased along with time; the psychological strain however remains related to negative perception of the Chernobyl catastrophe events. An integral score of the Chernobyl catastrophe negative reminiscence estimate after the passed MI was on average 25.1 ± 0.9 vs. 17.9 ± 0.9 in uncomplicated CHD ($p < 0.05$), whereas exceeding score 20 in 54% vs. 40% ($p < 0.05$). No consistent Chernobyl catastrophe of reminiscence intensity occurred for the passed time. Variation of negative reminiscence grade along with time was 0.021–0.054 ($p > 0.05$). Not a few study subjects complain on remaining of much irresistible negative feelings.

Combination of >25 cSv dose irradiation with non-radiation RF featured their aggressive nature potentiation. The exacerbation of “aggressiveness” of non-radiation RF was also found in a stable population of Syeverk city exposed to long-term low-dose irradiation [17].

All mentioned above indicates that along with possible direct radiation vascular damage and further atherosclerotic and fibrotic processes the traditional non-radiation factors were important potentiating each other in CHD genesis particularly the MI.

stances and compounds hazardous for human metabolism. The long-term impact of lead and other heavy metals resulted in an increase of cholesterol synthesis and predisposed to atherogenic dyslipidemia. Participation in a clean-up work was at most under the combination of IR, chemicals, unfavorable temperature regime etc.

In majority of the MI survivors the occupational activity was related

Morphology of coronary heart disease

Coronary atherosclerosis exceeding 50% was the main pathological finding in clean-up workers deceased from MI or related complications in 2003-2007. Left coronary artery involvement was more frequent (61.4%) along with more severe stenosis. Its luminal occlusion was $67 \pm 5.9\%$, of the right coronary artery — $56.6 \pm 4.3\%$. Lethal outcome from MI being recurrent in 23.9% respectively occurred in 39.6% of all death cases. Atherosclerotic coronary occlusion in recurrent MI was up to $65.5 \pm 6.5\%$ vs. $49.5 \pm 7.9\%$ in solitary infarction ($p < 0.05$). Two coronary arteries were involved in 43.5% of the recurrent MI death cases, in primary MI — of only one artery. Left coronary artery in repeated MI was found in 78.3% vs. 46.7% in a single MI ($p < 0.05$). According to the data for entire population [18] in case of 1 or 2 arteries injury the single MI was found in 62%, two and more infarctions — in 38%. In multivascular disease the solitary MI was found in 58%, two and more MI — in 42%. Aneurism was more often after the MI featuring abnormal Q-wave (Q-MI) of anterior localization. Proximal left coronary artery stenosis appeared being of more serious prognosis followed by a mortality rate 407.4%. Right coronary artery involvement was more favorable. Annual death rate reached 10-15% if three coronary arteries were involved.

Clinical appraisal of coronary heart disease in Chernobyl accident clean-up workers with history of myocardial infarction

Chest pain was of most impact on the chronic CHD course significantly worsening the prognosis and predisposing to repeated MI in clean-up workers after the initial MI. HF occurred twice more often after the Q-MI. The initial check-up of clean-up workers with history of MI has revealed the post-infarction angina in 92.1%. The 46.5% of clean-up workers suffering post-infarction angina survived the Q-MI and 45.5% — not Q-MI. Application of contemporary diagnostic tools provided the up-to-date approach in clarification of regularities in cardiovascular system remodeling under CHD after the survived MI, definition of abnormality priorities and assay of general risk of its recurrence and complications.

Electrocardiographic patterns. Ischemic episodes mainly painless, multifold increase of total diurnal ischemia duration, increase of ischemic episode number including those upon physical load, increase of single ischemic episode duration were consistent according to ECG DM for the postinfarction period vs. cases with no MI history. Myocardial ischemia was associated with HF of NYHA functional class (FC) II and over in 90.5%, with recurrent MI — in 91%. Ventricular and supraventricular extrasystoles were seen in 40% i.e. two-fold more often vs. no MI history. Number of extrasystoles per day, frequency of paired and group ones both with supraventricular tachycardia were also multifold more often. Therefore myocardial ischemia, high-grade ventricular rhythm disorders can be considered the markers of an unfavorable course of postinfarction period.

Basal heart rate and HRV abnormalities in postinfarction period are noteworthy. Basal heart rate was correspondingly over 70 min^{-1} exceeding 75 min^{-1} in more than 30% reflecting the elevated risk of heart rhythm disorders and sudden cardiac death requiring the respective interventions on correction. Upon the survived MI in heart rate 75 min^{-1} and over there were differences in a clinical course of chronic CHD. Number of angina

FC III cases after the Q-MI under heart rate $\geq 75 \text{ min}^{-1}$ was uppermost by 23.1% vs. $< 75 \text{ min}^{-1}$ cases. Signs of HF grade I & II were seen in 61.1% and 24.2% respectively in heart rate $\geq 75 \text{ min}^{-1}$ vs. 56.4% and 2.8% in $< 75 \text{ min}^{-1}$. Heart rhythm disorders were by 17.6% more often in heart rate 75 min^{-1} and over.

Decrease of statistical and spectrum DRV indices were seen in postinfarction period. Standard deviation of normal SDNN intervals reflecting general rhythm variability was decreased after the Q-MI by 9.2% ($p < 0.05$), SDNN-1 — by 6.4% ($p < 0.05$) vs. non-Q-MI and by 18.9% and 13.6% ($p < 0.05$) vs. clean-up workers with no MI history. HRV indices correlating with parasympathetic activity, RMSSD and pNN50% in clean-up workers after the MI were by 10.4% and 31.7% lower vs. non-Q-MI ($p < 0.05$) and by 13.6% and 36.9% vs. cases with no MI history ($p < 0.05$). Decrease of the HRV general spectrum power (Total Power — TP) $< 2000 \text{ msec}^2$ in clean-up workers after Q-MI and non-Q-MI was followed by a respective 29.8% and 10.5% increase of ventricular extrasystole cases and a 26.8% and 5.3% respective increase of KF signs. SDNN $< 100 \text{ msec}$, SDNN-i $< 40 \text{ msec}$, SDANN $< 55 \text{ msec}$, TP $< 2000 \text{ msec}^2$, and LF/HF < 0.95 units were associated with deterioration of the postinfarction period course, HF and HRD symptoms indicating the unfavorable time of day.

Peculiarities of myocardial remodeling. More pronounced postinfarction myocardial remodeling was found in clean-up workers suffering CHD after the Q-MI vs. non-Q-MI cases and subjects with no MI history. Remodeling had appeared already soon and lasted for months and years. LVH was seen in 98% with MMI exceeding 170 g/m^2 in 63.9% of them. There was a trend to the increased incidence of pronounced LVH by 12.1% after Q-MI. Hypokinetic zones and some LV posterior wall and interventricular septum segments thinning were registered 3.2 times more often vs. non-Q-MI. LV aneurism had developed in 17%. LVH was more severe in AH. MMI excess was 2-fold more in severe AH vs. moderate one. LA dilation $> 40 \text{ mm}$ associated with an increased AF incidence was seen in 60% of Q-MI survivors. Pronounced LVH predisposed to HF exacerbation, recurrent infarctions, AF, and ventricular extrasystole. There were correlations between MMI and HF FC by the NYHA ($r = 0.41$; $p < 0.01$), angina ($r = 0.47$; $p < 0.01$), AF ($r = 0.34$; $p < 0.05$), and ventricular extrasystole ($r = 0.3$; $p < 0.05$).

More pronounced LV geometry abnormalities were consistent in Q-MI survivors. An eccentric hypertrophy was seen in most of them, LV-dilation — in 30.6% that is 2-fold more vs. non-Q-MI and vs. no MI history. Significant impact on the incidence of recurrent MI, high-grade VE, and angina FC elevation was proved. Continuous exacerbation of LVH asymmetry was marked as an unfavorable factor in clean-up workers.

LV diastolic dysfunction (LVDD) occurred in a large majority of Q-MI survivors under all the types of myocardial remodeling. The isolated LVDD was seen in 66.2%. Systolic dysfunction was more often in an eccentric LVH. Pronounced LVH, systolic and diastolic dysfunction are defined as determinants of an unfavorable course of postinfarction period.

Postinfarction myocardial remodeling under $\text{MMI} \geq 170 \text{ g/m}^2$, chamber dilation, and LVDD were associated with deterioration of postinfarction period. MMI correlated with an angina FC ($r = 0.47$; $p < 0.05$). Eccentric LVH with dilation was accompanied by a high-grade VE and was consistent for the clean-up workers with MI history.

According to the physical tolerability assay the most significant limitation of a threshold power (W_{th}) was seen in patients after the survived Q-MI. Average difference vs. a compare group data were 18.9 watts. Highest limitation of the extent and duration of physical load was highest at that. Respective values in non MI history were lower by 30.1% ($p<0.05$) and 16.8% ($p<0.05$) respectively. ECG-signs of myocardial ischemia, angina attacks and combination of both above, BP elevation to the permissible threshold level were principal factors limiting the physical load tolerability upon the survived MI. Incidence of the ST-segment depression was 31.8%, of a high-grade extrasystole — 18.2%. Load was limited in 50% due to BP elevation up to the threshold permissible level.

Load elevation towards the threshold level was along with consistent elevation of heart rate, BP, and oxygen consumption. However an augmentation of the above parameters differed substantially in clean-up workers after MI. Heart rate augmentation under a load after Q-MI and non-Q-MI was 35.6% and 36.8% respectively vs. 54.7% ($p<0.05$) in subjects with no MI history. Heart rate increase per 1 watt of tolerated load in Q-MI survivors was 1.5-fold more vs. control. Increase of myocardial oxygen demand in postinfarction period was therefore at most due to an enormous heart rate elevation and correspondingly inadequate energy expenditures per unit of executed power. That is why an external heart work and an oxygen demand had increased. Finally all that had brought to ischemic events and/or excessive fatigability and physical exhaustion. The threshold power loading was provided with highest energy expenditure index i.e. 3.09 U being by 12.9% ($p<0.05$) higher vs. control group (no MI history). In case of higher heart rate and energy expenditure index augmentation with resulting in myocardial oxygen demand increase the amount of oxygen delivered to organs upon the survived MI was lower vs. control, whereas oxygen utilization occurred under the less effective circulation providing of a load, finally resulting in an inadequacy of increasing myocardial oxygen demand and its actual supply.

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12.3 FEATURES OF GENESIS OF HEART RHYTHM DISORDERS, CLINICAL PRESENTATION, STRUCTURE-FUNCTIONAL MYOCARDIAL ABNORMALITIES IN CHORNOBYL ACCIDENT WITHIN 25 YEARS

Heart rhythm disorders (HRD), their prevalence, peculiarities of genesis, and stipulated risk of hazardous cardiovascular events remain an important issue both for the entire population of Ukraine and especially for the clean-up workers. HRD makes an impact not only on the quality of life in clean-up workers but also on mortality especially the sudden cardiac death (SCD). Clean-up workers mortality rate has increased for the passed time 4 times [2]. About 13% within total mortality were due to SCD, from which 88% were stipulated by HRD.

Review of data for 9,423 clean-up workers admitted in-ward to Cardiology department in 1986–2009 (Fig. 12.9) revealed the HRD in 1,536 of them. Gradual increase of HRD incidence occurred from 2.2% to 35.5% since 1986 till 2009.

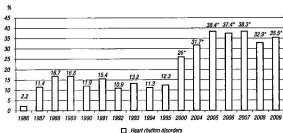


Fig. 12.9. Heart rhythm disorders incidence in Chernobyl accident clean-up workers (1986–2009 data).

Preconditions of heart rhythm disorders

Participation in Chernobyl catastrophe clean-up work in 1986, $ERD \geq 25$ cSv and rotational (watch-based) schedule of work were of top importance among the Chernobyl catastrophe factors preceding the HRD. ERD 10–24.9 cSv was established in 39.5% of HRD patients. HRD in clean-up workers who had worked under the rotational schedule since May 1986 was by 28.8% more often vs. in those since June–December 1986 and by 39.5% — since 1987 ($p < 0.05$) according to HRD incidence analysis depending on time period of participation in clean-up (Fig. 12.10).

More often the occupational activity of patients suffering HRD was related to cumulative impact of some xenobiotics. The 51.6% of HRD patients have had an occupational contact with cumulative effect of lead and other substances vs. 33.3% among having no history of HRD ($p < 0.05$). HRD incidence was higher among vehicle drivers, railway workers, welders, miners, chemical facility workers. Contact to xenobiotics i.e. lead tetraethide, welding aerosol, heavy metals, acetone-containing substances, pesticides is recognized as a prevalent stimulus of heart rhythm disorders.

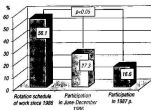


Fig. 12.10. Heart rhythm disorders incidence depending on time period of participation in clean-up after Chernobyl accident

Psychological strain was of not less concern here being related to negative reminiscences about the past events and socio-economical issues. Integral score of negative reminiscences about the Chernobyl catastrophe events in HRD patients was on average 26.3 ± 0.8 vs. 19.1 ± 1.3 in their absence ($p < 0.05$), whereas in 56% vs. 43% it was not more than 20 ($p < 0.05$). No significant change in an intensity of negative reminiscences about the bygone events occurred since last time.

Along with an impact of factors related to accident the non-radiation RFs

have also preceded the HRD onset i.e. tobacco smoking, compromised heredity, LVH, BP elevation, high total cholesterol (TCh).

Average age of clean-up workers at onset of HRD was 51.9 ± 0.9 years. In 21.4% the HRD have appeared in age up to 45 years that exceed the respective value for non-exposed Ukrainian population by 14.7%. Peak of HRD onset in clean-up workers was in 45–60 years (60.7%). After 60 the increment was stable meaning that HRD in clean-up workers was typically in a younger age.

Arterial hypertension preceded the HRD in 50%. After 45 years of age its incidence was 1.4 times more vs. data for years under 45. New AH cases gain upon HRD onset was 34.9%. LVH preceded the HRD onset for the most part of examined AH patients. They had MMI on average $173.3 \pm 5.4 \text{ g/m}^2$ exceeding values in normal BP by 34.2 g/m^2 ($p < 0.05$).

Body mass excess $\geq 25 \text{ kg/m}^2$ preceded the HRD onset in 84.4% being twofold more often in patients aged over 45. In 92.3% it was associated with other RF. As the body mass increase by 0.5–1.5 kg/year was typical after middle age then its stabilization can be surely useful in HRD prevention.

TCh level $\geq 5 \text{ mmol/L}$ was found in 73.4% of clean-up workers suffering HRD. At that up to 45 years it was no more than 5 mmol/L in 50.9%, whereas after that — in 27.3%. Hypertriglyceridemia however can be not less but rather more important factor of atherosclerosis and CHD and respectively HRD. Possible retardation or regression of atherosclerosis under the lipid-lowering therapy application substantiates the perspectives of tools implementation directed on this RF modification within HRD management scope in clean-up workers.

Tobacco smoking and alcohol consumption are of especial concern. Smoking was registered in 34.7% of examined HRD patients vs. 32.1% in no HRD. Smoking cessation was certainly one of tools of lifestyle modification to prevent HRD. Alcohol and caffeine consumption also result in AF risk increase. Alcohol consumption augmented the AF risk 1.8 times ($p = 0.009$) through the toxic effect directly on the LA otherwise through its volume increase being associated with high-density lipoproteins and triglyceride levels increase.

Hereditary predisposition to HRD was found in 27.7% of examined persons. It was 2.2 times more often among factors predisposing HRD origination in patients up to 45 years vs. after 45 years. Such regularity was seen in HRD cause review among young males having a long-term occupational contact with xenobiotics. Described above substantiates the importance within strategy of control in personnel recruiting/selection for nuclear industry.

Glucose intolerance and diabetes mellitus have also provoked the HRD onset. Elevated blood glucose level was found in 9.4%.

Compare of non-radiation factor incidence in age intervals up to and after 45 years in clean-up workers suffering HRD indicated the preponderance of smoking and compromised heredity in a young age vs. AH, BME, TCh, and diabetes mellitus after 45 years.

Traditional RF were found in 97.1%, two and more of them — in 81%. RF prevalence in Ukrainian population is several times lower [19] i.e. one RF is seen in 33.9%, two of them — in 28.2%. Subpopulation having three and more RF among the entire population was twofold less. In nuclear industry workers two RF were found in 22.3%, three and more — in 68.5% [20].

Irradiation in doses of 25 cSv and over in combination with non-radiation RF was peculiar with HRD incidence increase by more than 15% as a result of their interaction and "aggressiveness" potentiation. Study of a rather isolated population of Severst city exposed to long-term low-dose irradiation the amplification of traditional RF "aggressiveness" was also found [17].

Etiological and clinical structure of heart rhythm disorders

HRD analysis depending on etiology indicates that most pronounced disturbances found in HRD were stipulated by postinfarction cardiosclerosis and CHD combination with AH. Number of isolated VE and SVE per 1 study subject per round-the-clock, incidence and number of paired and group VE, supraventricular tachycardia episodes, and diurnal ischemia duration per 1 patient were significantly higher.

By etiology the HRD in clean-up workers have appeared under the CHD in 19.7%, AH — in 7.3%, in CHD combination with AH — in 50.3% ($p < 0.001$). The 16.1% of them had a history of MI. Other etiology factors had make up to 3.9%. Idiopathic HRD was found in 2.7%.

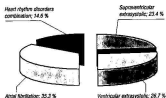


Fig. 12.11. Clinical structure of heart rhythm disorders in Chernobyl accident clean-up workers

Extrasystolia was most prevalent within HRD clinical structure in clean-up workers. Ventricular extrasystolic arrhythmia (EA) was in 26.7% and supraventricular one — in 23.4%. Combination of both types was registered in 14.6% (Fig. 12.11).

EA was found through ECG DM much more often. As the cardiac and general mortality rates were higher in case of the frequent or group VE in CHD and AH patients such HRD should be considered as forerunners of more unfavorable prognosis.

AF mainly its stable form was seen in 35.3% of clean-up workers. In 68% a chronic HF followed it. By prevalence the AF comes second only to EA. Age over 55 years, HF, and postinfarction cardiosclerosis were the predictors of a paroxysmal AF into the stable one. AF incidence in a general population is 0.1%, reaching 5% in persons older than 50 years and 10% after 80. AF is significantly linked to twofold relative risk increase of stroke [21].

Extrasystolic arrhythmia

Electrocardiography features. ECG DM is particularly useful in supraventricular and ventricular arrhythmia identification in clean-up workers. ECG DM in patients having VE is attributed to the A level study evidence according to the European society of cardiology (2009). It can be considered the method of choice in diagnostics of relatively rare EA and in quantitative assay of premature ventricular and supraventricular contractions revealed within ECG registration.

According to our data (Table 12.3) the excess of high-grade arrhythmia types and an increase of VE and SVE per 1 studied case by 57% ($p<0.05$) were the consistent features of EA in clean-up workers vs. non-exposed persons. Maximum number of VE per hour was more by 68%, whereas of paired SVE — by 51.1% ($p<0.05$).

Table 12.3. Extrasystolic arrhythmia features in Chernobyl accident clean-up workers according to electrocardiography daily monitoring

Indices	Groups	
	Clean-up workers having HRD, n=289	Non-exposed persons having HRD, n=82
Ventricular extrasystoles		
Extrasystoles per 1 examined person, M±m	2891±114*	1733.8±78
Maximum of extrasystoles per 1 hour	239.5±10.6*	141.9±14
Paired and group extrasystole frequency, %	51.1	46.7
Paired and group extrasystoles per 1 examined person, M±m	76.7±3.1	77.5±4.2
Supraventricular extrasystoles		
Extrasystoles per 1 examined person, M±m	1380.7±82*	825±62
Frequency of paired extrasystoles, %	62.2	63.4
Paired extrasystoles per 1 examined person	161.2±10.4*	82.4±9.5
Frequency of supraventricular tachycardia, %	51.2	42.7
Supraventricular tachycardia complexes per 1 examined person, M±m	30.8±4.1	28.4±4.2

*difference vs. non-exposed, $p<0.05$

Possible direct link of ventricular arrhythmias including the life-threatening ones to transient ischemia is of particular concern. In our practice the total duration of ischemia and of painless ischemia in clean-up workers suffering HRD was 22.1 ± 2.6 min and 15.8 ± 2.2 min vs. 8.9 ± 1.1 min and 6.3 ± 1.1 min in case of no HRD ($p<0.05$) and vs. 15.2 ± 2.5 min and 11.2 ± 1.2 min ($p<0.05$) in non-exposed persons. Number of diurnal ischemic episodes per 1 patient among the clean-up workers suffering HRD was 2.1 times higher vs. in its absence ($p<0.05$). There were close correlations between the number ($r=0.72$; $p<0.01$), duration ($r=0.74$; $p<0.01$) of myocardial ischemia for round-the-clock and the number of ventricular extrasystoles. LVH was found in all patients suffering episodes of myocardial ischemia. MMI there was 174.4 ± 4.1 g/m² vs. 163.2 ± 3.2 g/m² ($p<0.05$) in its absence. There were correlations between MMI and a number ($r=0.36$; $p<0.05$), duration ($r=0.38$; $p<0.05$) of myocardial ischemia episodes for a round-the-clock. These observations are in agreement with an opinion that myocardial mass augmentation in LVH becomes a substrate for relative insufficient coronary blood supply to myocardium especially under a load.

The heart rate increase in a rest state is an independent RF of cardiovascular disease and sudden death from arrhythmia. Clear link has been found between the heart rate and SCD which incidence was significantly higher in CHD with heart rate at a rest state of 75 min^{-1} and more. Number of patients having heart rate $\geq 75\text{ min}^{-1}$ among the clean-up workers suffering EA was reaching 40%.

According to HRV analysis in clean-up workers suffering EA the significant increase of sympathetic and parasympathetic activity, their dysbalance with sympathetic activity excess were found. Indices characterizing general HRV (SDNN, SDANN) in the clean-up workers suffering HRD vs. group with no HRD were significantly higher by 25.7% and 25.3% respectively. Significant increase of SDNN >170 msec, SDNN-i >90 msec, TP >8000 msec², LF/HF <0.95 Units was associated with VE & SVE diurnal number >3000 , HF signs that makes reasonable to consider these indices as predictors of HRD.

Structure-functional remodeling of left ventricle. LVH in clean-up workers suffering EA was found in 88.6%, in case of no HRD — in 86.6%, in non-exposed persons — in 89%. Severe LVH with $\text{MMI} >170$ g/m² was prevailing in clean-up workers suffering HRD reaching 53.7% vs. 39.5% in no HRD ($p < 0.05$), myocardial thickening over 14 mm was found in 19.4% and 11.7% respectively ($p < 0.05$) (Fig. 12.12). More pronounced LVH was consistent in patients with ventricular extrasystoles predominance.

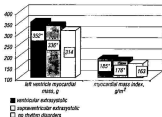


Fig. 12.12. Left ventricle myocardial mass in Chernobyl accident clean-up workers suffering extrasystolic arrhythmia

Left ventricle myocardial mass (LVMM) was exceeding in them the respective values in no HRD by 37.5 g, and its index — by 21.6 g/m² ($p < 0.05$). LVMM was higher in a prevalence of SVE and single VE vs. no rhythm disorders by 21.9 g, MMI — by 13.5 g/m².

There were direct correlations of the VE number with myocardial thickness ($r = 0.33$; $p < 0.05$) and MMI ($r = 0.3$; $p < 0.05$). Diurnal number of VE was twofold higher in severe LVH vs. moderate LVH ($p < 0.05$).

EDD index 31 mm/m² and more indicating to LV dilation and volume overload was found in 14.8% of clean-

up workers suffering HRD vs. 8.7% of no HRD history and vs. 15.8% in non-exposed persons. Diurnal VE number was there 2236 ± 282 being significantly over vs. cases of non-dilated LV 2.3 times ($p < 0.05$). There was a direct correlation of the VE number and EDD index ($r = 0.32$; $p < 0.05$).

The antero-posterior LA size >40 mm was associated with SVE and further — with AF. Diurnal number of SVE in case of LA >40 mm was 1.9 times more vs. in non-dilated LA ($p < 0.05$). A direct correlation of SVE number and LA size ($r = 0.33$; $p < 0.05$) was found.

LV diastolic dysfunction was seen in 72.6% of the clean-up workers suffering HRD vs. 65% in no HRD ($p < 0.05$) and 78% in non-exposed persons. Abnormal relaxation was found in 52.6%, 58.3%, and 48.7% respectively. Pseudonormalization of transmitral flow was seen mainly in severe LVH among the 20% of clean-up workers suffering HRD vs. in 6.7% in their absence ($p < 0.05$) and 29.5% in non-exposed. Diastolic function disorders were more pronounced in case of SVE vs. VE. The E/A ratio in pseudonormal transmitral flow was there 1.44 ± 0.02 vs. 1.33 ± 0.01 in VE ($p < 0.05$). Time

of LV isovolumetric relaxation was less by 24 msec, deceleration time — by 10 msec ($p<0.05$). Number of SVE in diastolic dysfunction was by 39% over the respective value in a normal transmitral flow. Diastolic function disorders and resulted LA hypertension in LVH can induce the AF paroxysms. More pronounced disorders of diastolic function in SVE vs. group with no HRD could be induced by a significant LA dilation, the antero-posterior size of which in pseudonormal type of transmitral flow was 43.2 ± 0.5 mm in SVE vs. 40 ± 0.3 mm ($p<0.05$) in no HRD.

Systolic LV function was not abnormal in majority of examined persons. Ejection fraction less than 45% was found in 5.9% of clean-up workers suffering HRD vs. 10.9% in non-exposed and 1.3% in no HRD. Higher number of extrasystoles was registered including the high-grade VE in EF<45%. There was an inverse correlation between the VE and EF ($r=-0.35$; $p<0.05$).

Through the review of myocardial structure/functional abnormalities in EA depending on their etiology the more pronounced disorders were found in postinfarction cardioclerosis and CHD combination with AH. Severe LVH, concentric type of LV remodeling in EA being stipulated by CHD combination with AH, left myocardial chambers dilation in postinfarction cardioclerosis were characteristic here. There was a significant increase of myocardial thickness and mass, more pronounced disorders of LV diastolic function with pseudonormal transmitral flow in 25–27% of cases. Systolic function disorders were peculiar for the cases of EA stipulated by postinfarction cardioclerosis. More profound abnormalities of cardiac structure and function were associated with a significant increase of isolated VE and SVE number per 1 examined person for around-the-clock, of incidence and number of paired and group VE, and supraventricular tachycardia episodes.

Physical load tolerability was lowered in every third clean-up worker suffering EA. Threshold power (W_{th}) in case of paired and group VE was by 17.3% lower vs. cases of less than 10 extrasystoles diurnally ($p<0.05$). The extent of executed work there was by 34.9% lower, load duration — by 21.9% ($p<0.05$). There was an inverse correlation of W_{th} and SVE number. The clean-up workers having SVE tolerated much lower physical load for a lesser time vs. non-exposed persons ($p<0.05$). Dependence of physical load providing on the disease being an etiology of EA was established. The W_{th} , extent of executed work, and load duration were lower in AH and after the survived MI. The lowest W_{th} value was in the MI survivors in association with AH. It was by 32.8 watts less vs. cases of CHD with no AH ($p<0.01$). W_{th} correlated with and angina FC, HF ($r=-0.52$; $p<0.001$). Age, paired and group VE, angina FC, survived MI, LVH were the meaningful factors of physical working capacity decrease in clean-up workers suffering HRD. Incidence of EA onset under load was 8.3 times higher vs. group with no HRD ($p<0.05$) and 1.3 times vs. non-exposed. Headache, dizziness, and dyspnea were most prevalent among other reasons of loading test interruption.

Atrial fibrillation

The AF occupies 35.3% within HRD structure in clean-up workers. AF symptoms are not pathognomonic.

According to ECG DM data the significant increase of extrasystole number per 1 examined person with prevailing of VSE $\geq$ 500 diurnally, of maximum number of extrasystoles per 1 hour by 36%, of paired and group ventricular extrasystoles per

1 examined person by 145% and of supraventricular ones — by 113% ($p < 0.05$) was registered in clean-up workers suffering a paroxysmal AF vs. the non-exposed persons. Average duration of a single ischemic episode and diurnal ischemia duration were by 35% over the ones in no HRD.

LVH was more pronounced in clean-up workers suffering paroxysmal AF than in no AF; severe LVH was seen in 50%. MMI increase vs. no RCD cases was 14.4 g/m^2 ($p < 0.05$). Myocardial thickening by 14 mm and more was found in 26% of clean-up workers suffering AF vs. 11.7% in no HRD and 10.5% in non-exposed.

LA was dilated in majority (62%) of patients being in 19% associated with dilated LV. Diastolic function was abnormal in 42.9% of patients under the preserved LV systolic function. Changes of absolute values of the latter were exceeding the respective indices in no HRD as in all types of LVDD.

Physical load tolerability was decreased in 42.9% of clean-up workers suffering paroxysmal AF. In association with AH the W_{ex} extent of executed work and load duration were lower. Age ($r = -0.37$; $p < 0.05$), angina FC ($r = -0.52$; $p < 0.001$), LA size ($r = -0.31$; $p < 0.05$), LVH ($r = -0.42$; $p < 0.05$) were the meaningful factors of tolerability decrease towards physical load in the clean-up workers suffering AF. SBP elevation up to the submaximal level (47.6%) was the most frequent cause of load interruption. General fatigue, dyspnea were among other reasons of load test interruption.

Heart rate at a rest state was important in the HF risk assay under the stable AF. In stable AF the average rate of ventricular contractions was by 27.9% over ($p < 0.05$) the values in no HRD. There were 86% of the clean-up workers having stable AF in whom the heart rate was 75 and more per minute being 50.8% more than in no AF. Ventricular contraction rate of 90 per 1 minute was registered in more than a half of the examined persons. ST-segment displacement for more than 1 mm below the isoline was registered in 12% vs. 7.5% in no HRD.

LVH was diagnosed in all clean-up workers suffering stable AF vs. 91.9% in no HRD. Severe LVH was present in 76.2%. MMI exceeded the values in no HRD group by 28.6 g/m^2 ($p < 0.05$). LV hypokinetic wall motion was found in 56% of clean-up workers suffering stable AF. Portions of wall dyskinesia appearing in focuses of ischemia and cardiosclerosis can be the source of thrombus formation resulting in an increase of cardioembolic stroke risk.

LA cavity increase over 40 mm was registered in 92.5% of clean-up workers suffering stable AF vs. 31% in a group with no HRD. LA cavity increase preceded the LV cavity increase in 33 clean-up workers suffering stable AF. Average antero-posterior size of LA in clean-up workers suffering AF was reaching $47.7 \pm 0.8 \text{ mm}$. LA dilation can be both the source and the consequence of AF. LA cavity increase and LVMM were considered being independent RF of AF in AH. AH in 76.8% of the clean-up workers suffering AF indicated to the leading role of AH along with CHD in its genesis.

Compensatory role of LVH in AF especially under the insufficient heart rate control was quickly exhausted and soon the cardiac output was provided by the LV cavity dilation. Late remodeling of LV was accompanied by a progressing dilation of its cavity and eccentric hypertrophy development in 56% of examined persons. LV dilation was in 82% of clean-up workers suffering stable AF and an eccentric LVH vs. 16% ($p < 0.05$) in a concentric LVH.

Systolic dysfunction with EF < 45% was found in 25% of clean-up workers suffering stable AF. Statistically significant EF decrease by 17.6% and ΔS — by 23.1% ($p < 0.05$) were revealed vs. group with no HRD. There was a reason to believe that cardiac systolic function disorders were an independent predictor of thromboembolic complications in case of AF on non-valvular origin.

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CHAPTER 13 THYROID GLAND AND METABOLISM

13.1 INTEGRATION OF IMPACTS AND DAMAGE LEVELS

Despite 24 years have passed since the Chernobyl NPP accident the vast amount of problems related to its health consequences not just remains important but has become even on the front burner. Instead of an expected fade of thyroid disease splash among the various population groups of survivors the consecutive increase of incidence occurs according to the annual statistics of morbidity and mortality [1]. Moreover endocrine disorders possess in general a significant place within the entire morbidity structure of the survived population of Ukraine. And just the system of internal secretion organs and specifically its disorders in a great measure impact the quality of life and life span of the patients. It is stipulated by a tricky morphology of endocrine glands and multilevel nature of its interaction pathways with other functional systems, both with actually a part in regulation of vital functions [2].

The Chernobyl NPP accident in 1986 was followed by an environmental release of numerous chemical elements avid to several organs of endocrine system. Both short- and long-living isotopes (e.g. ^{131}I , ^{90}Sr , ^{137}Cs , ^{226}Ra , ^{238}U , ^{239}Pu etc.) exerted the damage of central and peripheral endocrine organs that had predisposed to the transient or long-term remote disorders of their function. The above named radionuclides upon incorporation appeared being the main source of radiation exposure. Their detrimental effect was amplified by the external γ -irradiation. The absorbed radiation dose was partly received within few days or months after the accident, whereas the other one – for years that makes difficulty to estimate its negative impact.

In general it is worth keeping in mind that it is unreal to foresee and survey all the pathways of radiobiological effects at a level of multicellular systems, as each of the sub-systems has the specific sensitivity and individual dependence on dose of radiation [3].

There are three groups of responses to radionuclide incorporation: physical ones, biophysical processes and general biological abnormalities. Physical reactions occur during all the time of radionuclide presence in tissues. The same is peculiar to biophysical events. Then tissue and cell general biological abnormalities occur and are observed not only during radionuclide stay in human body but for many years upon that [4]. The well-known radiation effects and mediated reactions are the source of damaging radionuclide effects. Radiation damage evolves in systemic illnesses if numerous physiological systems and structural levels are involved [4].

Among the population survived after the Chernobyl NPP accident the most prevalent diseases are thyroid ones (40–52%), obesity and body mass excess (52% and 33% respectively), diabetes mellitus (DM) (4–6%), metabolic syndrome X (MSX) (30–40%), osteopenic syndrome (22%), and reproductive disorders (40–45%), whereas in the entire population of Ukraine the respective incidence and prevalence values are much lower according to the files of Ukrainian Ministry of Public Health [2, 5, 6]. This situation is the results of a complex impact of negative factors of the accident, and firstly of the radiation. In a comprehensive view the integrated impact of external γ -exposure and an internal radiation from incorporated radionuclides had injured the various branches of humoral regulation both of central endocrine organs (secondary and thirdly regulation levels) and peripheral endocrine organs and tissues (primary level). Radiation injury there occurred through activation of genetic predisposition under combined impact of environmental hazards.

Environmental deficiency of some microelements is historically known for the most part of Europe and also in Ukraine. Two such microelements i.e. iodine and selenium are involved in thyroid metabolism, and their deficiency leads to iodine-deficient diseases, namely a diffuse goiter when thyroid cellular mass increases to absorb more iodine. Under such circumstances the thyroid of survivors was capable to accumulate a lot of radioactive iodine within a few months upon the Chernobyl accident in 1986. Such accumulation appeared being much more vs. persons with a sufficient iodine supplementation before the accident. Excessive incorporation and organification of radioactive iodine resulted in severe exposure of thyroid. Children were at that the most critical group of survivors as more intensive metabolism is peculiar to them in general [2].

AS it was mentioned above the estimate of an unfavorable effect of radiation after the Chernobyl NPP accident is a challenging issue as the doses were absorbed within both months and years. The most pronounced abnormalities in the immediate days and week after the accident were found in thyroid because of extra high radioiodine content in the air. Also the mentioned iodine deficiency had predisposed to intensive radioiodine accumulation with resulting destruction of thyrocytes. This event has provoked the onset of an acute thyroiditis in Chernobyl NPP accident clean-up workers who had received high radiation doses (over 1000 mSv). A bit later the immediate launch of thyroid radiation effects occurred featuring thyroiditis and later the malignant thyroid neoplasms emerged. The long-term transient euthyroid hyperthyroxinemia was the effect within first 3–5 years in other survived persons having 250–1000 mSv thyroid doses. Later it resulted in predisposition to the chronic thyroid disease, namely first of all to thyroiditis and nodular goiter, and other endocrine disorders.

Radiation had damaged the cellular genetic apparatus resulting in chromosome instability, somatic mutations and as a consequence in abnormal reparation of the exposed cellular populations, inability to synthesize an adequate amounts of hormones and other biologically active compounds, and finally in cellular death and connective tissue proliferation [7]. More over cellular membrane abnormalities emerged followed by abnormal receptor interaction of hormones, intracellular physiological pathways predisposing to hormonal tissue resistance. Unfavorable impact of Chernobyl accident factors on the endocrine system had led to hormonal disorders in energy pathways regulation of body mass, to abnormal behavioral reactions, increase of body mass excess cases, oxidative

stress with resulting dyslipidemia, DM and other abnormalities (see later). Some of such consequences are systemic ones namely the insulin- and leptin-resistance syndromes, featuring an aggressive course, resistance to management and association with clinically significant cardiovascular (arterial hypertension, coronary heart disease), neuropsychiatric (dyscirculatory encephalopathy, psychoorganic syndrome) and other disorders.

Further 5 years (1992–1996) the risk of thyroid disease in Chernobyl NPP accident survivors had increased about nine-fold and of DM — about two and a half times. Annual rate of endocrine disease incidence in clean-up workers was about 3–5-fold higher vs. the adult population of Ukraine in a whole. Some kind “rejuvenation” of such disease occurs being otherwise typical for the older people. According to the SRU files for 68145 persons surveyed in 1988–2009 the incidence of non-cancer thyroid disease was prevalent through the chronic (autoimmune) thyroiditis, nodular goiter, and primary (acquired) hypothyroidism (Fig. 13.1). Prevalence of chronic thyroiditis since 1997 continued to increase in clean-up workers, whereas in Kyiv city population it remained stable (figures 13.2, 13.3). The most robust increase of thyroid disease prevalence was registered in clean-up workers who had been less than 20 in 1986. External (total body) radiation doses within 0.25–1 Gy range are recognized the significant factor of chronic thyroiditis and nodular goiter genesis in clean-up workers of 1986–1987 service period and population evacuated from the 30-km zone.

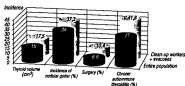


Fig. 13.1. Incidence of thyroid disease in clean-up workers of 1986–1987 service period and evacuees from the 30-km zone (2006 year data) vs. an entire population of Ukraine not exposed to radiation

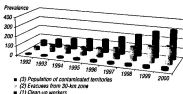


Fig. 13.2. Prevalence of chronic thyroiditis in Ukraine among the Chernobyl NPP accident survivors of various age (Ministry of Public Health files, adults and children, values per 10000 population)

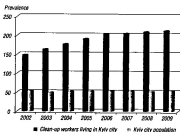


Fig. 13.3. Prevalence of chronic thyroiditis in Chernobyl NPP clean-up workers and Kyiv city population (adults and children, values per 10 000 population)

Parenchyma of endocrine organs is regarded as low (slowly) renewable tissue type. Cellular population there are considered relatively resistant to a direct radiation impact (except high radiation doses) but are radiosensitive for the remote effects.

Data on radiation effects on other endocrine glands and tissues have been recently (1997–2010) received by the staff of SCRM. Such kind of research was not made before because of specific difficulties [8]. Progress in recognition of hormonal interaction pathways highlighted the new aspects of the Chernobyl NPP accident unfavorable factors impact in health. Just reviewed in details further here obesity and body mass excess, MSX, and osteopenia are of enough relevance in clean-up workers.

13.2 CENTRAL DISORDERS OF ENDOCRINE REGULATION

According to the cutting edge data the abnormal hormonal regulation of energy metabolism is found in survivors having body mass excess [5, 8]. Specific disorders of cerebral regulation of neuroendocrine and motivation processes, adipocyte-hypothalamic information interactions with neurochemical (hormonal) dysfunction resulting in abnormalities of eating behavior, psychological state and motivation are of especial concern. Serum content of the α -melanocyte-stimulating hormone (MSH) produced by cerebral structures is normally (in persons not exposed to Chernobyl NPP accident factors) elevating proportionally to body mass increase followed by serum content rise of leptin synthesized by adipose tissue. This is a physiological reaction focused on a decrease of food consumption in overweight. However under the same circumstances but in Chernobyl NPP accident survivors having body mass excess or obesity and even more pronounced hyperleptinemia the MSH secretion is unchanged (Table 13.1) [5]. As MSH is an important component of melanocortin system tightly involved in energy adaptation pathways the described abnormality is a marker of energy supplementation disorders of hypothalamic-pituitary complex in survivors [5]. These disorders in turn appear as an insufficiency (or an absence) of peripheral hormonal stimulation (insulin and leptin) on neuroendocrine structures of arcuate nuclei and, consequently – as no expression of pro-opiomelanocortin (POMC) and cellular resistance to POMC in hypophysis. All that leads to excessive accumulation of adipose tissue, appearing of an abdominal and/or total obesity, and MSX onset followed by a wide range of the respective hormonal/metabolic disorders [5].

Table 13.1. Serum α -melanocyte-stimulating content hormone depending on body mass index

Studied parameters	Chernobyl NPP accident survivors, age			Subjects being out of the accident, age		
	18.5-24.9	25-29.9	≥ 30	18.5-24.9	25-29.9	≥ 30
Number of persons	36	69	137	35	41	109
Average BMI value	22.84 $\pm$ 1.56	27.71 $\pm$ 1.31	33.93 $\pm$ 3.03	21.23 $\pm$ 3.49	28.49 $\pm$ 1.02	35.02 $\pm$ 4.04
α -MSH (pmol/L)	16.37 $\pm$ 13.07	13.72 $\pm$ 22.63	15.04 $\pm$ 19.50	9.68 $\pm$ 7.14	18.17 $\pm$ 15.32	25.14 $\pm$ 29.91

BMI – body mass index, kg/m²; α -MSH – α -melanocyte-stimulating hormone. Values presented as Mean

Several unfavorable environmental factors have had an impact on the reproductive system in survivors. For example, menstrual cycle disorders increased 2–3-fold in females. Intensification of menses was typical within first few years upon the accident, whereas clinical cases of oligomenorrhea and amenorrhea, infertility and polycystic

ovary syndrome became more frequent 5–6 years later. Incidence of various grade erectile dysfunction had increased dramatically (by 60–70%) in men. Initially within the very first years after the accident the serum sex hormones and pituitary gonadotropine content was increased followed than by a decrease of their concentrations to lower threshold levels against the background of pituitary dysfunction onset (increase of basal levels of follicle-stimulating and/or luteinizing hormones). Occurrence of a precocious hypogonadotropic hypogonadism was and is the final in some of such cases. Cases of prolactin concentration increase or lactation onset under its normal concentrations were also found.

Along with the non-stochastic effects of the unfavorable environmental factors a robust increase of the stochastic disease (cancer) of endocrine tissues was revealed in Chernobyl NPP accident survivors after the accident. Detailed description of that can be found in many scientific papers [9, 10].

So, a staged development of non-stochastic endocrine disease corresponding to pathophysiological alterations in the central and peripheral parts of endocrine system was surveyed after the Chernobyl NPP accident (Table 13.2).

Table 13.2 Non-stochastic endocrine disease after the Chernobyl NPP accident

Period	Stage	Time interval	Reactions, processes, diseases
0 (initial)	Functional strain resulted from a qualitative alimentary deficit	before 26.04.1986	Hyperplasia of endocrine (hormone-producing) cells predisposing to excessive avidity of isotope accumulation
1	Primary reaction to altering agents (unfavorable accident factors)	26.04.1986–08.1986	Increase of peripheral hormone concentrations in blood as a result of partial cellular damage
2	Compensated hyperproduction of peripheral hormones	09.1986–1989	Intensification of peripheral hormone synthesis against an absence of reaction of central regulation i.e. disorders of feed-back links due to lack of releasing factor and tropic pituitary hormone synthesis
3	Subclinical disorders of endocrine tissue function	1990–1995	Recovery of central regulation of hormone synthesis, decrease of functional capacity of peripheral endocrine tissues, onset of subclinical thyroid disease and other endocrine organs
4	Clinical presentation of radioinduced endocrine disease	1996–2010	Splash of endocrine system disease against a serious decrease of peripheral endocrine tissue functional capacity and disorders of central hormonal regulation

13.3 PERIPHERAL DISORDERS OF ENDOCRINE REGULATION

Experimental data are available on a functional state of pancreatic islet β -cells after the high-dose and lethal-dose total external γ -radiation inducing an acute radiation syndrome (ARS) of various grades. Beta-cell incretory activity becomes increased with extreme abnormalities linked to periods of primary reaction and eruptive phase of ARS [11]. Under a short-term exposure to relatively low doses the staged changes of insulin

production occurred i.e. its production decrease early upon exposure and increase after a while followed by a postponed normalization. Increased insulin production in an exposed person is considered a result of excessive release of contra-insulin hormones: glucocorticoids, adrenocorticotrophic hormone, somatotropin, angiotensin II, norepinephrine, and free fatty acids.

In its turn after the 5 and 15 Gy local irradiation of abdominal part of male rats the DM is a remote in time radiation disease (dose-dependent onset) along with an increased pancreatic cancer risk [12]. The staged changes of blood insulin levels after a single-time 1 Gy γ -irradiation are explained in a few experimental works [13, 14]. The decreased by 23% insulin content was found in isolated islets of Langerhans under glucose concentration 5 mmol/L in a medium and by 20% in case of 15 mmol/L respectively after a month upon exposure. Seven months after the irradiation an increase of insulin content occurred in the islets under the same glucose content in a medium. Normalization of indices were found only at the end of 12th month. The exposed animals were not different from non-exposed ones by any other parameters for the entire time of survey. Such deviations of insulin concentrations i.e. level lowering in 1 month were observed in rats upon the internal irradiation with 0.3 Gy absorbed dose. The profound decrease occurred at the background of simultaneous serum corticosterone elevation. A bit increase of insulin level was observed also in rats upon 40 days since 5 Gy total irradiation against serum corticosterone excess and moderate lowering of thyroxine and epinephrine concentration both with significant hypotestosteronemia. Hyperinsulinemia appeared in animals just after a 0.1–0.5 Gy total irradiation. Hormone concentration decrease was observed after 5 Gy irradiation. Epinephrine elevation increased only after 2–5 Gy of radiation.

Disorders of pancreatic endocrine function under low radiation doses were scarcely studied, and post-radiation hyperinsulinemia pathway is not clear [15]. Increased sensitivity to low radiation doses was registered in mice with hereditary predisposition to DM [16]. DM appears in beagle puppies with no hereditary predisposition to the disease upon exposure to integral radiation doses up to 88.3 cGy in a range of time since birth (up to 1 year) [17].

13.4 PERIPHERAL ENDOCRINE AND METABOLIC DISORDERS

Carbohydrate and lipid metabolism

Even a low-dose ionizing radiation is known from experimental studies as resulting in abnormal hormone interaction at a membrane level in the target tissues

Increased DM incidence appeared being a milestone finding in Chernobyl NPP clean-up workers and 30-km zone evacuees after the accident [18]. Key role of insulinresistance (InR) in DM pathogenesis clearly indicates at that to its importance under and after the radiation exposure. Data on pancreatic dysfunction in human under InR impact are available in few scientific papers being relevant to high-dose exposure. InR and hyperinsulinemia were found in persons exposed to head radiotherapy in childhood for brain cancer. Decrease of a spontaneous insulin secretion under the arterial hypertension (AH), hypercholesterolemia due to low-density lipoproteins (LDLP) in

combination with obesity and glucose intolerance (GII) were observed in such subjects too [19]. Hyperinsulinemia against the background of normal glycemia was found in its turn among persons 5–18 years old after radiotherapy in acute or chronic leukemia [20]. Mentioned abnormalities are considered a result of local high-dose irradiation despite none of patients acquired the ARS.

Noteworthy that an endogenous hyperinsulinemia against relative lowering of C-peptide content was found in children with body mass excess being explained by a decreased insulin clearance, and also in kids with normal body mass but a point gene mutation responsible of proinsulin to insulin conversion. Such mutations can occur as stochastic effects after radiation exposure. Moreover some data on insulin levels and insulin receptor function, long-term increase of contra-insulin hormones where hypercortisolemia was found even 10 years upon the Chernobyl NPP accident, both with cellular membrane and membrane-binding structure abnormalities resulted from oxidative stress after radiation brings the opinion of abnormal hormonal sensitivity including one to insulin under such circumstances.

Examination of 5–12 years old children for a long time residing on radiation-contaminated territories after the Chernobyl accident revealed a hyperinsulinemia in both boys and girls along with hyperandrogenia in latter. Noteworthy that hyperinsulinemia in childhood is a key risk factor of MSX in adulthood. Moreover serum content elevation of prolactin and aldosterone, lower daily urine excretion of catecholamines and signs of hypocorticism are also found in such cases. Similar abnormalities of function of hormonal system of adaptation expressed among other through a prolonged hypercorticism were found in experimental animals upon a low-dose radiation. All mentioned hormones are contra-insulin ones and their long-term elevation was obviously involved in the insulin secretion abnormality.

It is a principal point that obesity is a InR marker. And insulin sensitivity in human is decreased by up to 40% in case of an individual body mass excess of 35–40%. Obesity and body mass excess issue is rather important in general in persons exposed to radiation. Just the MSX syndrome according to Reaven's criteria implying an android obesity, was found in 48.1% of the randomly selected Chernobyl NPP clean-up workers according to the data by RCRM. Here is noteworthy the fact that MSX incidence within a long-term survey had increased from 15% in 1990–1995 to the mentioned above value in 1996–2006. Basal concentrations of insulin, C-peptide, glucose, triglycerides increase along with other abnormalities of hormonal homeostasis i.e. thyroid-stimulating hormone (TSH), leptin, calcitonin (CT), cortisol content elevation and total thyroxine, parathyroid hormone (PTH) and testosterone lowering and of carbohydrate/lipid metabolism within MSX genesis. Therefore the issue of obesity in general and of MSX in particular is clearly important in Chernobyl NPP accident survivors. The etiopathogenetic role of radiation is of especial concern here. Health disorders in children of parents exposed to radiation after the Chernobyl accident can be stipulated by some neuro-endocrine abnormalities at a hypothalamo-pituitary-thyroid level that can finally lead to endocrine organ diseases.

Obesity is widely prevalent in the acute lymphoid leukemia (ALL) patients. Head exposure, female gender and hereditary predisposition are the risk factors here. The phenomenon of leptin insensitivity, growth hormone (GH) deficiency, damage to the

central nervous system zones responsible for appetite regulation and eating behavior are worth attention here among a wide range of pathways proposed to explain the obesity prevalence upon head irradiation. Significant limitation of body mass excess in remote term after radiation therapy is reported in cease of a synthetic GH analog octerotide administration. The absolute values of non-fat body tissues in young adult ALL patients receiving head radiation therapy were comparatively less vs. of age- and BMI-matched controls. Serum leptin content at that was significantly higher. Coefficient of a leptin concentration per unit of adipose tissue mass was higher vs. control values with high difference depending on serum GH concentration. It means the reversed dependence on highest values calculated in case of most pronounced GH deficiency. Hyperleptinemia and its link to GH abnormalities is considered a result of radiation damage of hypothalamic structures.

Experience of administration of GH preparations in case of its deficiency indicates to a close link between the leptin and GH synthesis/effect. An average GH level to some extent defines the leptin content probably through modification of the adipose tissue mass and its distribution. Replacement GH therapy in growth-retarded children is followed by the leptin level lowering, at that its concentration decrease correlated with growth pattern within first year of treatment. According to another study results the relative risk of obesity after head radiotherapy with over 20 Gy dose was 2.59 vs. control in females (95% CI = 1.88–3.55) and 1.96 in males (95% CI = 1.33–2.57) from 1765 adults who survived after the childhood ALL and 2565 adult siblings matched by age and ethnicity. Relative risk of obesity was the highest in females when the ALL had been diagnosed at age of 0–4 years (average 3.81; 95% CI = 2.34–5.99). Obesity risk was not linked to chemotherapy. Such an effect is a consequence of the age-dependent radio-induced hypothalamic-pituitary disorders. It means that ionizing radiation in younger age distorts the leptin receptor sensitivity in ventromedial parts of growing and developing hypothalamus.

Studies are important here as relatively lower doses of head radiation therapy in childhood leukemia were linked according to regression analysis to the higher incidence of obesity (15%) vs. 7% in higher absorbed doses. Obesity was defined by the BMI standard deviation score (BMI-Z-score) calculated with taking into account the BMI distribution asymmetry depending on an age (BMI-Z >2 corresponds to an obesity, BMI-Z >3 means a severe obesity).

Decrease of insulin, C-peptide and calcium-regulating hormones concentration along with corticotropin, cortisol and prolactin elevation were found 8–12 weeks after the Chernobyl NPP accident in exposed children.

Incidence increase of disease associated with endothelial dysfunction i.e. type 2 DM, arterial hypertension, coronary heart disease (CHD), discirculatory encephalopathy (DEP) and chronic cerebrovascular disease were surveyed in parallel with body mass excess in Chernobyl NPP accident survivors. There was a dependence on age too.

Cardiovascular disease is a most serious disorder in females that is much dependent on body mass and to some extent on the age, whereas cerebrovascular and thyroid disease is mainly associated with age. Incidence of estrogen-dependent disease appears higher along with body mass excess in females featuring metrofibroma (up to ~9% of cases) which development is of low dependence on an age.

Type 2 DM risks among various population groups are higher in females and depend on a body mass according to the results of UKPDS (United Kingdom Prospective Diabetes Study), HDS (Hypertension in Diabetes Study), FIELD (Fenofibrate Intervention and Event Lowering in Diabetes) and some other studies. Among the Chernobyl NPP clean-up workers such a dependence on body mass and age was found both in men and women. Higher type 2 DM incidence in females (4.43%) vs. males (3.24%) corresponds to a typical pattern of distribution in the entire population of Ukraine (6/4 ratio).

Disease which origination can be linked to radiation in Chernobyl NPP accident survivors have been identified through the compare of indices to respective values in the compare group (Fig. 13.4). The highest risks are demonstrated (except thyroid disease) for type 3 DM, CHD and DEP.

Excess of body mass in clean-up workers makes the most robust impact on such dependency onset because MSX besides the arterial hypertension, free fatty acids elevation and hypercoagulation is followed by dyslipidemia, hypertriglyceridemia and atherogenous cholesterol of low-density and very low-density lipoproteins. Such abnormal blood lipid profile predisposes to a further InR exacerbation, intensification of endothelial damage that leads to more severe arterial hypertension to which the females are more resistant. Precisely these negative processes develop just under disorders of lipid metabolism (dyslipidemia) and excessive level of the free fatty acids and triglycerides that was found in adult study subjects (Tables 13.3, 13.4).

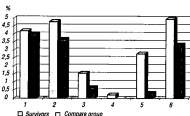


Figure 13.4. Incidence ratio of the most prevalent disease revealed in the Chernobyl NPP accident survivors and in the compare group 1 — diabetes mellitus, type 2; 2 — autoimmune thyroiditis; 3 — hypothyroidism; 4 — thyroid cancer; 5 — coronary heart disease; 6 — discirculatory encephalopathy

Triglyceridemia patterns were significantly dependent in survivors on body mass, being prone to exacerbation in females. The latter is probably linked to menopause and dramatic decrease of estrogen synthesis (estrogens exert anti-androgen properties).

Moderate acceleration of RBC sedimentation was found in survivors that under no infection or other source can indicate the inflammation process activation being related to alternative free fatty acids and cytokine level elevation namely the eicosatetraenoic (arachidonic) acid and tumor necrosis factor- β (TNF- β) due to their accelerated release from adipose tissue at the background of body mass excess (proinflammatory state as a sign of MSX).

InR as a MSX pathogenetic basis was followed in survivors by disorders of carbohydrate metabolism i.e. excessive glycemia (7.3 ± 5.1 mmol/L in males and 8.2 ± 3.7 mmol/L in females) that was not significantly linked neither to age nor to body mass.

Table 13.3. Comparison of lipid metabolism indices in survivors having various body mass (Mto)

Indices	BMI 16.1-24.9 kg/m ² (1)	BMI 25-29.9 kg/m ² (2)	BMI ≥30 kg/m ² (3)	t P _{1,2}	t P _{1,3}	t P _{2,3}
	Females					
n	19	28	41			
Total cholesterol, mmol/L	5.4±1.8	5.6±1.5	6.5±1.6	1.04 0.32	2.63 0.01	2.18 0.02
β-lipoproteides, U	51.9±16.8	54.2±14.4	69.3±25.8	2.84 0.01	0.78 0.44	1.55 0.13
Triglycerides, mmol/L	0.4±0.1	1.4±0.1	1.8±0.8	2.79 0.01	2.65 0.01	2.78 0.02
Indices	Males					
n	17	32	43			
Total cholesterol, mmol/L	6±1.5	9.7±4.2	5.9±1.1	0.324 0.75	0.94 0.37	1.38 0.18
β-lipoproteides, U	59.2±19.9	53.3±19.8	62.3±17	1.044 0.33	0.41 0.69	1.03 0.31
Triglycerides, mmol/L	3.0±0.1	1.7±0.1	2.1±0.7	2.45 0.02	2.84 0.01	2.45 0.03

P_{1,2}, P_{1,3}, P_{2,3} – significance of difference between survivors having various body mass

Table 13.4. Comparison of lipid metabolism indices in survivors of various age (Mto)

Indices	Age years				t P ₁	t P ₂
	34-49		≥50			
	females	males	females	males		
Number of survivors	48	45	42	47		
Total cholesterol, mmol/L	6.0±1.6	8.1±4	6.3±1.7	6.1±1.1	2.82 0.08	0.07 0.73
β-lipoproteides, U	60.6±24.6	59.2±22.1	67.3±24.9	55.4±17.6	0.92 0.3	0.16 0.39
Triglycerides, mmol/L	1.7±0.9	2.1±0.7	2.4±0.3	1.7±0.1	2.53 0.03	0.14 0.52

P₁ – significance of difference between females of various age groups; P₂ – significance of difference between males of various age groups

Carbohydrate metabolism was abnormal in clean-up workers having InR in a vast majority of cases. InR grade defined by HOMA index both with C-peptide serum level significantly ($r=0.32$; $p<0.05$) dependent on body mass being lower in females (InR was not found in normal body mass). Serum leptin was more elevated in females suffering InR followed by endogenous insulin level and HOMA value increase.

There is a dissociation between insulin and C-peptide concentrations despite both are parts of a proinsulin molecule undergoing splitting to insulin and C-peptide in equimolecular quantities i.e. both compounds have one precursor. Phenomenon can't be explained in particular by autoimmunity, as the meaningful titer of insulin antibodies was found only in few patients. We consider possible difference of metabolic degrading of both molecules.

Bone metabolism

Epidemiological study results have indicated some peculiarities of clinical course of bone-muscular disease in Chernobyl NPP personnel and persons of various age exposed to low-dose ionizing radiation. Mineral density indices of trabecular and compact bone tissue components according to the photon densitometry are significantly lower in persons exposed to radiation vs. respective values in control indicating to meaningful prevalence of osteopenic syndrome among the examined subjects. Clinical and case history data on bone-muscular system state in the study cohort have revealed a significant increase of spinal osteochondrosis and large joints arthrosis. Some features of clinical course of spinal osteochondrosis as mainly vegetative-visceral and neuro-reflective syndromes both with migrating bone pain in large joints and tubular bones. Disorders of calcium and phosphorus homeostasis as various severity D-vitamin deficiency and hypoparathyroidism signs were found in Chernobyl NPP clean-up workers by Belarusian researchers. Stable cephalgia, cognitive disorders, pain in limbs and joints were the most prevalent complaints.

Dividing cells i.e. pre-osteoblasts and osteoblasts are under the highest risk of radiation damage. These cells produce and secrete to intercellular space the collagen fibril polymers on which surface the mineralizing takes place. Osteoblast dysfunction leads to slow-down of hydroxyapatite crystal nucleation. Some abnormalities of hydroxyapatite crystal in solid tooth tissue and alveolar ridge were found in persons having the high absorbed radiation doses. Abnormal ratio between the phosphate- and carbonate anions in favor of the latter against relative increase of amide groups stipulating lower durability of crystal structure was found. Accelerated elimination of the phosphate anions from bone leads to increase of urine pyrophosphate content indicating the systemic process.

Complex of hormonal-metabolic disorders found in the Chernobyl NPP clean-up workers in proximate years after the accident can be considered an adaptation-compensatory response to radiobiological stress. Genetically specified norm of reaction is of evolutionary-historical nature and human organism not always can adequately react to new rates of environmental change that becomes an important factor of radiation disease onset. In response to the stable radiation osteopenic syndrome genesis the compensatory laboratory hypoparathyroidism and calcitonine excess occurs against the background of hypercortisolemia and elevated serum estradiol content. The response is focused on preservation of mineral component of bone matrix and maintenance of stable serum calcium and phosphorus concentration.

Onset of an osteopenic syndrome and its severe form i.e. the osteoporosis in Chernobyl NPP accident survivors in remote terms is linked to the phenomenon of decrease of bone mineral density and resistive characteristics, premature bone aging more pronounced in young and mid-aged persons resulted from combined impact of total external irradiation and local effect of osteotropic and myotropic radionuclides i.e. ^{90}Sr , ^{134}Cs , ^{137}Cs . Hormonal dysregulation are known being of an important role in abnormal osteogenesis featuring an osteopenic syndrome origination.

According to the newest data the significant progressing decrease of serum parathyroid hormone content along with calcitonin elevation was found in Chernobyl NPP clean-up workers (Table 13.5).

Table 13.5. Basal serum concentration of hormones in almost healthy men (1), in clean-up workers with absorbed radiation doses of 0.5–1 Gy in 1986–1990 (2), in 2002–2005 (3), and in ARS convalescents in 2002–2005 (4) (M±m)

Hormones	1	2	3	4
Parathyroid hormone, ng/L	54.0±1.12 (111)	20.78±2.71* (63)	3.97±0.47* (75)	4.87±0.78* (21)
Calcitonin, ng/L	2.48±0.13 (140)	5.42±0.98* (23)	8.75±0.63* (64)	12.39±3.29* (20)
Cortisol, nmol/L	245.0±17.1 (20)	533±33.0* (37)	321.19±17.12* (65)	371.82±44.36* (17)
Insulin, pmol/L	63.2±14.3 (25)	128.6±8.7* (72)	68.3±5.0 (71)	61.5±7.6 (17)

Number of examined subjects is given in brackets; * — significant difference vs. almost healthy subjects.

Analyzing the PTH and CT concentration abnormalities one can put forward an opinion that a stable compensated hypoparathyroidism aimed on preservation of the bone mineral density (BMD) occurs in clean-up workers after the impact of radioenvironmental factors of the accident. It is recognized that decrease of PTH production and secretion predisposes to limitation of bone matrix resorption and calcium ions release to blood. Excess of CT secretion also slows down the resorption of bone tissue, stimulates biosynthetic activity of osteoblasts and osteocytes, and intensifies bone deposition of mineralized calcium. Residual moderate hypercortisolemia 18–19 years upon exposure to radiation can to some extent depress the bone tissue remodeling due to decrease of intestinal calcium reabsorption and intensified urinary excretion. An additional point is that cortisol interaction with cytoplasmic glucocorticoid receptors of osteoblasts leads to slow-down of protein and collagen synthesis and depression of RNA synthesis. One should bear in mind that elevated cortisol levels are still registered in clean-up workers even in remote terms upon radiation, being however significantly lower vs. in the nearest years after the accident. The

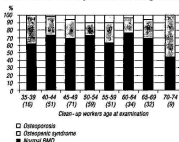


Fig. 13.5. Incidence of osteopenic syndrome depending on age of the Chernobyl NPP clean-up workers (number of patients in each group in brackets)

revealed long-term abnormalities of PTH and CT secretion also in remote terms after the external radiation (and possibly as a result of chronic impact of osteotropic radionuclides) remain being a reflection of stable systemic compensation focused on preservation of mineral and organic component of bone tissue i.e. on retardation of osteopenic syndrome. The latter arises as a result of energy deficit stipulated by expenditures due to an increased strain of several physiology systems and processes in exposed organism.

BMD decrease ($T < -1$) was found in ~31% of clean-up workers having 0.5–1 Gy absorbed radiation doses, with osteoporosis ($T < -2.5$) been found in ~6.5% of them. Osteopenic syndrome and osteoporosis were diagnosed in 27.5% and 5.8% respectively of various severity grade ARS convalescents. There was no therefore any difference of osteopenia and osteoporosis incidence between the named groups, just as of an average bone stiffness (STF), i.e. BMD was not dependent both on the level of an absorbed radiation dose and an age of survivors (Fig. 13.5).

Noteworthy that there was neither any correlation between BMD and PTH or CT in serum of clean-up workers, nor between BMD and BMI. Calcium, phosphorus, and magnesium concentrations were at that normal both under again normal or abnormal BMD indicating to the strong phylogenetically established homeostasis maintenance of these elements (Table 13.6).

Table 13.6. Serum concentrations of total calcium, non-organic phosphorus and magnesium in patients having normal bone mineral density (1), osteopenia (2), and osteoporosis (3) ($M \pm m$)

Parameter	Groups			p_1	p_2
	1	2	3		
Calcium, mmol/L	2.29±01 (215)	2.27±02 (76)	2.34±04 (19)	>0.5	>0.1
Phosphorus, mmol/L	0.99±01 (186)	1.01±02 (67)	1.07±04 (19)	>0.5	>0.5
Magnesium, mmol/L	0.92±01 (91)	0.93±02 (34)	0.89±03 (13)	>0.5	>0.25

p_1 – significance of difference between Groups 1 & 2, p_2 – between groups 1 & 3, number of examined persons is given in brackets

Osteopenic syndrome was diagnosed in ~10% of females and in ~4.5% of male subjects among the Chernobyl NPP clean-up workers having relatively low absorbed radiation doses and among the residents of radionuclide-contaminated territories having annual radiation dose over 5 mSv. Noteworthy women were more prone to disorders of phosphorus/calcium metabolism with the respective prevalence value of 5–10%. However according to the ultrasound densitometry data an abnormal bone Stiffness index was more frequently (~20%) found in females to a considerable extent depending on age and body mass (Tables 13.7, 13.8).

Noteworthy the obese female survivors have normal bone stiffness ($85.3 \pm 13.3\%$) whereas women with lower BMI values are peculiar with a progressing decrease of a stiffness (to $72 \pm 9.9\%$ & $68.5 \pm 16.7\%$ respectively, $p_{1,3} < 0.05$ & $p_{2,3} < 0.05$). The lowest speed of ultrasound was found in the normal body mass females (101.2 ± 11.5 dB/MHz) proving the protective role of a body mass excess on osteopenic syndrome genesis due to elevated estrogen content produced by adipose tissue. Estrogen excess maintains the enough calcium content in a bone (especially upon menopause). Impact of other factors in body mass excess is also possible. Similar trend is peculiar for males having relatively low absorbed radiation doses.

So the low BMI value is an important risk factor of osteopenic syndrome, whereas its elevation particularly to the obesity levels can be of compensatory or protective nature that corresponds to our study results. The latter demonstrate a significant correlation

between leptinemia level and a bone tissue mass in females with no however any link between leptin concentration and osteopenia markers apart of gender. The revealed interdependence between leptinemia value and bone tissue mass in females is quite natural keeping in mind the leptin receptors in bone tissue cells.

Table 13.7. Ultrasound densitometry in survivors depending on body mass ($M \pm \sigma$)

indices	BMI			P1,2	P1,3	P2,3
	18.5-24.9 kg/m ² (1)	25-29.9 kg/m ² (2)	≥30 kg/m ² (3)			
Females						
Number of survivors	13	22	36			
BUA, dB/MHz	101.2±11.5	109.9±11	114.7±10.9	>0.05	<0.05	>0.05
SOS, m/sec	1503±34.2	1520±15.2	1532±25.8	>0.05	<0.05	>0.05
Stiffnes, %	68.5±16.7	72±9.9	85.3±13.3	>0.05	<0.05	<0.05
T-score	-2.6±0.3	0.5±0.3	-1.1±1	<0.05	<0.05	<0.05
Z-score	-2.1±0.2	0.5±0.1	0.4±0.1	<0.05	<0.05	>0.05
Males						
Number of survivors	10	26	28			
BUA, dB/MHz	119±12	120.4±14.3	122.7±6.6	>0.05	>0.05	>0.05
SOS, m/sec	1560±25.3	1331±518.2	1540±42.6	<0.05	>0.05	<0.05
Stiffnes, %	86.4±14.2	87.7±15.9	95.7±13.4	>0.05	<0.05	<0.05
T-score	-0.7±0.1	-1.5±1.2	-0.7±0.5	<0.05	>0.05	<0.05
Z-score	-0.1±0.1	-0.6±0.1	-0.5±0.1	<0.05	<0.05	>0.05

BMI — body mass index; BUA — broadband ultrasound attenuation; SOS — speed of sound; $P_{1,2}, P_{1,3}, P_{2,3}$ — significance of difference between survivors having various body mass

Table 13.8. Ultrasound densitometry in survivors depending on age ($M \pm \sigma$)

Indices	Age, years			
	34-49		≥ 50	
	females	males	females	males
Number of survivors	33	38	38	26
BUA, dB/MHz	97.7 $\pm$ 11	122.5 $\pm$ 12.6	114.3 $\pm$ 10	120 $\pm$ 12
SOS, m/sec	1489 $\pm$ 24.1	1398 $\pm$ 437.2	1534 $\pm$ 24.1	1545 $\pm$ 36.3
Stiffness, %	63 $\pm$ 11.4	89.6 $\pm$ 14.2	84.7 $\pm$ 11.5	94.8 $\pm$ 16
T-score	0.1 $\pm$ 0.1	-0.8 $\pm$ 0.2	-1.2 $\pm$ 0.8	-1 $\pm$ 0.3
Z-score	-0.3 $\pm$ 0.2	0.7 $\pm$ 0.1	0.2 $\pm$ 0.1	-0.6 $\pm$ 0.1

BMI — body mass index; BUA — broadband ultrasound attenuation; SOS — speed of sound; $P_{1,2}, P_{1,3}, P_{2,3}$ — significance of difference between survivors having various body mass

No abnormal serum concentrations of calcium, phosphorus, or alkaline phosphatase were found under normal parathyroid function in survivors of both genders having low radiation doses.

Osteopenic syndrome was found in ~30% and osteoporosis — in ~1% of the thyroid cancer patients (differentiated thyroid carcinoma) who receive a life-long thyroxine replacement therapy after total thyroidectomy. BMD decrease was significantly more often

in females vs. men (~43% and ~14% respectively, $p < 0.001$). The significant difference of the decreased BMI incidence was found in TSH concentrations 0.1–0.5 mU/L vs. TSH over 0.5 mU/L (Table 13.9).

Table 13.9. Average ($M \pm m$) thyroid-stimulating hormone concentrations, age, body mass index values and duration of L-thyroxin administration in persons having normal bone mineral density, osteopenic syndrome and osteoporosis

Indices	Normal BMD (n=128)	Osteopenic syndrome (n=55)	Osteoporosis (n=2)	P _{1,2}	P _{1,3}	P _{2,3}
TSH (U/L)	1.7±0.4	1.2±0.5	0.4±0.2	>0.1	<0.001	>0.1
Age (years)	45.9±1.4	46.7±2.5	61.0±3.0	>0.1	<0.001	<0.001
BMI (kg/m ²)	27.0±0.5	24.4±0.7	26.4±4.9	<0.01	>0.1	>0.1
Duration of L-thyroxin therapy (years)	6.6±0.3	7.9±0.5	9.0	<0.05	<0.05	>0.1

BMI — body mass index; BMD — bone mineral density; TSH — thyroid-stimulating hormone

Thus the TSH concentration, age, BMI value, and duration of a replacement thyroxin medication can effect the BMD. Multiple regression analysis has testified the impact of gender, age, BMI value and replacement therapy on the BMD. There was a positive correlation of the T-criterion value and male gender and BMI, whereas a negative correlation was found towards age and thyroxinotherapy duration. BMI is of a supreme positive effect on the T-criterion value ($\beta=0.342968$). Contrary to univariate analysis no data were received for TSH values. The phenomenon can be explained by a significantly different duration of thyroxin therapy. Not only BMI value and replacement therapy can make effect on BMD but also the menarche age and menopause duration. The latter index is of supreme reverse effect. Provided data coincide with results of other studies pointing to insufficient evidence of a negative impact of thyroxin therapy on BMD in differentiated thyroid cancer patients.

So, under no prognostic value of TSH concentration in osteopenia risk (BMD decrease) not the fact of thyroxin therapy is of importance but the consequences of surgical or combined management of a differentiated thyroid cancer, time since primary intervention, BMI value and duration of menopause.

Increase of an adipose tissue amount occurs upon radiation exposure under the impact of several genetic, environmental and other factors directed on bone tissue protection and radioresistance amplification, followed however by some unfavorable processes (Fig. 13.6).

Effect of ionizing radiation on the organic component of bone tissue is a subject of several studies where through the infrared spectroscopy application the organic matrix deformity was found as a result of C-O bond opening and relative increase of its general mass. The compromised organic matrix in its turn loses the ability to retain the ions of mineral salts exacerbating therefore demineralization. Estimation of radiation role in the process and essay of application efficacy of extracellular matrix biomarkers are the important issues in clarification of their pathogenesis. Therefore collagen type I metabolism was studied in the Chernobyl NPP clean-up workers exposed to 0.15–7.1 Gy of radiation. Aminoterminal peptide of procollagen type I (PINP) and carboxyterminal telopeptide of collagen type I (ICTP) were assayed in serum.

In the Chernobyl NPP clean-up workers survivors (1–7.1 Gy radiation doses) the PINP level was significantly higher vs. control group of non-exposed persons indicating to collagen accumulation on the extracellular matrix after the high-dose radiation impact. Some indices of densitometry reflecting not only the mineral density but amount, size and space orientation of a trabecular bone tissue appeared being significantly lower in ARS convalescents. Radiation therefore makes negative effect on both non-organic and organic component of bone tissue extracellular space. Rather close link was found through the correlation-regression analysis between radiation dose and ICTP content ($r=0.67$; $p<0.05$) in the Chernobyl NPP clean-up workers who had survived the ARS.

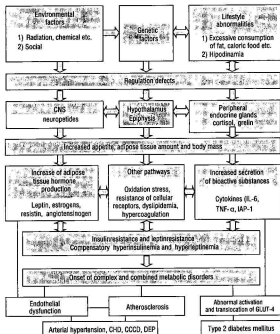


Fig. 13.6. Simplified flowchart of insulinresistance, leptinresistance and somatic disease pathophysiological pathways in the Chernobyl NPP accident survivors

The Chernobyl NPP clean-up workers having 0.15–0.99 Gy radiation doses the similar however less significant link was found ($r=0.48$; $p<0.05$) that was again testified the radiation impact on collagen type I metabolism. The latter protein synthesis and degrading were distorted under the radiation impact leading to abnormal bone tissue organic matrix.

Conclusion

1. There is a staged onset of non-stochastic endocrine diseases after the Chernobyl NPP accident.
2. Thyroid disease, obesity, body mass excess, type 2 diabetes mellitus, metabolic and osteopenic syndromes are the most prevalent endocrine regulation and metabolic disorders in population that had survived after the accident.
3. The most robust increase of thyroid disease prevalence is found in clean-up workers who ere under 20 years old in 1986.
4. Abnormal pathways of hormonal regulation of energy metabolism were found in the Chernobyl NPP accident survivors having body mass excess.
5. Incidence of insuliareistance and hyperinsulinemia is higher in the Chernobyl NPP clean-up workers and evacuees from the exclusion zone.
6. There is a dissociation between insulin and C-peptide concentrations in the Chernobyl NPP accident survivors.
7. Decrease of BMD (osteopenic syndrome) is found in remote term in about every third Chernobyl NPP clean-up workers that have 0.5–7.1 Gy absorbed radiation doses, and osteoporosis as a severe form of osteopenic syndrome is diagnosed in 6% of cases.
8. Ionizing radiation at dose over 0.5 Gy have had a negative impact on the organic bone tissue component with an abnormal metabolism of type I collagen in particular as the respective marker level is correlating to radiation dose.

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CHAPTER 14 ANTIOXIDANT SYSTEM, OXIDATIVE MODIFICATION OF PROTEINS AND LIPIDS

Progressing increment of somatic morbidity in persons exposed to an impact of the Chernobyl catastrophe factors requires an assay of biochemical nature of the appearing abnormalities. The scope of available experimental and clinical evidence indicates that origination of reactive oxygen species (ROS) and initiation of lipid peroxidation (LPO) both with oxidative protein modification (OPM) are the principle pathogenetic pathways of a wide range of disorders [1, 2].

Any reaction of an organism to stressful factors under normal conditions can lead to a short-term increase of ROS. It is stipulated by the adaptive response to extreme conditions with ROS being secondary messengers in a transmembrane signaling [3, 4].

There are two types of known intracellular ROS. Extremely active low-weight relatively short-living radicals i.e. intermediate products of incomplete one-, two- or three-electron reduction of oxygen molecule (superoxide anion-radical, hydrogen peroxide, hydroxyl-radical) and oxygen molecules in singlet state, nitric oxide, peroxyxynitrite, hypohalite are the first type ones (Fig. 14.1).

Less destructive, larger and long-living radicals are attributed to the second type being produced through the first-type ones interaction with cellular biomolecules [7]. Oxygen, its derivatives (superoxide- and hydroxyl-radicals) and hydrogen peroxide are the most important reactants in aerobian cells.

Abnormal consequences are known here as an excessive accumulation of ROS, peroxides and their secondary products i.e. in a state named oxidative stress. At that a damage of the most critical polymers occurs i.e. of nucleic acids, proteins and lipids [8].

Metabolic disorders such as glycolysis and oxidative phosphorylation activity decrease, depression of protein and nucleic acid synthesis, abnormalities of various enzymatic processes occur as a result of hydroperoxides, unsaturated aldehydes, malondialdehyde and other toxic LPO products accumulation.

Principal effect of the free-radical oxidation (FRO) products towards protein inactivation lies in interaction with their molecular SH-, NH₂-, CH₃-groups that can lead to protein structure distortion, appearing of protein-protein cross-linking and protein fragmentation [9]. As a result the multifunctional protein activity (enzymatic, regulatory, involvement in matrix synthesis and ion transport) appears decreased or hampered, whereas some of that predispose to mutations or become autoantigens [10]. Noteworthy

Noteworthy that more and more data are available in scientific literature on the issue of biological activity of compounds produced in FRO and LPO reactions under an adaptation response within organism physiological reactions. These minor but obligate biological processes are linked to several important functions i.e. synthesis of eicosanoid hormones (prostanoids and leucotrienes) and iodotyronines, and to providing of inflammatory, immune and apoptotic reactions. Having high reaction capability and selectivity of biological effect the LPO products can be the component that limits organism's resistance to deleterious environmental factors. It occurs through modification of physical/chemical properties of cellular membranes, of membrane-bound and lipid-dependent enzymes, and of neuroendocrine and immune system reactivity.

Active products produced after irradiation are known to initiate and accelerate the free-radical LPO tissue reactions amplifying the spontaneous processes. Just after the radiation impact on biological system the antioxidant reserve loss occurs, antioxidants are utilized, disintegrated and inactivated within neutralization of active products of radiation, the antioxidant enzymes lose their activity [16, 17].

Pathogenetic pathways initiated immediately in the acute period of a direct contact to ionizing radiation continue their function being supported by a negative factors complex (e.g. environmental, economical, psychoemotional) and decrease of reserved capabilities of regulatory systems. LPO activation stipulated by a chronic stress and exhaustion of AOS reserves lead to exacerbation of pathological processes induced directly by radiation.

The presented here section provides summarization of a long-term studies of pro-oxidant/antioxidant balance in persons exposed to the Chornobyl catastrophe factors.

The indices of OPM i.e. content of basic or neutral 2,4-dinitrophenylhydrazones, of LPO — compounds with isolated double bonds (CIDB), diene or oxodiene conjugates (DC & ODC respectively), thiobarbituric acid active products (TBA-AP) and Schiff's base, both with AOS parameters i.e. activity of red blood cells (RBC) SOD (E-SOD), of RBC CAT (E-CAT) and of plasma CAT (P-CAT), glutathione reductase, selenium glutathione peroxidase, and content of reduced glutathione were assayed to estimate the state of maintenance systems providing the prooxidant/antioxidant balance. An integral index of antioxidant state was calculated $(E-SOD \times E-CAT / TBA-AP)$ i.e. the AOS factor (F_{AOS}). Content of reactive sulfhydryl groups (SH-groups), midmolecule (medium molecular weight) peptides (MMP) and activity of lysosomal enzymes β -galactosidase β -glycosidase were assayed. Indices from almost healthy population groups matched by age and gender being not exposed to ionizing radiation were applied as a control.

The following groups were involved in the study within 1992–2004 period: 1) various severity grade acute radiation syndrome (ARS) convalescents, whose somatic status within post-accident period featured the incidence increase and trend to chronic course of digestive and circulation organ diseases, decrease of mental and physical working capacity, transformation of neuro-autonomous psychoneurological disorders into the organic neurovascular disease; 2) the accident consequences clean-up workers suffering cardiovascular, digestive, and broncho-pulmonary disease after radiation doses of 0.2–1 Gy received in 1986–1987; 3) the Chornobyl NPP personnel; 4) persons inhabiting and working in the exclusion zone; 5) children living on the radionuclide-contaminated territories.

Stable elevation of DC and ODC content under no TBA-AP level increase was found in various severity degree ARS survivors (Table 14.1). The most robust alterations of LPO products content were at that in 1994–1995.

Table 14.1. Lipid peroxidation indices in the various severity degree acute radiation syndrome convalescents (M±m)

Year of study	N of persons examined	Compounds with isolated double bonds (E_{232} /mL)	Diene conjugates (E_{232} /mL)	Oxidized conjugates (E_{232} /mL)	TBA-active products (nmol/mL)
Control group					
1992–1996	30	1.89±0.18	0.79±0.11	0.38±0.06	3.65±0.11
ARS not confirmed					
1992–1993	20	3.38±0.25*	1.44±0.12*	0.68±0.10*	3.20±0.60
1994–1995	33	3.39±0.40*	1.66±0.11*	0.75±0.07*	3.58±0.43
ARS grade 1					
1992–1993	13	3.65±0.51*	1.54±0.18*	0.62±0.05*	3.10±0.41
1994–1995	13	3.69±0.13*	1.56±0.10*	0.89±0.05*	3.01±0.19*
1996	23	2.85±0.31*	1.26±0.11*	0.60±0.04*	3.91±0.46
ARS grade 2, 3					
1992–1993	14	3.64±0.27*	1.39±0.15*	0.50±0.06*	3.28±0.32
1994–1995	14	3.97±0.17*	2.06±0.12*	0.91±0.06*	3.43±0.21
1996	11	2.78±0.35*	1.04±0.10*	0.49±0.04	4.59±0.78

*significant difference vs. control group values ($p<0.05$).

Assay of the AOS enzymatic branch state is of highest diagnostic value under a long-term activation of the FRO processes. The AOS enzymatic branch state efficacy is of a decisive role in prooxidative / antioxidative balance maintenance here. Applied analysis of the antioxidative protection system in ARS convalescents indicated to decrease of RBC anti-oxidative protection key enzymes activity i.e. E-SOD and E-CAT (Table 14.2).

Table 14.2. Antioxidative protection system indices in the convalescents of various severity grade acute radiation syndrome (M±m)

Year of study	N of persons examined	Superoxide dismutase (U/mg Hb)	Catalase (μmol/min × mg Hb)	Glutathione peroxidase (μmol/min×L)	Glutathione reductase (μmol/min×L)
Control group					
1992–1996	30	4.37±0.37	1820.0±60.0	746.0±40.0	605.0±32.0
ARS not confirmed					
1992–1993	20	2.60±0.50*	1002.0±78.0*	845.0±75.0	550.0±50.0
1994–1995	33	3.60±0.34*	1738.0±60.0	901.0±37.0	570.0±30.0
ARS grade 1					
1992–1993	13	3.24±0.43	1141.0±85.0*	858.0±72.0	450.0±51.0
1994–1995	13	3.60±0.42*	1099.0±70.0*	849.0±45.0	440.0±45.0
1996	23	3.21±0.46*	1242.0±108.0*	743.0±53.0	542.9±62.4
ARS grade 2,3					
1992–1993	14	3.50±0.74	1093.0±77.0*	746.0±30.0	449.0±45.0
1994–1995	14	5.47±0.38	1382.0±60.0*	711.0±38.0	427.0±38.0
1996	11	3.01±0.37*	1183.0±85.0*	853.0±115.0	636.6±58.2

*significant difference vs. control group values ($p<0.05$).

State of maintenance systems of prooxidative/antioxidative balance was assayed in 576 Chernobyl NPP staff members aged 30–58. Data analysis indicated that along with a trend to TBA-AP content elevation the TBA-AP increase up to 100% was found in 34% of examined persons (Table 14.3).

Table 14.3. Lipid peroxidation and antioxidant system indices in Chernobyl NPP personnel (M±m)

Year of study	N of persons examined	TBA-active products (nmol/mL)	Superoxide dismutase (U/mg Hb)	Catalase (μmol/min × mg Hb)	Anti-oxidative state factor (units)
Control group					
1992–1996	30	3.65±0.11	4.37±0.37	1820.0±60.0	2428.0±90.0
ChNPP personnel					
1993	215	4.49±0.54	4.06±0.20	1443.0±21.3*	1693.1±74.8*
1994	313	5.09±0.54	4.72±0.12	798.6±22.7*	984.5±62.1*
1995	48	4.89±0.34	4.65±0.22	780.0±32.0*	741.7±75.0*

*Significant difference vs. control group values ($p<0.05$).

E-CAT activity decrease by 21–57% was found in AOS state assay in 82% of the examined persons, that had a significant impact on a depression of antioxidant protection. The most severe decrease of F_{AOS} (two-fold) was registered in 65% of cases.

LPO and AOS indices were analyzed in Chernobyl NPP personnel depending on a length of employment. Studied population was divided in 3 groups: 1) professionals who had started to work at a power plant before the accident and have continued to work at the time of study, 2) workers of 1986–1987 period, 3) workers since 1990. As shown in Table 14.4 the most pronounced alterations (TBA-AP content increase, E-CAT and E-SOD activity lowering along with F_{AOS} value decrease) were in the first group of professionals employed before and after the accident.

Table 14.4. Lipid peroxidation and antioxidant system indices in Chernobyl NPP personnel depending on a length of employment (M±m)

Indices	Control group	NPP personnel		
		group I* (n=34)	group II* (n=34)	group III* (n=18)
TBA-active products (nmol/mL)	3.65±0.11	5.87±0.42*	6.26±0.77*	4.40±0.54
Superoxide dismutase (U/mg Hb)	4.37±0.37	3.08±0.31*	3.33±0.35*	3.52±0.50*
Catalase (μmol/min × mg Hb)	1820.0±60.0	686.7±67.2*	1035.5±100.9*	1034.4±140.5*
Antioxidative state factor (units)	2428.0±90.0	387.7±48.0*	566.9±87.6*	701.5±117.2*

*service record since before 1986 (group I), in 1986–1987 (group II), and since 1990 (group III); *significant difference vs. control group values ($p<0.05$).

The data received indicate to an abnormal prooxidative/antioxidative equilibrium with oxidation processes prevalence with an expenditure of AOS enzymatic components leading to increase of the aggressive secondary LPO product levels in the examined persons. Noteworthy that the most pronounced manifestations of an oxidative stress were in the group of professional employed at Chernobyl NPP before and after the accident vs. the compare group that can be linked to an effective consecutive prophylactic and curative arrangements applied in the first ones. The revealed metabolic disorders can lead to the pronounced destructive alterations, damage of cellular and intracellular

membranes, free-radical modifications of critical biopolymers and be a pathogenetic source of various diseases.

Significant vs. control group level increase of CIBB by 78%, of DC by 120% and of ODC by 71% was in clean-up workers examined in 1995–1996 (radiation doses 0.5–0.8 Gy) who suffered cardiovascular, nervous and hepatobiliary diseases.

Among the anti-oxidative protection enzymes the E-CAT activity was significantly lower (by 20%) whereas E-SOD remained within control values (Table 14.5).

Table 14.5. Indices of lipid peroxidation and antioxidant system in Chernobyl NPP accident clean-up workers; studies of 1995–1996 period (M±m)

Indices	Control group (n=30)	Accident clean-up participants (n=121)
LPO indices		
Compounds with isolated double bonds (E_{232} /mL)	1.69±0.8	3.01±0.26*
Diene conjugates (E_{232} /mL)	0.79±0.11	1.76±0.19*
Oxidodiene conjugates (E_{270} /mL)	0.38±0.05	0.65±0.09
TBA-active products (nmol/mL)	3.65±0.11	3.49±0.27
AOS indices		
Superoxide dismutase (U/mg Hb)	4.37±0.37	4.23±0.37
Catalase (μmol/min × mg Hb)	1820.0±60.0	1446.5±70.2*
Antioxidative state factor (units)	2426.0±90.0	2001.0±31.0*

*significant difference vs. control group values ($p<0.05$).

Review of individual values here indicated that E-CAT activity decrease was in 40% of examined persons, E-SOD activity lowering – in 31%. F_{AOS} lowering was found in 55% of study subjects indicating to the decrease of adaptation capabilities of an organism.

Indices of oxidative homeostasis were studied within 1995–1996 period in 173 males aged 38–55 who had been for a long time employed in the Chernobyl exclusion zone. Increase of CIBB content by 78%, of DC by 123% and ODC by 71% was found in 1995 year of study, whereas TBA-AP levels remained within control range in a whole for the entire study group (Table 14.6).

Table 14.6. Lipid peroxidation indices in the exclusion zone long-term employees (M ± m)

Indices	Control group	Exclusion zone employees	
Year of study		1995	1996
n	30	104	69
Compounds with isolated double bonds (E_{232} /mL)	1.69±0.18	3.01±0.26*	3.20±0.12*
Diene conjugates (E_{232} /mL)	0.79±0.11	1.76±0.19*	1.44±0.08*
Oxidodiene conjugates (E_{270} /mL)	0.38±0.05	0.65±0.09*	0.68±0.04*
TBA-active products (nmol/mL)	3.65±0.11	3.49±0.27	4.46±0.28

*significant difference vs. control group values ($p<0.05$).

Similar trends of the product content were surveyed in 1996 year of study, at that DC and ODC content increase was found in 80% of cases, and TBA-AP — in 40% of them.

At a such level of a FRO process activation the AOS enzymatic branch provides no adequate antioxidant protection represented with a decline of F_{AOS} values indicating to decrease of organism adaptation capacities (Table 14.7).

Table 14.7. Antioxidant system indices in the exclusion zone long-term employees (M±m)

Indices	Control group	Exclusion zone employees	
Year of study		1985	1996
n	30	104	69
Superoxide dismutase (U/mg Hb)	4.37±0.37	4.35±0.33	3.29±0.18*
Catalase (μmol/min × mg Hb)	1820.0±60.0	1455.4±82.0*	1321.0±60.0*
Antioxidative state factor (units)	2426.0±90.0	2012.0±28.0*	1193.0±69.0*

*significant difference vs. control group values ($p<0.05$).

Lowering of F_{AOS} values was found in 55% of examined persons in 1995. Next year the decreased E-SOD activity was found in 43%, E-CAT — in 65%, and F_{AOS} — in 82%.

Glutathione system assay in 1996 had revealed the normal values of glutathione reductase activity (812.0 ± 37.0 & 746.0 ± 40.0 μmol/min×L, respectively for the exclusion zone employees and a control group; $p>0.05$) and amount of reduced glutathione (607.0 ± 35.0 & 605.0 ± 32.0 μmol×L respectively; $p>0.9$).

GPx activity increase (239.0 ± 10.0 & 152.0 ± 16.0 μmol/min×L respectively; $p<0.05$) was found in 90% of study subjects that was possibly linked to E-CAT activity lowering and switching of antioxidant protection function to glutathione system, as just the GPx acts similarly to CAT in hydrogen peroxide degrading.

Estimate of lysosomal membranes through lysosomal enzymes β-glucosidase and β-galactosidase activity assay was held at the next study period. Both enzymes are the markers of subcellular structures as β-glucosidase is tightly bound to lysosomal membranes and β-galactosidase – to their matrix. Function of lysosomes as cellular organelles involved in the emergency states overcoming defines the universal mode of lysosomal apparatus role in pathogenesis of a range of pathological processes.

Robust increase of lysosomal enzyme activity i.e. β-glucosidase (36.0 ± 7.1 & 13.9 ± 0.6 μmol/min×L respectively; $p<0.05$) and β-galactosidase (60.9 ± 5.1 & 17.6 ± 1.0 μmol/min×L respectively; $p<0.05$) was found meaning the increased lysosomal membrane permeability.

Serum MMP content is recognized as a criterion of endogenous intoxication severity. MMP concentration in all studied subjects ($n=100$) was elevated (0.51 ± 0.12 and 0.28 ± 0.10 respectively; $p<0.05$). As it was found earlier the MMP concentration excess for 25–30% above normal threshold can lead to their specific interaction with nuclear genome and inhibition of reparation and transcription. MMP at such concentrations can therefore induce the non-specific biochemical disorders. Nonexceptionally that E-SOD and E-CAT hypoactivity is stipulated by their low concentration due to inhibition of synthesis under the MMP impact.

Increased content of OPM products i.e. of basic 2,4-dinitrophenylhydrazones (1.87 ± 0.04 & 0.91 ± 0.28 E₃₂₀/mL respectively; $p<0.05$) was revealed in serum of 27 persons in 1997.

There were 104 clean-up workers aged 34–59 (46.7 ± 2.1) years suffering chronic cholecystitis (ICD10 code K81.1; clean-up workers group) and 22 almost healthy persons not engaged in a clean-up work (control group) all being examined in 2000–2002. Documented radiation dose of an external radiation was not exceeding 0.35 Gy.

It was found that an activation of the free-radical processes is present with accumulation of DC (1.207 ± 0.046 & 0.883 ± 0.092 E_{232}/mL , here and further respectively for the group of patients and the control group, $p < 0.05$) and ODC (0.739 ± 0.032 & 0.536 ± 0.044 E_{276}/mL , $p < 0.05$) against the background of an increased CDB content (2.495 ± 0.074 & 1.843 ± 0.113 E_{220}/mL , $p < 0.01$) in the second decade after the Chernobyl NPP accident among the accident clean-up workers. Increase of the individual values of mentioned compounds was registered in 27% and 34% respectively reflecting the polymorphism of LPO processes course.

At that the discrepancy between production and utilization of lipid peroxides had led in 27% of the clean-up workers to accumulation of the lipid peroxidation end-products i.e. Schiff's bases. In our opinion it is the sign of an unfavorable course of the free-radical oxidation as such compounds are the structural core of lipofuscins i.e. markers of cellular dystrophy processes. It is confirmed by an abnormal content of the protein oxidative modification products — 2,4-dinitrophenylhydrazones of basic or neutral nature.

Under the activation of free-radical processes the distorted ratios between lipid and protein oxidative modification processes are expressed in an abnormal correlation of TBA-AP and neutral 2,4-dinitrophenylhydrazones content ($r_s = -0.025$, $p > 0.8$; and $r_s = 0.699$, $p < 0.03$, respectively for clean-up workers group and control group) and of TBA-AP and basic 2,4-dinitrophenylhydrazones ($r_s = -0.129$, $p > 0.3$ and $r_s = 0.711$, $p < 0.03$ respectively). Described phenomenon can be the reflection of a long-term activation of free-radical processes and be linked to an abnormal content of specific pro-oxidants at various points of oxidative stress.

It is known that AOS state is of a key role in prooxidative/antioxidative equilibrium maintenance. Functional capabilities of the AOS enzymatic branch which components slowly acquire the activity under FRO processes activation but feature a comparably higher reserve due to the direct component regeneration are of a decisive importance in the prevention of negative consequences of chronic oxidation stress. It was found that the abnormal percent of the AOS enzymatic branch specific components was within range of 8–44%: E-SOD activity was decreased in 35% of the clean-up workers, activity of E-CAT was decreased in 3% and increased in 5% of them, activity of ceruloplasmin was increased in 17%, and P-CAT activity was elevated in 44%. SH-group content was lowered in 83% of examined persons.

In a whole the activity of RBC antioxidant enzymes E-SOD and E-CAT among the clean-up workers group was decreased by 31% ($p < 0.001$) and by 16% (Table 14.8).

At that there was an incoordination of enzymatic activity as no correlation was found between E-SOD and E-CAT activity being otherwise present in control ($r_s = 0.137$, $p > 0.08$ and $r_s = 0.735$, $p < 0.02$ respectively).

Also the abnormalities of AOS function were registered in the patients featuring a decreased F_{AOS} (706.3 ± 60.6 & 1284.5 ± 220.7 units, respectively for clean-up workers and control group, $p < 0.05$). It can be a consequence of an enzymatic disorganization of

cellular membranes under the prooxidant shift being confirmed by a changed ratio of E-CAT and P-CAT activity (1.753 ± 0.139 & 3.036 ± 0.338 units, respectively, $p < 0.05$).

Table 14.8. Antioxidant system indices in Chernobyl accident clean-up workers suffering chronic cholecystitis (2000–2002 survey)

Parameter	Control group		ACUP group	
	n	M ± m	n	M ± m
In RBC				
Superoxide dismutase (U/mg Hb)	22	4.471 ± 0.294	98	$3.097 \pm 0.148^{***}$
Catalase ($\mu\text{mol/min} \times \text{mg Hb}$)	22	1038.2 ± 78.5	98	873.7 ± 30.4
In plasma				
Ceruloplasmin (mg/L)	22	210.9 ± 13.5	63	242.7 ± 9.7
Catalase ($\mu\text{mol/min} \times \text{mg Hb}$)	22	401.1 ± 43.8	59	$645.9 \pm 50.4^{**}$
Sulphydryl groups ($\mu\text{mol/L}$)	22	405.2 ± 7.33	59	$267.1 \pm 11.2^{***}$

, * – significant difference vs. control group values ($p < 0.01$ & $p < 0.001$ respectively).

Noteworthy under an unstable clinical remission of chronic cholecystitis against the background of dysfunction of AOS enzymatic branch the manifestations of prooxidant/antioxidant imbalance were found in 64% of the clean-up workers. Therefore two types of prooxidant/antioxidant imbalance have been described through the analysis of study results.

In a first one i.e. in dysfunction of an antioxidant protection with partial hyperlipoperoxidemia the increased content of ODC (by 33%, $p < 0.03$) and TBA-AP (by 35%, $p < 0.001$) had occurred against E-SOD activity by 35% ($p < 0.001$) and a SH-group content by 37% ($p < 0.001$) decrease, both with P-CAT activity elevation by 102% ($p < 0.02$). F_{AOS} value was also decreased by 64% ($p < 0.01$). Synchronicity of E-SOD and E-CAT effects was unchanged ($r_s = 0.516$, $p < 0.02$), whereas E-CAT/ E-SOD ratio of activity elevation by 30% was insignificant. Relative activity of RBC antioxidant enzymes and ceruloplasmin levels were not significantly different from the control group values. Most of such type of abnormalities was found in case of the disease exacerbation incidence up to 3–4 per year (Fig. 14.2).

In another variant i.e. antioxidant system failure with accumulation of lipid and protein oxi-

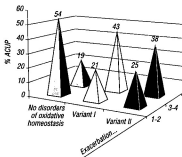


Fig. 14.2. Prooxidant/antioxidant equilibrium disorders in clean-up workers depending on exacerbation incidence of a chronic cholecystitis. Variant I – antioxidant system dysfunction with partial hyperlipoperoxidemia; Variant II – antioxidant system failure with accumulation of protein and lipid oxidative modification products.

derivative modification products the increased level of neutral 2,4-dinitrophenylhydrazones (by 15%, $p<0.05$), DC (by 82%, $p<0.001$), ODC (by 91%, $p<0.001$), and Schiff's bases (by 43%, $p<0.01$) had occurred against the decreased absolute (by 25%, $p<0.05$) and relative ($E-SOD/DC$ – by 41%, $p<0.02$) $E-SOD$ activity indices, the decreased SH-group content (by 315, $p<0.001$), elevated ceruloplasmin (by 38%, $p<0.01$), and P-CAT activity (by 45%, $p<0.05$). Described abnormalities of antioxidant enzymes did not prevent the F_{AOS} value decrease (by 49%, $p<0.05$).

Conducted studies therefore have revealed the profound disorders of prooxidative/antioxidative equilibrium in persons employed in the exclusion zone within a long time, namely the OPM and LPO activation with high accumulation of intermediate products of lipoperoxidation, depression of antioxidant protective system with pronounced hypoactivity of its enzymatic branch, intracellular membrane destabilization, and signs of endogenous toxicosis. All the described above can be a pathogenetic background of vital activity disorders in remote period after the Chernobyl accident.

At a moment there are enough data already indicating to stable disorders of pro-oxidation/antioxidation equilibrium in children of various age exposed to the Chernobyl NPP accident factors. Namely an increase of content of intermediate LPO products and $E-SOD$ hypoactivity were found in 1996 among children residing in Zhytomyr and Kyiv regions having several chronic somatic diseases in remission (chronic gastroenteritis, cholecystocholangitis, tonsillitis, multiple caries) (Table 14.9).

Table 14.9. Lipid peroxidation and antioxidant system enzymatic activity indices in children 12–14 years of age living in radionuclide contaminated territories of Ukraine; year 1996 study (Mmm)

Indices	Almost healthy children		Somatic pediatric patients	
	Kyiv regions (n=35)	Zhytomyr regions (n=20)	Kyiv regions (n=45)	Zhytomyr regions (n=25)
LPO indices				
Diene conjugates (E_{232}/mL)	1.22±0.08	0.98±0.14	1.58±0.11	1.54±0.12*
Oxidodiene conjugates (E_{278}/mL)	0.62±0.09	0.57±0.09	0.70±0.07	0.62±0.08
TBA- active products (nmol/mL)	2.84±0.14	2.88±0.13	4.68±0.13*	4.60±0.15*
AOS indices				
Superoxide dismutase (U/mg Hb)	3.83±0.30	3.00±0.37	2.80±0.34*	2.68±0.30
Catalase ($\mu mol/min \times mg$ Hb)	1400.3±98.9	1345.8±98.5	1407.8±5.8	1439.4±92.9

*significant difference vs. values in the group of almost healthy children ($p<0.05$).

Increase of radiation induced thyroid disease prevalence among the pediatric population of contaminated territories is one of the most important health consequences of the Chernobyl NPP accident. Comprehensive survey of children 10–17 years of age residing in Rokytno district Rivne region (Stare Selo village) was held in 2001–2003. Two groups of children were selected for the precise examination.

The first group included 30 children born in 1986–1987 being exposed to radiation in prenatal or early postnatal age and residing in the named above region since accident. Those children were exposed to impact of radioactive iodine on thyroid (dose range 0.75–2.5 Gy) against living under the continuous iodine deficiency coupled with an unfriendly environment after the Chernobyl catastrophe (soil contamination by radioactive isotopes).

The second group included 30 kids born after the Chernobyl NPP accident and continuously living under the iodine deficiency and environmental radioactive contamination but having no impact of radioiodine on thyroid.

Reference group was represented by the children born in 1987–1991 been not exposed to radioactive iodine and continuously living under the severe iodine deficiency (Lviv province) but under no radioactive environmental contamination.

Analysis of study data on lipid peroxidation has led to identification of by 48% excess of ODC content both with by 22% CIBD content lowering in children exposed to radioiodine impact and now suffering chronic thyroiditis vs. reference group values (Table 14.10).

Table 14.10. Lipid peroxidation products in serum of children residing in Rivne and Lviv regions; 2001–2003 years of study (M±m)

Residence (year of birth)	n	Compounds with isolated double bonds (E_{232}/mL)	Diene conjugates (E_{232}/mL)	Codienes conjugates (E_{232}/mL)	TBA-active products (nmol/mL)
Children with chronic thyroiditis					
Rivne region (1984–1986)	9	1.738±0.147*	0.874±0.132	0.663±0.061*	2.508±0.167
Rivne region (1987–1989)	10	1.904±0.161	1.154±0.135	0.643±0.087	2.180±0.301
Lviv region (1988–1990)	10	2.239±0.160	1.158±0.088	0.449±0.046	3.226±0.464
Children from chronic thyroiditis risk group					
Rivne region (1984–1986)	9	1.892±0.152	1.085±0.133	0.611±0.101	2.599±0.351
Rivne region (1987–1989)	11	2.001±0.114	1.300±0.130**	0.682±0.085**	2.574±0.272
Lviv region (1988–1990)	10	1.956±0.099	0.875±0.102	0.387±0.040	3.000±0.473
Children with diffuse non-toxic goiter Grade I-A – I-B					
Rivne region (1984–1986)	8	1.775±0.159*	0.979±0.129	0.589±0.109	2.689±0.648
Rivne region (1987–1989)	11	1.811±0.129*	1.007±0.101	0.489±0.098	3.262±0.423
Lviv region (1988–1990)	12	2.233±0.161	0.991±0.083	0.490±0.047	3.587±0.364

*, **significant difference vs. values in the group from Lviv region ($p<0.05$ & $p<0.01$).

Disorders of LPO processes in children from Rivne province were represented also by an abnormal ratios of primary and intermediate lipid peroxidation products with relative prevalence of ODC levels: ODC/CIBD — 0.397 ± 0.050 and 0.202 ± 0.018 units ($p<0.001$), ODC/DC — 0.950 ± 0.249 and 0.391 ± 0.036 units ($p<0.001$), and TBA-AP/ODC — 4.376 ± 0.787 and 8.620 ± 2.129 units ($p<0.05$) respectively for children from Rivne and Lviv regions born in 1984–1986.

Table 19.10 shows the significant DC and ODC content increase in case of chronic thyroiditis risk in children from Rivne region not exposed to radioactive iodine vs. values in the reference group. In children exposed to radioiodine the DC level increase by 24% and ODC — by 58% was insignificant.

Secondary LPO products were elevated vs. CIBD level in Rivne region residents both exposed to radioactive iodine and those born later (ODC/CIBD — 0.324 ± 0.047 , $p<0.05$; 0.339 ± 0.036 , $p=0.001$ & 0.196 ± 0.020 units respectively for the children from Rivne region born in 1984–1986 & 1987–1989 and those from Lviv region born in 1988–1990).

CIDB content was lowered in children having a diffuse non-toxic goiter being born in 1984–1986 and in 1987–1989 from Rivne region vs. kids from Lviv region ($p < 0.05$ for both groups). The latter have had an elevated DC and ODC content vs. the available oxidation substrate i.e. CIDB.

It was clear from the study results that Rivne region children had an elevated level of basic OPM products in all groups independently from the disease present vs. groups of nosological control from Lviv region (Table 14.11).

Table 14.11. Products of oxidative protein modification in serum of children resident in Rivne and Lviv regions; 2001–2003 study years (M±m)

Residence (year of birth)	n	Products of oxidative protein modification			
		neutral		basic	
		E_{330}/mU	E_{330}/mU	E_{330}/mU	E_{330}/mU
Children with chronic thyroiditis					
Rivne region (1984–1986)	6	1.709±0.550	1.731±0.522	0.876±0.301	0.564±0.105***
Rivne region (1987–1989)	7	1.770±0.669	1.803±0.617	1.686±0.600	0.776±0.308**
Lviv region (1988–1990)	9	1.673±0.401	1.487±0.171	0.532±0.075	0.108±0.024
Children from chronic thyroiditis risk group					
Rivne region (1984–1986)	3	1.360±0.312	1.410±0.272	0.450±0.300	0.677±0.115*
Rivne region (1987–1989)	9	1.172±0.300	1.250±0.296	0.999±0.291	0.804±0.152***
Lviv region (1988–1990)	10	1.788±0.244	1.921±0.291	0.767±0.153	0.103±0.035
Children with diffuse non-toxic goiter Grade I-A - I-B					
Rivne region (1984–1986)	4	1.915±0.643	2.145±0.636	1.290±0.308	0.596±0.166**
Rivne region (1987–1989)	12	1.890±0.472	2.373±0.631	1.677±0.314	0.610±0.146**
Lviv region (1988–1990)	10	2.164±0.356	2.530±0.410	1.154±0.208	0.173±0.028

*, **, *** – significant difference vs. values in the group of children from Lviv region ($p < 0.05$, $p < 0.01$ & $p < 0.001$ respectively).

Analysis of study results on antioxidant protection state has revealed the decreased E-SOD activity in children from Rivne region suffering chronic thyroiditis (Table 14.12).

Despite both groups were not different by this parameter, and E-CAT activity decrease was not significant, the F_{AOS} as an integral parameter was decreased in children exposed to radioactive iodine.

Abnormal antioxidant properties of blood plasma were found at that. Just the content of a principal antioxidant plasma enzyme ceruloplasmin (that owing to cupric ion content provides the four-electron reduction of O_2^- directly to a water molecule) was decreased in both groups i.e. by 32% ($p < 0.01$) in children exposed to impact of radioactive iodine and by 27% ($p < 0.05$) among those with no experience of radiiodine exposure. The described phenomenon was against the background of decreased content of a blood plasma reactive SH-groups.

The E-SOD activity was also decreased in children from Rivne region having chronic thyroiditis risk, whereas the decrease was even more significant in persons exposed to radiiodine vs. not exposed ones.

Table 14.12. Antioxidant system state in children born resident in Rivne and Lviv regions; 2001–2003 study years (Mm)

Residence (year of birth) n	Superoxide dismutase (U/mg Hb)	Catalase (U/mmol × mg Hb)	Antioxidative state factor (μmol)	Ceruloplasmin (mg/L)	Sulphydryl groups (μmol/L)	Reduced glutathione (μmol/L)
Children with chronic thyroiditis						
Rivne region (1984–1986) n=9	2.113±0.259***	1110.0±189.3	910.7±160.3*	237.3±20.7**	441.5±28.7*	385.3±54.8
Rivne region (1987–1989) n=10	2.354±0.278***	1018.6±106.2	1298.9±239.0	256.1±23.7*	418.8±7.52**	256.4±45.9
Lviv region (1988–1990) n=10	5.228±0.579	1244.5±219.2	2576.5±712.0	348.9±13.9	665.4±57.5	355.4±28.2
Children from chronic thyroiditis risk group						
Rivne region (1984–1986) n=10	2.051±0.127***	864.3±96.2	838.2±177.7*	249.3±12.0*	520.0±108.4	283.7±39.6
Rivne region (1987–1989) n=11	3.103±0.394**	1058.5±107.6	1337.7±163.1	280.3±21.1	447.0±17.6**	343.9±36.6
Lviv region (1988–1990) n=10	4.693±0.207	1101.0±150.6	2469.5±669.6	314.5±20.2	638.3±44.3	390.0±35.6
Children with diffuse nontoxic goiter grade I-A–I-B						
Rivne region (1984–1986) n=8	3.264±0.359	1065.0±139.9	1672.0±246.7	261.2±26.4**	403.6±27.3*	255.3±5.33*
Rivne region (1987–1989) n=11	2.878 ±0.239**	920.3±93.7*	976.7±123.5*	273.1±18.7***	452.4±65.1*	258.7±34.1*
Lviv region (1988–1990) n=12	4.047±0.266	1351.0±172.2	1666.5±384.4	371.3±16.4	623.4±32.4	484.1±47.4

*, **, *** – significant difference vs. values in the group of children from Rivne region ($p<0.05$, $p<0.01$, $p<0.001$ respectively); * – significant difference between the groups of children from Rivne region ($p<0.05$).

At that the E-CAT activity decrease by 22% had led to abnormal blood antioxidant properties in children from Rivne region born in 1987–1989 in whom the $FAOs$ was decreased by 37% both vs. Lviv region group values and Rivne region persons not exposed to radioactive iodine.

Ceruloplasmine content was decreased (by 21%) in this group too along with a trend to SH-groups and reduced glutathione content lowering by 17% and 27% respectively.

There was a decreased by 30% content of SH-groups in Rivne region children not exposed to radiiodine vs. group of kids from Lviv region.

Ceruloplasmine, SH-groups, and reduced glutathione content was decreased in children having a diffuse non-toxic goiter grade I-A–I-B residing in Rivne region.

The identified abnormalities of oxidative protein/lipid modification and antioxidant protection, both with revealed oxidative destruction against the background of hypothalamic-pituitary-thyroid system strain and iodine deficiency are the real triggers of thyroid disease onset and development.

Summarizing the results of a long-term studies it should be noted that prophylactic check-ups of persons exposed to Chernobyl NPP accident factors are to be focused on case identification of the initial metabolic abnormalities aiming the compilation of groups for the further survey and application of extra curative and prophylactic arrangements.

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CHAPTER 15 BRONCHOPULMONARY PATHOLOGY

The Chernobyl catastrophe has put before clinical medicine and radiation protection a new problem — the effects of external ionizing irradiation and radionuclides inhalations on bronchopulmonary system of clean-up workers of the accident and the sufferers population under the influence of other modifying factors.

Parameters of whole body and bronchial system irradiation of people injured after the Chernobyl NPP accident, provide extreme non-uniformity of distribution in time and space of the absorbed and effective equivalent doses at low levels, and also the change of physical and chemical structure of inhaled radionuclides mixture in various terms after the accident [1].

Dose loadings on respiratory organs and, thus, expected consequences of internal irradiation of the clean-up workers were defined by air polluting radionuclides from the beginning of accident up to the end of 1988. Thus, it is expedient to allocate some periods in liquidation of Chernobyl NPP accident from the clinical-dosimetric point of view [2–4].

At the first period from the moment of accident till May, 10, 1986, in formation of of radioactive substance emission a leading role played dispersed of nuclear fuel as a result of explosions April, 26 and May, 3 1986 and the expiration of steam of fragmental radionuclides from the fuel which has stayed in mine of a reactor, at its heating up to 1500–2000°C during the burning of graphite active zone.

The second period began from second half of May, 1986 and was finished with the termination of construction "Object Shelter " in November, 1986.

The third period includes the end 1986–1988 when a leading role in pollution of the atmosphere played secondary dusting, caused by natural processes of wind rise of radioactive particles from a surface of ground and activity of people in the contaminated territories. At this stage significant receipts of radioactive substances in respiratory organs was possible to expect in persons, involved to deactivating works on the industrial site of Chernobyl NPP.

One of the distinctive features on the Chernobyl NPP accident is the thrown out into environment not only fission products, but also nuclear fuel (^{238}U , enriched ^{235}U), and radionuclides with the induced activity. Thus, the radioactivity in air was both in gaseous, ionic-molecular state, and as a radioactive aerosol — "hot particles". Last were formed by two ways: as a result of explosion of reactor and subsequent warming up

("firm hot particles") and sedimentation of radionuclides thrown out in the atmosphere with evaporated water ("liquid hot particles") [2-4].

At inhalation of radionuclides the problem of spatial distribution of irradiation dose within lungs and radiation damage mentions about forty various types of cells, among them bronchial epithelium, vessels endothelium, cells of connecting tissue and some others, updated local stem cells located in the same place [5-8]. Under radionuclides inhalation only small share of cells, body or a tissue are damaged. The damaged and lost cells always are distributed by casual image in space and in time.

The aerosols of radioactive substances formed after Chornobyl NPP accident, are extremely various on the physical and chemical properties by virtue of what have various biological transportability. Lungs are critical body organs for aerosols of Zirconium, Niobium, Ruthenium, Cerium, Neptunium, Caesium [2-4].

The irradiation of bronchopulmonary system during the first ten days, when emissions into the atmosphere proceeded, occurred as a result of inhalation of radionuclides contained in the air. Dose creating role of emergency radionuclides on every arrived to lungs unit of activity provided the account of the chemical form, the activity which has got in the environment and half-life period. At the given stage, from positions of bronchopulmonary system irradiation ^{133}I , ^{106}Ru , ^{144}Ce had the greatest value [9]. Then emissions from the reactor and quantity of radioactivity in air had sharply decreased. Secondary rise of the radioactivity which had dropped out on ground by the wind or as a result of industrial activity was considered insignificant. Therefore, further lungs irradiation occurred mainly not in the inhalation way, but by external irradiation from radioactivity laying on a surface.

The most essential value for bronchopulmonary system in means of radiation safety in the remote terms have long-living transuranium elements (TUE): $^{239-241}\text{Pu}$, ^{241}Am , ^{243}Cm . Isotopes of these elements seldom meet separately from each other and present in an environment in the combinations reflecting specificity of their reception [2]. In view of numerous and variability of TUE structure as an example is enough to consider one of most biologically significant and well investigated elements — plutonium. At inhalation receipt of the specified isotopes of plutonium the general laws of distribution of particles are kept depending on active median aerodynamic diameter, however, micro distribution of radionuclides is extremely non-uniformly. The significant amount of radioactive substances is defined in lung parenchyma, in bronchial integumentary epithelium, and in due course, and in deeper layers of bronchial tubes walls. Postponed TUE can leave the respiratory system in several ways. Thus, the part of them will penetrate directly from lungs (after dissolution in a tissue liquid) directly into blood. This fraction of radionuclides is transported into other organs and tissues, mainly, into liver, bone, or excretes. The second fraction collected by macrophages leaves lung by mechanisms of bronchial and tracheal clearance and is swallowed with sputum. The third fraction translocates through lymphatic vessels up to regional lymph nodes where it delays, but the part of it, eventually, can reach system circulation [2, 3].

Despite of it, the highest concentration of plutonium are always found out in lungs and tracheobronchial lymph nodes [2, 3]. The mentioned above mechanism of radioactive particles deducing for the account of tracheobronchial clearance, is fair for many insoluble and poorly soluble compounds, and also «hot» particles. Thus, as well as at

inhalation of radioactive short living aerosols, there is an irradiation of bronchial tissue by the particles which are staying in respiratory ways. About 90% of all radionuclides are concentrated in a layer of slime above surface epithelium, which makes possible an irradiation of basal cells even by α -particles [2, 3].

Accident at the Chernobyl NPP was followed by the increase of morbidity in lung diseases of the clean-up workers, evacuated people and inhabitants of contaminated territories [1–4].

Results of the long-term (1996–2009) pulmonology monitoring of 16,133 clean-up workers of Chernobyl NPP accident of 1986 in the Out-Patient Clinic of Radiation Register of RCRM testify to the reliable incessant increase of morbidity for chronic bronchitis (ChB) and chronic obstructive pulmonary disease (COPD) among the marked contingent of patients (Fig. 15.1).

Analysis of relative radiation risks (RR) from data of deep clinico-epidemiological inspection (1992–2004) of 7,665 men — COPD patients clean-up workers of 1986–1987 with the different doses of total-body external irradiation (Fig. 15.2.) educed reliable connection of development of this pathology with influence of ionizing radiation in doses above 0.25 Sv (control was laid down by patients with doses lower 0.05 Sv).

These data on the whole coincide with the results of clinico-epidemiological researches on the base of SRU and Russian national radiation register [5–7], that testify the one of the leading places of respiratory organs pathology in the structure of general morbidity.

During 1988–2010 the complex pulmonological study was carried out in 3,376 clean-up workers of Chernobyl NPP accident, males in the age of 21–62. Middle age of them was 39.7 ± 8.5 . The doses of the external irradiation documentary at 63% of surveyed group, were in a range 5–85 cSv, on average, 19.7 ± 4.2 cSv. More than 80% from them took part in clean-up works in April — October 1986. The clean-up workers of later terms (1987–1988) were included into the group only in the case when they

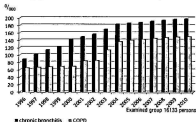


Fig. 15.1. Results of long-term pulmonological monitoring clean-up workers of the Chernobyl catastrophe at the RCRM outpatient department

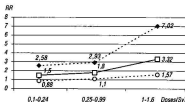


Fig. 15.2. Relative radiation risks (RR) and 95% confidential interval for chronic obstructive pulmonary disease morbidity in patients — clean-up workers of Chernobyl NPP accident of 1986–1987.

participated in decontamination of premises and platforms of Chernobyl NPP, works on "burial place" of materials and the equipment in 30-km exclusion zone.

The "hot" particles incorporation into the lungs were verified by whole body counter technology for 10 individuals (predominately ^{137}Cs , ^{60}Co) (Table 15.1).

Table 15.1. Desimetric characteristic of patients, who participated in works for liquidation of consequences of Chernobyl NPP accident and undergone inhalation incorporation of "hot" particles

No	Patient code	Birth year	Radioisotope	Activity	Dose, mSv	Date of examination
1	A-v A.I.	1957	^{137}Cs	4300 Bq	250–1160	21.02.2002
2	B-ch Ya.O.	1940	^{60}Co	308 Bq	21–38	27.03.2002
3	G-i S.A.	1952	^{137}Cs	7226 Bq	400–1900	24.01.2002
4	K-ko I.M.	1981	^{60}Co	160 Bq	50–170	01.10.2002
5	K-i V.G.	1947	^{60}Co	585 Bq	40–65	08.02.2002
6	R-v V.V.	1945	^{60}Co	581 Bq	37–59	20.12.2000
7	T-b Yu.Yu.	1952	^{137}Cs	520 Bq	30–135	17.04.2002
8	F-v Yu.I.	1941	^{60}Co	464 Bq	25–42	10.06.1999
9	Yu-ko V.A.	1946	^{60}Co	804 Bq	55–90	14.01.2002
10	G-n S.N.	1958	^{137}Cs	120 Bq	10–37	08.05.2002

Among the basic forms of chronic lung pathology of clean-up workers COPD dominated — 73%. The asthmatic patients were 11%, ChB was registered in 16% of observed patients.

The manifestation of bronchopulmonary pathology in an early period after irradiation was characterized by syndrome of upper airways expressive irritation. Both during the works and in the nearest period after works in zone of Chernobyl NPP clean-up workers had cough syndrome without sputum excretion. The subsequent currency of disease was characterized by «poverty» of clinical picture with progressive development of obstruction and formation of dyspnea. Later on the pathology got features of COPD with the appropriate clinical triad: cough, sputum, dyspnea in a combination with the obstructive and mixed ventilating disorders [1–4].

Obstructive disorders of lung ventilation (65%) are most typical for this group of patients. Isolated decrease of vital capacity (VC) was marked at 6% of patients. The mixed ventilation disorders were revealed at 13% of patients. The normal level of ventilating parameters was fixed only in 16% cases and, basically, corresponded to ChB.

The bronchoobstructive syndrome for clean-up workers was transformed from the obstruction of small bronchi and hypotonic dyskinesia of membranous part of trachea to total bronchial obstruction with the low level of reverse obstruction [2–5]. In 1988–1992 the most often syndrome was the isolated obstruction of small bronchi, rank two was the hypotonic dyskinesia of membranous parts of trachea and the main bronchi, and rank three was the syndrome of generalized obstruction (Table 15.2). During 1993–1997 the redistribution in structure of bronchoobstructive syndromes had happened with growth of generalized bronchoobstruction syndrome frequency to 45%. In 1998–2002 period the structure of bronchoobstructive syndromes remain nearly the same (Table 15.2). For today the total (generalized) bronchial obstruction prevails, rank two has a syndrome of isolated obstruction of small bronchi, rank three — hypotonic dyskinesia membranous part of a trachea and the main bronchi.

Table 15.2. Structure of bronchoobstructive syndromes of clean-up workers of Chernobyl MPP accident, who probably undergone radionuclides inhalation (%)

Bronchoobstructive syndromes	Observation periods			
	1983–1992	1993–1997	1998–2002	2003–2009
Isolated obstruction syndrome of small bronchi	52	38	34	13
Hypotonia pars membranacea trachea et bronchus	28	17	13	8
Syndrome of generalized obstruction	20	45	53	79

The current general characteristic of respiratory disorders using lung tests (spirometry for bronchial airflow, bodyplethysmography for lung volumes, single breath for lung diffusion) in COPD patients, who probably undergone radionuclides inhalation, are presented on Fig. 15.3.

The study of respiratory disorders in clean-up workers and nonclean-up workers groups suffered for COPD with using of modern Lung Function Tests showed the significant differences in lung volumes and diffusion lung capacity. The vital capacity, expiratory reserve volume and lung diffusion levels in group of COPD patients clean-up workers were significantly low comparatively with group of COPD patients nonclean-up workers.

Endoscopic research of tracheobronchial tree ($n=2,543$) allowed to establish, the typical development of a mucous membrane atrophy (73%), as a thinning of mucous membrane, sharper in comparison with norm of cartilaginous skeleton evidence, exhaustion of vascular pattern. In 20% of patients the opposite bronchofiberscopic pathological changes of bronchial mucous were revealed — hyperplastic, consisting in a thickening, growth and hypostasis of mucous membrane, narrowing of segmentary and subsegmentary bronchi ostiums, the blurry vascular pattern. The combined form of macromorphological changes — atrophy in proximal parts in a combination with hyperplasia in distal parts of bronchial tree was revealed in 7% of patients. Thus, endoscopical studying of bronchial mucosa membrane pathological changes testified to essential prevalence of atrophic changes in the surveyed contingent of patients [7, 8].

The basic endoscopic type of bronchial mucosa changes was catarrhal-sclerotic, accompanied with deformation of tracheobronchial tree — 84%. This type of changes was characterized by fine light pink longitudinal gentle circularly-directed connective scars of a mucous membrane mainly at ostiums of segmentary and subsegmentary bronchi. Prevalence of catarrhal-sclerotic changes of mucous membrane corresponding to atrophic form of endobronchitis. Isolated scler-

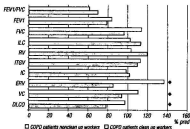


Fig. 15.3. Characteristic of respiratory disorders by Lung Function Tests in COPD patients, who probably undergone radionuclides inhalation. *the differences between observed group are significant ($p < 0.05$).

rotic changes of bronchial mucous were registered in 16% cases of supervision, catarrhal ones — in 4%.

According to classification of J.Lemoine [9], the expressiveness of endobronchial inflammatory process was assessed as 0 stage in 19%, 1st stage — 59%, 2nd stage — 13%, 3^d stage — 9%. During the first ten years (1988–1997) of endoscopic monitoring the 0 (23%) and the 1st (68%) stage of endobronchial inflammatory process expressiveness prevailed, but in 1998–2009 — the 1st (51%) and 2nd (32%) stage. These data were corresponding to the purulent changes of endobronchial secretion: from on average 17% cases in 1988–1997 to 61% cases in 1998–2009.

Results of endoscopic endobronchial study in clean-up workers of Chernobyl NPP accident, suffering from COPD showed, that primary endoscopic variant of endobronchial disorder was chronic diffuse 1st stage of inflammation atrophic endobronchitis with catarrhal-sclerotic type of mucosa changes. In the remote postaccidental period the purulent changes of endobronchial secretion prevailed [3–5, 10].

Echocardiography based cardiac function was evaluated in 2009–2010 in 357 male COPD patients, clean-up workers of 1986 and 72 patients of comparable control group without effect of ionized radiation. Age was (58.1±5.6). Doses of external exposure for first group were in diapason of 1–50 cSv. COPD patients from the group of clean-up workers had significant difference in Left Ventricular Evacuation Function (LVEF) level (38.1±5.6%) with control group (47.2±8.5%). More than 70% patients of main group showed features of chronic *cor pulmonale*, followed by diastolic dysfunction (58.7% in main group, 32.4% in control group) and left ventricular hypertrophy (64.1% in main group, 34.1% in control group). Mild pulmonary artery hypertension (PAH) was detected in approximately half of patients of main group (53.1%) and in 24.8% in control group, moderate PAH in 19.6% patients of main group and 13.2% of control group. Thus COPD patients clean-up workers had significantly more hard clinical course of concomitant cardiovascular pathology than COPD patients of control group [11].

The study of immunity in COPD in radiation exposure were carried out during 1988–1997 (1st subgroup, n=109) and 1998–2009 (2nd subgroup, n=117) for 236 clean-up workers of 1986–1987 (basic group). Middle age was (43.6±3.8) years. Duration of the disease according to anamnestic data was (9.4±3.2) years. Significant clinical distinctions between groups were not established. Individual dose levels were within the limits of 12–64 cSv.

The control group was made by practically healthy persons living in conditions of a natural radiating background.

Differentiated blood cell count did not find significant differences between the basic and control groups, except reliably higher erythrocytes sedimentation rate among patients from the basic group.

Statistically significant distinctions in CD3⁺, CD8⁺ and CD22⁺ lymphocytes counts were revealed. Changes in T-system were accompanied by increase of relative quantity of T-suppressors at deficiency of the general population of T-cells, and decrease of regulatory subset ratio CD4⁺/CD8⁺ (Fig. 15.4). The tendency to the increase of T-active/cytotoxic lymphocytes number was marked in them as well as essential increase of CD22⁺ lymphocytes quantity and disimmunoglobulinemia.

The researches that have been carried out for the 2nd subgroup of patients have shown, that patients had changes, both in content of the basic subsets, and in internal correlation of immune system communications (Fig. 15.4).

The revealed changes of cellular link were not accompanied by significant deviations of the content of serum immunoglobulins IgA, IgG, IgM, except for reduction of IgA level in the group of patients with COPD. Besides it, at patients with COPD in the remote period after clean-up works substantial increase of the content of large and small circulating immune complexes was established.

Thus, in cases of COPD clean-up workers had marked changes of cellular and humoral immunity. These changes during supervision over patients varied and, quite often, were different, in dependence of term after participation in clean-up works. During 1988–1997 the COPD occurred on a background of reduced quantity of T-lymphocytes which was accompanied by decrease of expression on cell surface functionally active receptors — CD3, CD2, HLA-DR. Decrease of CD3⁺ T-lymphocytes content in the surveyed patients of the 1st subgroup correlated with the increased number of somatic mutations in the T-cellular receptor locus that testified to value of radiation-induced processes in formation of functional insufficiency of T-cells. Immune deficiency in this period in clean-up workers with the highest dose level was accompanied by formation of atrophic forms of endobronchitis in COPD that can reflect morphogenetical value of immune changes.

For the 2nd subgroup of patients the parity T- and B-links ratio was marked. CD3⁺ cells content in patients with COPD was higher, than in the control. These changes were characteristic as well for other groups of observed patients in the remote period after irradiation and were accompanied by a decrease of radiation-induced somatic mutations number up to norm. At the same time, in this period increase of circulating immune complexes was found out, which had not been marked in patients at earlier terms. The possible reason was restoration of B-link functioning in reply to antigenic stimulation. Clinical course of COPD was accompanied by raised CD8⁺ lymphocytes count whereas the majority of researchers in the remote period after irradiation marked decrease of number and functional activity of T-suppressors. Probable explanation is the intensity of cytotoxic reactions just as it observed in groups of the individuals who had survived nuclear bombing and in participant of clean-up works with other kinds of a somatic pathology [12, 13].

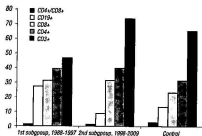


Fig. 15.4. Dynamics of the basic subset of lymphocytes change at patients with COPD after participation in clean-up works of Chernobyl NPP accident

Flow cytometric analysis of bronchoalveolar lavage leukocytes was performed in 62 patients exposed in 1986–1987. Leukocytes were identified by CD45 common leukocyte antigen expression, three-part differential was used for granulocytes, lymphocytes and monocytes/macrophages discrimination. Antibody panel included CD3, CD4, CD8, CD10, CD13, CD14, CD16, CD19, CD33, CD45, CD56, HLA-DR. Additional staining of intracellular matrix was performed with anti-cytokeratin. Squamous metaplasia and cell atypia of bronchial epithelium in the cases of atrophic and sclerotic changes were associated with high cytokeratin expression. Granulocytes exhibited high CD45 expression. Prevalence of CD8⁺56⁺ cytotoxic cells was shown among the lymphocytes with high HLA-DR expression while CD4⁺ subset size was significantly lower than in peripheral blood samples. Quantitative studies of antigen expression showed similarity of average figures with the peripheral blood for most of the differentiation antigens while HLA-DR expression on CD8⁺ cells and monocyte/macrophage cells was elevated. Somatic mutations study does not reveal variant lymphocytes lacking T-cell receptor. Chronic progradient course of disease showed lower signs of cytotoxic cell activation and granulocytes counts. Increase of circulating immune complexes concentrations was not associated with significant activation of B-cells from broncho-alveolar lavage (BAL). Differences in subset organization revealed by flow cytometric analysis could indicate activated cell tropism to lung tissue and also be the markers of an involvement of cytotoxic mechanisms in the formation of late post-irradiation bronchopulmonary disorders [14].

Summarizing this study, it is necessary to specify the stages of COPD immune mechanisms after participation in clean-up works with the prevalence of radiation-caused immunological deficiency at the first stage and cytotoxic, and also immune complex reactions in the period of the remote effects [12–14].

When studying dynamics of a bronchi mucous membrane structural changes, patients — participants of clean-up works were conditionally divided into two subgroups, depending on time of the postaccidental researches: 1988–1997 (1st group) and 1998–2003 period of supervision (2nd group).

The received results allowed to verify structural conformity to clinical symptoms of pathomorphosis of COPD in clean-up workers of Chornobyl NPP accident who undergone radionuclides inhalation. Those can be systematize as peculiarity of microvessels damage, infringement of fibrillogenesis and modification of inflammatory chronisation [2–5, 10].

Transformation of regenerative processes is defined as infringements of epithelium repopulation kinetics with pathology, both proliferation and differentiation. According to the structural complex analysis one of the most typical attributes describing infringement of cellular population kinetics of bronchus mucous membrane in clean-up workers, is basal-cellular hyperplasia (Fig. 15.5).

Its expression did not decrease with increase of term of supervision (1988–1997). High proliferative activity of basal cells was kept and at change adjoining basal membrane, down to it hyalinosis (Fig. 15.6), i.e. on conditions that infringement of mechanisms of plastic, information and power provision of proliferation mechanism. To possible pathological transformation of basal cells genome testified their nucleus atypia, found out in some cases, shown by increase of the sizes, change of the form, carioteci

winding, hyperchromia, mosaic lying of chromatin.

Hypertrophied filaments were frequently present at cambial elements cytoplasm, therefore it is possible to assume their primary planocellular differentiation (Fig. 15.7). In this case the high frequency of planocellular metaplasia superficial bronchial epithelium at the individuals, exposed to radiation was seen. Metaplasia of multiseriate cylindrical epithelium into multilayered plane epithelium was shown at the ultra structural level by change of the cells form and degree of their differentiation, infringement of intercellular contacts, and nucleus atypia as well.

At cytoplasm of plane epitheliocytes small electronic dense granules were frequently present containing, probably, keratohyalinum. Besides, in plane cells individual secretory granules were found out similar to mucoid ones (Fig. 15.8). A secret emission thus was carried out not in a lumen of a bronchi tube, but into intercellular spaces. Superficially located cells were completely deprived of cilia, having only rare microfibers. It is logical to assume, that the described phenomena testify for the benefit of «perverted» planecellular differentiation of mature elements of bronchus superficial epithelium — goblet and cilia cells. Determination of this process by secretory function is not excluded, because goblet cells in tracheas and bronchial epithelium make secretion cyclically. Having excreted the contents on a surface of a mucous membrane, they get a kind, not characteristic as for ciliated, and mucous cells. In such condition on their surface appeared irregularly located microfibers.



Fig. 15.5. Basal-cellular hyperplasia of superficial epithelium of mucous membrane of a bronchus. Stained with hematoxylin and eosin. $\times 400$

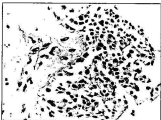


Fig. 15.6. Hyaline basal membrane and dysplastic changes of proliferative superficial epithelium of a bronchus. Stained with hematoxylin and eosin. $\times 400$

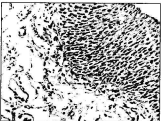


Fig. 15.7. Planocellular metaplasia of superficial epithelium of a bronchus mucous membrane. Stained with hematoxylin and eosin. $\times 200$

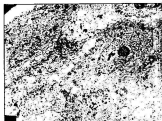


Fig. 15.8. Small electron dense of epithelites of a bronchus superficial epithelium. Microphoto $\times 7000$.



Fig. 15.9. Heterogeneous on density granules in cytoplasm of a mucous cell of bronchial superficial epithelium. Microphoto, $\times 11000$.

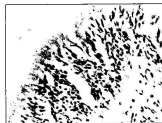


Fig. 15.10. Associative hyperplasia of basal and cilia cells of bronchial superficial epithelium. Stained with hematoxylin and eosin. $\times 400$.

Pathological changes of nucleus with rare figures of mitosis in sites of planocellular metaplasia were submitted in the greater degree, than in locus of basal-cellular hyperplasia. In supervision 1988–1997 planocellular metaplasia of bronchus superficial epithelium was combined with hyalinosis of it basal membrane. Apparently, these two processes are mutually conditioned.

In 1st clinical group the local hyperplasia of mucous cells took place only in some occasions and it was connected with basal-cellular hyperplasia, having the tendency to increase the frequency of occurrence during supervision. Recycling mucous epitheliocytes contained granules of heterogeneous density and zones of essential condensation of a secret except for homogeneous ones (Fig. 15.9).

These facts, probably, testify to change of a chemical compound of the mucus, produced by epithelium, produced by epithelium, that causes infringement a functional consistency of bronchi both due to reduction of a secret production, and in connection with its qualitative transformations. As a whole it promotes severe course of a disease and it resistance to the standard treatment. The bronchial secret plays the important role in the maintenance of the respiratory system homeostasis due to the participation in maintenance of the effective clearance mechanism, and due to bacteriostatic properties and the ability to condition air as well.

In earlier terms of supervision (1st group) the sites of bronchial superficial epithelium, deprived of cilia cells, were typical (in the presence of cambial elements hyperplasia). Probably, it is a special case of modification or switching of a genom site, which determine

the "cilia" orientation of cambial elements of epithelium differentiation. After 1997 combinations of basal and cilia cells hyperplasia demonstrated and become more and more typical (Fig. 15.10).

It was remarkable, that cilia epitheliocytes had normal ciliar apparatus only in few individuals. At the same time various changes of them were mentioned: merge of separate cilia into groups, desquamation of superficial cuticle, disorganization of basic filaments inside the cilia, polymorphic cytoplasmical diverticuli on apical cell surface. At the majority of patients of 1st group the full reduction of cilia took place. In supervision 1998–2003 hyperplastic changes both cilia and microfibers (Fig. 15.11) were revealed. Probably, these changes in even can cause the partial restoration of cilia cells function.

Prevalence of cilia apparatus damage and infringement of accumulation and emission of mucus, certainly, reduce protective properties of a mucous membrane, certainly, reduce protective properties of a bronchial mucous membrane, promote penetration of microbodies and synchronization of an inflammation. For all period of supervision (1988–2003) in three clean-up workers severe dysplasia of bronchial superficial epithelium was revealed, in one — carcinoma in situ, and also in fourteen cases the first time diagnosis for central cancer was established. However, deformations of epithelium basal membranes (as an extracellular attribute of dysplasia) were found out much more often, especially in patients, who had stayed for the long time (more than a year) in a zone of increased radiation (Fig. 15.12).

Dysplastic changes of epithelium were found both in sites of planocellular metaplasia, and outside of them.

Thus, at the clean-up workers suffering from COPD, infringements of a sequence of phenotypical expression of proliferated potentialities of bronchial mucous membrane superficial epithelium cells were found out.

More than in half of supervisions it was possible to verify the «tracks» which were settled down above epithelium and lamina propria of a bronchial mucosa by means of histosautoradiography with the help of photosensitive emulsion. Presence in a material of the

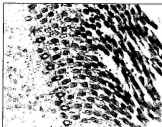


Fig. 15.11. Hyperplastic changes of cilia in fibillar cells bronchial superficial epithelium. Microphoto. $\times 1400$

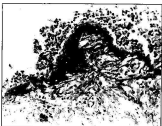


Fig. 15.12. Expressed deformation and sclerosis of a bronchial epithelial basal membrane. Stained by method MSB. $\times 100$

same bronchial bioplate of various kinds of radioautographs confirmed the presence of non-uniform mix radionuclides. In bronchial bioplates of the control group, received from patients with COPD till April, 1986, sources of ionizing radiation were not found out.

During all investigated period damages of microcirculation vessels of bronchial mucous membrane were verified.

Changes which have been found out in microvessels of a bronchus mucous membrane in the early period of supervision, differed by the form and intensity and testified, first of all, to their alteration. Intravessel damages and damages of vessel walls and surrounding tissues are marked too.

The greatest prevalence among intravessel infringements of microcirculation was revealed with aggregation of elements of blood, mainly erythrocytes. As a rule, it had the extreme image — sludge at which erythrocytes part hemolysis occurred, or erythrocyte blood clots were formed. In some cases adhesion of erythrocytes to endotheliocytes (Fig. 15.13) was marked. At a lumen of vessels macrovesicles were present, generated by a membrane such as plasmatic one.

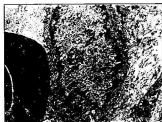


Fig. 15.13. Adhesion of erythrocytes to endotheliocytes in a microvessel of a lamina propria of a bronchial mucous membrane. Microphoto, $\times 15000$



Fig. 15.14. Squamotic, necrobiotic changed endotheliocytes in a blood vessel lumen in lamina propria of bronchial mucous membrane. Microphoto, $\times 1500$.

ated by a membrane such as plasmatic one. «Erythrocytic shadows» which are described by many researchers in vessels after radiating impact, had other kind. There were destroyed elements of blood, cellular detritus, squamotic necrobiotic changed endotheliocytes (Fig. 15.14) myelinoids etc. in intravessel space.

Changes of endothelium were characterized by dystrophic process. Vacuolisation of cytoplasm, accumulation of lisosomiclooking bodies, osmophilic and myelin-like structures, crystallization of mitochondria, expansion of perinucleus spaces etc. took place. In the early period of research, in two supervision, the expressed changes of endotheliocytes nucleus were found. In one case karioteka formed deep invaginations due to which in nucleoplasma cytoplasmical inclusions were formed in nucleoplasma (Fig. 15.15), and in the other — dramatic alteration of nuclei with their inversion, partial lysis of karioteka and vessel lumen occlusion (Fig. 15.16) was marked.

At some patients it is marked significant condensation of cytoplasmatic matrix at which were hardly distinct endocellular organoids. The increase of

luminal and basal surfaces of microvessels due to formation of numerous of digitate cytoplasmic diverticula of endotheliocytes was regarded by us as compensatory reaction to decrease of the transcapillary exchange, the insignificant quantity of micropinocytotic vesiculi testified to it.

One more prominent feature of microvessel elements channels of patients — clean-up workers was multilayer and fragmentation of vascular basal membrane, probably, connected to constant change of endotheliocyte population (Fig. 15.17). It is necessary to emphasize, that similar changes of basal membrane of microvessels are described at senile age (after 80 years).

In some cases of the second period of observation microfilaments were in a zone of endotheliocytes accumulated (Fig. 15.18). Expression of given symptom still needs the explanation.

It was interesting also to note the absence at studied individuals of one of inflammatory process characteristic attribute — neutrophilic leukocytes adhesion to endotheliocytes and diapedesis. Microhaemorrhages released in the lamina propria of bronchial mucous membrane were probably connected with traumatic procedure of bioplate extraction, but not with disruption of vascular wall integrity. Typical perivascular changes were characterized by insignificant cellular infiltration and by perivascular or diffuse sclerosis (Fig. 15.19).

In first five years after accident a presence of myelin-like structures in perivascular space and in lamina propria of bronchial mucous membrane was often marked.

In some supervision of the last period the tendency to increase of

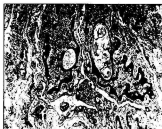


Fig. 15.15. Deep invaginations of karyotexa with formation of cytoplasmic inclusions in endotheliocyte nucleoplasm. Microphoto. $\times 11000$.

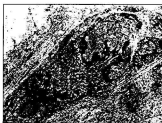


Fig. 15.16. Partial lysis of endotheliocyte karyotexa and vessel lumen occlusion in a lamina propria of bronchial mucous membrane. Microphoto. $\times 13300$.

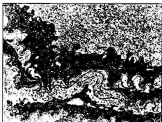


Fig. 15.17. Multilayer and fragmentation of vascular basal membrane. Microphoto. $\times 20000$.



Fig. 15.18 Hypertrophied microfilaments in a perikation zone of endotheliocytes a microvessel of lamina propria bronchial mucous membrane. Microphoto. $\times 3800$.

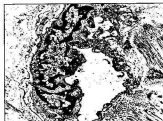


Fig. 15.19 Perivascular sclerosis of lamina propria of a bronchial mucous membrane. Microphoto. $\times 1100$.



Fig. 15.20 Extended sclerosis of bronchial wall. Stained by method van-Gieson. $\times 30$.

capillaries quantity in subepithelium layer of bronchi was found. That can be explained as structurally functional adaptation of organs under pathological conditions of functioning (hypoxia).

Wide spectrum of transformations of bronchial mucous membrane vascular structures, and also the various degree of their expressiveness in those or other cases, allows to assume both the direct influence of various damaging factors on microcirculatory vessels, and indirect, dependent on specific body features. Nevertheless, characteristic alteration of endotheliocytes in early terms of observation does not exclude direct damaging influence of radiation on these cells.

The convincing pathomorphological proofs were received that on the basis of chronic endobronchitis progressing in the individuals, exposed to radiating influence, lay the sclerotic changes of bronchus walls. Deep infragments of fibrillogenesis first of all were present at lamina propria of their mucous membrane. At the majority of patients — clean-up workers, as opposite of nosologic control, both widespread sclerotic processes in deeper layers of bronchial walls (Fig. 15.20) and fibroplastic deformations of epithelial basal membranes were found.

In those supervision where the bronchial bioplate volume allowed to estimate a condition of muscular plate and submucous layer, superfluous collagen creation in these microregions was identified.* Sclerotic processes in a muscular plate were accompanied by its atrophy and local hypertrophy, and in submucosa layer — dystrophic, atrophic and local hyperplastic changes of glands. Expressiveness of sclerosis grew in time course: sclerotic changes,

except for separate observation, were inherent almost to all patients with the over five years anamnesis of disease (2nd group).

The structural substratum of chronic inflammatory process in bronchial mucous membrane of patients — clean-up workers, in comparison with control parameters, was characterized by smaller intensity of inflammatory-cellular infiltration. Its qualitative structures including lymphocytes, leukocytes, plasmatic and mast cells, monocytes and macrophages were met extremely seldom. In the 1st group of patients lymphocytes and leukocytes prevailed, and in patients of 2nd group the quantity of mast cells was essentially increased. The low content in infiltrates of immunocompetent cells testified for the benefit of hyporeactive course of inflammation in exposed individuals. The transformed manifestations facilitate microbic invasion (Fig. 15.21). The fact of frequent detection of fungoid infection during second period of observation is worthy of notice [14, 15].

Taking into account, that planocellular metaplasia and cellular atypia are precancer conditions, inspection of clean-up workers of Chornobyl NPP 1986–1987 demands pulmoneological vigilance [15].

Studying of a bronchi mucous membrane structural changes in clean-up workers of 1986–1987 have shown the negative relationships between proliferation of the transformed epithelial cells and activation of immunity. We observed groups included 20 COPD patients (with FEV1 41–64% pred), 6 lung cancer and 10 controls to reveal possible association between expression of airway epithelial cell matrix proteins, lymphocyte activation and gene expression in COPD and lung cancer. Lavage and peripheral blood cells were analyzed by flow cytometry with monoclonal antibodies to Vimentin, Cytokeratin, BER-EP4, EGFR, Ki-67, HER-2 and lymphocyte activation antigens, gene expression by RT-PCR. In COPD cases a combination of normal expression of EGFR, HER2 was shown with low Ctk⁺, Vim⁺ and BER-EP4⁺ cell numbers. Lymphocyte activation was associated with overexpression of BAX, MAPK14 (p38) and absence of TGFR gene expression. Divergences in expression of proteins of cellular matrix of Ki — 67, HER2 it is not educed between the radiation-exposed and non-irradiated persons. In lung can-

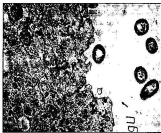


Fig. 15.21. Microorganisms at apical surfaces of damaged bronchial tubes superficial epithelium. Microphoto. $\times 12000$.

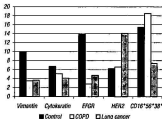


Fig. 15.22. Distribution of bronchoalveolar lavage cells expressing cytoskeleton matrix and cytotoxic lymphocyte proteins.

cer cases high Ki-67⁺ and HER2⁺ epithelial cells indices were accompanied with low EGFR⁺, Cdk⁺Vim⁺BER-EP4⁺, low percentage of CD25⁺, CD38⁺ and bcl2⁺ lymphocytes with absence of BAX and MAPK14 overexpression (Fig. 15.22.) [16].

Obtained data support the hypothesis of the lower reactivity of peripheral blood and airway lymphocytes to the epithelial cell cytoskeleton proteins in COPD and cancer. Association of the cytotoxic cells activation in COPD with bcl2 and mitogen-activated protein-kinase genes could represent immune response to secondary factors including infection.

Conclusion

Radiation-dosimetric and technological features of Chernobyl accident have caused a situation at which lungs and respiratory ways were one of the main targets-tissues responsible for development of radiation pathology under the influence of external sources of radiation and inhalation of radionuclides. The analysis of the dosimetric and clinical-epidemiological data testifies that the basic group of risk on realization of stochastic and non stochastic effects of influence of ionizing radiation and inhalations of radionuclides in conditions of Chernobyl accident are the clean-up workers 1986–1987.

The ChB and COPD in clean-up workers 1986–1987 manifested during the first 3–7 years after participation of patients in execution of postaccidental works.

The analysis of anamnestic data showed that at clean-up workers during the works and in the nearest period after works in zone of Chernobyl NPP coughing syndrome without attributes of sputum was present. The subsequent course of disease was characterized by "poverty" of a clinical picture with progressive development of obstruction syndrome and formation of dyspnea. In the later period the pathology got features of COPD.

Endoscopic investigation of mucous membrane of a tracheobronchial tree among clean-up workers, suffering from COPD has established, that to the basic endoscopic version of affection of bronchi was the chronic diffuse atrophic endobronchitis, 1st degree of inflammation with catarrhal-sclerotic change of mucous membrane.

Pathomorphological studying of bronchobiopsates allows to systematize structural equivalents of clinical pathomorphosis of COPD at patients — clean-up workers as transformation of regenerative processes, special damage of microvessels, disorders of fibrillogenesis and modification of inflammatory chronization. Taking into account, that planocellular metaplasia and cellular atypia are precancer conditions, investigation of clean-up workers 1986–1987 demands pulmononcologic alarm.

It is necessary to note the vicissitude in formations of immunologic reactions at patients with COPD after participation in the accident consequences clean-up works with prevalence of radiation-caused immunodeficiency at the first stage and cytotoxic, as well as immunocomplex reactions in the remote period.

Thus, the given data testify, that for victims of Chernobyl NPP accident (first of all clean-up workers) presence bronchopulmonary pathology such as COPD with some special clinical, endoscopic, pathomorphological and immunological features is typical. It requires the creation of adequate methodology for diagnostics and treatment of bronchopulmonary diseases for the given contingent.

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CHAPTER 16 MALE REPRODUCTIVE HEALTH

The territory of Ukraine is highly saturated with nuclear power plants (NPP). The exploitation of the NPPs is not so safe because an unforeseen loss of radionuclides sometimes may occur. In this connection in April 1986 the whole world became a bystander of the great catastrophe on Chornobyl NPP which resulted in wide scale pollution of territories by different radionuclides. The short-lived iodine radioisotopes were only active for a few months while the stable radioisotopes of strontium, caesium and plutonium penetrated in soil, rivers, plants and animals and thereby spread far beyond the confined zone of the accident [1]. Presently the special radiodosimetric maps are available which delineate strictly the areas with high, middle and low radiation pollution.

It is well known that ionizing radiation is very harmful for highly proliferative tissues and dividing active cells. The human reproductive system, specifically the male germ cells, belongs to this category. Therefore different anomalies of the male reproductive system may emerge owing to the short or long-term exposures to ionizing radiation.

Fecundation is the main event leading firstly to the multicellular embryo and then to the born child. Its realization in the female oviduct requires the simultaneous of egg cell along with spermatozoon, the latter being delivered from outside by sperm ejaculate. Primarily, the spermatozoa are formed in testes in the course of two meiotic divisions from spermatocytes, which in turn originate from spermatogonia. The time span for restoring spermatozoa stock in human is 72 day long.

Successful fertilization requires a sperm plasma membrane with normal integrity and function. The numerous functions of spermatozoid membrane are related to cell metabolism, for maintaining sperm motility, capacitation, acrosome reaction and sperm-oocyte interaction [2]. One of the key processes in human fertilization is the acrosomal reaction, usually triggered in sperm upon their binding to the zona pellucida of the oocyte. As a result of it the acrosomal content is released, that enable penetration of the sperm into the oocyte.

The final function of the sperm cell is to fuse with the egg plasma membrane. At the moment of fusion it ceases to be a sperm and becomes part of a newly formed cell, the zygote. The fusion between sperm and egg plasma membranes is the culmination of a sperm journey that relies on both sperm motility and the interaction between the sperm surface and the various environments that it encounter during its progression towards the egg [3].

The early phase of disruption of the integrity of the spermatozoid membrane is characterized by the loss of phospholipids asymmetry due to translocation of phosphatidylserine from the inner to the outer leaflet of the plasma membrane [4]. This transloca-

tion of phosphatidylserine is one of the earliest features of cells undergoing apoptosis. Spermatozoa showing evidence of phosphatidylserine exposure were found in human semen at higher levels in infertile men than in normal sperm donors.

Normal spermatogenesis depends on the efficiency of apoptosis. Approximately 25-75% of germ cells degenerate in the adult mammalian testis. Consequently, the basal apoptotic cell population in the ejaculated sperm might result from abortive apoptosis of sperm that escaped the elimination mechanism operating during spermatogenesis [5].

DNA-damage due to apoptosis has been found to occur in spermatozoa of poor quality as measured with the terminal deoxynucleotidyl transferase (TdT)-mediated UTP-nick and end label (TUNEL) or Comet assay. The presence of DNA fragmentation in ejaculated spermatozoa is more evident in atypical forms, confirming that morphology and sperm count correlate with testicular function.

Elevated percentages of apoptotic spermatozoa have also been found after infections of the reproductive tract, cancer and other pathologies [6]. However, scarce data have been published concerning the mechanisms involved in cell death of ejaculated sperm from normospermic donors. This is important to elucidate especially in those pathologies where normal spermatozoa are exposed to non-physiological damaging agents, for example elevated levels of reactive oxygen species (ROS) and radiation [7]. ROS are a known inductor of apoptosis in somatic cells and in maturing spermatozoa at the testicular level, radiation is responsible for chromosome aberrations and DNA fragmentation in spermatozoa.

Microscopic and physicochemical examinations of semen are routinely applied for the diagnosis of male infertility. Even though this conventional analysis of semen gives considerable information, its limitations for the prediction of male fertilizing ability are well recognized. Thus, the application of new and more reliable technologies is needed [8].

A variety of tests have been recently proposed to explore new parameters in sperm cells. Assays based on flow cytometry (FCM) are potentially of great interest, because of their capacity to increase the accuracy, discriminating power, and speed of semen analysis. Moreover, FCM allows the simultaneous measure of several biological characteristics at the single-cell level as well as the automatic analysis of several thousand cells in a few seconds, thus providing statistically reliable samples. Therefore, the stainability of DNA with intercalating dyes and the mitochondrial membrane potential of sperm cells evaluated by FCM appear to be useful markers related to fertility potential [9].

Concerning sperm chromatin, the presence of spermatozoa possessing a nuclear chromatin less tightly compacted than in normal mature spermatozoa has been reported in samples from infertile donors, in comparison with those from fertile men. Several assays, mainly based upon the DNA intercalating fluorescent compounds (acridine orange, ethidium bromide, propidium iodide), have been developed to evaluate by FCM the presence and the number of such abortive spermatozoa [10].

The propidium iodide staining procedure allowed noting in semen the presence of cells at different maturative stages, the technique being equally effective on cells separated from seminal fluid, on ejaculates *in toto*, and on sperm cells frozen in liquid nitrogen as well as on fresh sperm cells [10].

Commonly, the percentage of immature cells is significantly higher in semen samples from infertile donor compared to control ones. The percentage of haploid im-

mature spermatozoa is negatively correlated with cell concentration in semen, and this phenomenon is particularly evident in semen from oligozoospermic subjects [11]. Apart from this, some authors have reported that sperm cells with a chromatin less condensed than in normal mature spermatozoa represent spermatozoa in which histones have not been replaced by protamines during spermiogenesis. This phenomenon, partially responsible for the increase in fluorochrome stainability, has been associated with infertility. Moreover, it was reported that spermatozoa which bind more fluorochrome contain endogenous DNA nicks, thus explaining anomalies in the chromatin packaging process occurring during spermiogenesis in some patients. The endogenous nicks have been postulated to be necessary to facilitate the protamination of DNA, but they disappear once the maturative process has been completed. The presence of endogenous nicks in ejaculated human spermatozoa might be related to apoptosis [12].

It was shown that FCM and light microscopy detect different alteration in sperm cells and that the maturative defect of the chromatin is not mirrored by changes in cell morphology [13]. Thus, it appears that sperm chromatin analysis by FCM could represent an independent assay, valuable for diagnosis of pathological condition not detectable by conventional analysis. Therefore, the results obtained by FCM concerning the nuclear maturity by propidium iodide and the analysis of mitochondrial membrane potential by rhodamine 123 or cationic dye JC-1 may provide reliable data that can be used for diagnosis of male infertility and further understanding of the cellular and molecular basis of these disorders. Furthermore, elucidation of phosphatidylserine translocations in plasma membrane by Annexin V-FITC analysis on flow cytometer is of great use for quantification of apoptic spermatozoa in semen samples [14].

According to the radiobiologic law, established, by Bergonje and Tribando, in the 20th century, ionizing radiation first of all damages physiologically active dividing cells, which are present in abundance in highly proliferative male germinal epithelium. Ionizing radiation produces highly reactive free radicals which cause lipid peroxidation and membrane damage. In contrast to other cells, spermatozoa do not possess an effective antioxidant system of lipid defense because of little catalase activity. This may cause severe damage to spermatozoon structure as it was shown by several authors [15].

Ionizing radiation is very harmful for germ line cells because of their high proliferative activity. Thereby different cell injuries may occur causing the loss of fertility potential and male infertility development. Regarding high radiation pollution of some territories in Ukraine, the pursuing of the research concerning the evaluation of male infertility risk in Ukrainian population will be of great concern and necessity.

The present research aimed in evaluation quality and fertility potential of sperm collected from donors originating from radiation-polluted regions of Ukraine, namely Zhytomyr, Kyiv, Ivano-Frankivsk and control Poltava region, respectively. Apart from this, the role of radiation component in sperm damaging was to be assessed.

Materials and methods

Patients and Regions

Semen samples were obtained from 128 patients in the age interval 33 ± 6 years who have been subjected to examination in specialized hospital for inhabitants of radiation polluted territories affiliated to RCRM (Kyiv, Ukraine). All subjects were subdivided in

4 groups, namely: group I — inhabitants of Poltava region ($n=29$), group II — inhabitants of Ivano-Frankivsk region ($n=32$), group III — inhabitants of Kyiv region ($n=34$) and group IV — inhabitants of Zhytomyr region ($n=33$). Every region includes the Central city of the same name with suburbs as well as the satellite villages and small towns. According to the SRU and radioecological data Poltava region is considered as clean on convention zone because its territory is free from radionuclides. Meanwhile Ivano-Frankivsk region is regarded as low polluted territories. Kyiv region belongs to moderate radiation polluted whereas Zhytomyr region is a highly radiation polluted one. Consequently, the accumulated radiation dose for subjects from group I was calculated to be approximately zero, for group II — equal to 1 cGy, for group III — 5 cGy and for group IV — 10 cGy, respectively [16]. All subjects were asymptomatic for genito-urinary infection. All of them gave informed written consent for participation in cross-sectional studies.

Semen analysis

Freshly ejaculated semen was obtained by masturbation into sterile container after 3–4 days of sexual abstinence. Ejaculates were allowed to liquefy at 37 °C for 30 min. Semen profile was assessed by light microscopy according to the procedure proposed by the World Health Organization [17]. The variables taken into account were: volume of ejaculate (ml), liquefaction, colour, smell, pH, round cell concentration ($\times 10^6/\text{ml}$), sperm concentration ($\times 10^6/\text{ml}$), forward motility (%), morphology (%), concomitant cells (leucocytes, epithelium cells)

Flow cytometry analyses

All FCM analyses were performed by flow cytometer PAS (Partec, Germany) equipped with a single 488-nm argon-ion laser. FL1 (FITC) signals were detected through a 530/30 nm band pass filter, FL2 (PI) signals were detected through a 585/42 nm band pass filter. 20 000 events were recorded in list mode and analyzed using "FlowMax" software (Partec, Germany). The sperm population was gated on the basis of the linear forward (FSC) and side-scatter (SSC) properties.

Determination of viable sperm, apoptosis development and necrosis

Annexine V apoptosis detection kit I (BD Pharmingen, USA) was used to detect the translocation of PS from the inner to the outer leaflet of the spermatozoa plasma membrane [4]. An aliquot of semen specimen (50 μl of sample containing $0.5 \times 10^6/\text{ml}$ sperm) was added to 145 μl of binding buffer prepared according to the manufacturer's instruction, plus 5 μl of Annexin V-FITC, and incubated at room temperature for 10 min. Samples were then washed once with binding buffer, centrifuged at 600 g for 10 min, and, finally, the pellets were resuspended in 200 μl of binding buffer containing 1 $\mu\text{l}/\text{ml}$ of PI. The FITC-labelled spermatozoa were analyzed by flow cytometer.

The different labelling patterns in the bivariate PI/Annexin V-FITC analysis identify the different cell populations (Fig. 16.1). We qualified sperm as normal (negative Annexin V and PI), early apoptotic (positive Annexin V and negative PI), apoptotic (positive Annexin V and positive PI) and necrotic (negative Annexin V and positive PI). We defined as the apoptotic index the ratio between the positive Annexin V negative PI sperm and the total living sperm (PI negative).

Determination of ROS by flow cytometry

Intracellular generation of O_2^- was estimated using dihydroethidine (DHE) of "Sigma" production, a membrane permeating uncharged probe that reports overall cellular O_2^- production through oxidation to ethidium bromide and emitting red fluorescence [18]. ROS generation was induced by the addition of DHE to spermatozoa in suspension with concentration 10^7 cells per ml to give a final concentration of DHE equal 2 mM, SYTOX Green a cell viability stain with green fluorescence being also added to the final concentration 0,05 mM. Then the spermatozoid suspension was incubated for 15 min at 37°C, centrifuged for 5 min at 600 g and resuspended in Biggers-Whitten-Whittingham (BWV) medium. The latter consisted of 95 mM NaCl, 44 mM sodium lactate, 25 mM $NaHCO_3$, 20mM HEPES, 5,6 mM D-glucose, 4,6 mM KCl, 1,7 mM $CaCl_2$, 1,2 mM KH_2PO_4 , 1,2 mM $MgSO_4$, 0,27 mM sodium pyruvate, 0,3% (w/v) BSA, 5 U/ml penicillin and 5 mg/ml streptomycin.

Assessment of mitochondrial membrane potential by flow cytometry

Fluorometric assessment of mitochondrial membrane potentials was made on the basis of mitochondrial specific probe Rhodamine 123 (Sigma) (R123) [9]. The latter is a cationic compound that excites at 488 nm and emits at 515–575 nm (green fluorescence). It accumulates in the mitochondria as a function of transmembrane potential, of R123 concentration and of sperm numbers. A combination of R123 and propidium iodide has been shown to readily discriminate between living and dead spermatozoa. For the assay to the spermatozoid suspension with concentration 2×10^7 per ml was added 12 μ l of 2.99 mM PI in Tyrodes Salt Solution/ml BWV plus 3 μ l of 0.53 mM R123 in DMSO/ml BWV. Samples were incubated for 30 min at 37°C. This was followed by centrifugation for 5 min at 600 g and resuspension in BWV.

Statistical analysis

Comparison of data for different donors' group was made using analysis of variances, Anova, and unpaired Student's t-test with amendment of Bonferroni. Confidence intervals for mean values were identified using t-statistic at $P=0.95$ and standard errors. All statistical tests were two sided and $p < 0.05$ was considered statistically significant [19, 20].

Results

The data on sperm quality of donors from different regions of Ukraine who participated in randomized cross-sectional studies concerning fertility potential of spermatozoa are presented in Table 16.1.

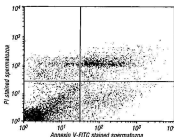


Fig. 16.1. Annexin V/propidium iodide (PI) bivariate analysis used to identify the different cell subpopulations. The lower left quadrant of each graph contains Annexin V negative/PI negative viable, non-apoptotic sperm. The lower right quadrant shows Annexin V positive/PI negative early apoptotic sperm. The upper right quadrant represents Annexin V positive/PI positive apoptotic sperm. The upper left quadrant contains Annexin V negative/PI positive sperm necrotic sperm.

Table 16.1. Sperm quality in different groups of donors

Group number, N	Region's name	Volume of ejaculate (ml)	Concentration of spermatozoa ($\times 10^6/\text{ml}$)	Sperm normal motility, %	Sperm low motility, %	Sperm immotility, %	Degenerative sperm, %
I	Poltava, (control)	3.8 $\pm$ 0.1	127 $\pm$ 12	52 $\pm$ 3	28 $\pm$ 3	20 $\pm$ 2	17 $\pm$ 2
II	Ivano-Frankivsk (1 rem)	2.8 $\pm$ 0.2*	85 $\pm$ 5*	43 $\pm$ 2*	32 $\pm$ 2	25 $\pm$ 1*	26 $\pm$ 1*
III	Kyiv (1-5 rem)	2.0 $\pm$ 0.2*	95 $\pm$ 6*	43 $\pm$ 1*	28 $\pm$ 2	29 $\pm$ 1*	31 $\pm$ 2*
IV	Zhytomyr (5-10 rem)	1.6 $\pm$ 0.3*	60 $\pm$ 4*	43 $\pm$ 1*	22 $\pm$ 1*	38 $\pm$ 1*	36 $\pm$ 2*

$p < 0.05$, significant difference with control

It is seen that mean value of ejaculated volume gradually diminishes from 3.8 ml in Poltava region to 1.0 ml in Zhytomyr. Meanwhile the mean value of sperm concentration in ejaculate was shown to be highest in the samples from Poltava's inhabitants and to be equal to 127 million/ml. Interestingly, for Kyiv this index was more than for Ivano-Frankivsk, although the smallest magnitude was detected for Zhytomyr being equal to 60 million/ml. Similar mean values of normal sperm motility were found in all 3 radiation polluted regions, while for Poltava's inhabitants the utmost meaning of the parameter was established to be equal 52%.

In the most radiation polluted region — Zhytomyr the sperm samples were characterized by a large proportion of degenerative spermatozoa along with immobile sperms. In Ivano-Frankivsk and Kyiv these variables exceeded those for Poltava but were significantly less than for Zhytomyr. In the Table 16.2 the data concerning the subpopulations in sperm samples from different donors are presented.

Table 16.2. Determination of sperm subpopulations in different donors' groups using Annexin V and PI staining

Group number, N	Region's name	Annexin V-PI ⁻ , %	Annexin V-PI ⁺ , %	Annexin V-PI ⁺ , %	Annexin V-PI ⁺ , %
I	Poltava, (control)	70 $\pm$ 14	10 $\pm$ 7	11 $\pm$ 8	9 $\pm$ 6
II	Ivano-Frankivsk (1 rem)	64 $\pm$ 11	10 $\pm$ 8	15 $\pm$ 8	10 $\pm$ 4
III	Kyiv (1-5 rem)	61 $\pm$ 10	11 $\pm$ 7	17 $\pm$ 11	12 $\pm$ 7
IV	Zhytomyr (5-10 rem)	54 $\pm$ 9	11 $\pm$ 8	20 $\pm$ 13	15 $\pm$ 10

Sperm viability ($V^-/PI^- + V^+/PI^-$) was the highest in the samples from Poltava's donors, the mean value being 80%. For donors of Ivano-Frankivsk and Kyiv the differences in sperm viability were insignificant, while for Zhytomyr poor sperm viability was established. The quantity of necroses in spermatozoa tended to increase slightly with the rise of the mean value of accumulated dose for studied regions. Changes for non-viable apoptotic spermatozoa were more pronounced. Early signs of apoptosis development in spermatozoa were shown to progress gradually between Poltava and Zhytomyr. The apoptotic index for sperm samples was highest in Zhytomyr and lowest in Poltava (Fig. 16.2.)

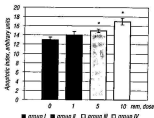


Fig. 16.2 Impact of regional differences in the means for population radiation dose accumulation on the apoptotic index of sperm samples from different regions' inhabitants. *statistically significant difference with control (0 rem), i.e. radiation free region of Poltava

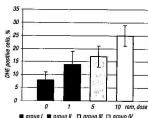


Fig. 16.3 Influence of regional differences in the means for population radiation dose accumulation on ROS production in semen samples from different regions' inhabitants. *statistically significant difference with control (0 rem), i.e. radiation free region Poltava

Raise in accumulated radiation dose was associated with elevation of ROS production in sperm samples (Fig. 16.3).

ROS production for Zhytomyr donors which accumulated 10 rem was threefold more than for Poltava's donors (0 rem) and almost twofold more than for donors from Ivano-Frankivsk (1 rem). We found differences in the mitochondrial potential ($\Delta\psi$) of spermatozoa too. The total $\Delta\psi$ tended to drop depending on the mean value of radiation accumulated dose, for Kyiv and Zhytomyr the mean values being significantly less than for Poltava (Fig. 16.4).

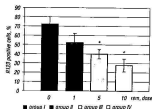


Fig. 16.4 Impact of regional differences in the means for population radiation dose accumulation on mitochondrial potential of spermatozoa in semen samples from different regions' inhabitants. *statistically significant difference with control (0 rem), i.e. radiation free region Poltava

Discussion

There are two crucial targets for ionizing radiation detrimental effects in living cells. First of all, radiation affects DNA that results in damaging nucleotides and accumulation of single along with double strand breaks in DNA structure [1, 6, 10]. However, in spermatozoon DNA presents in nucleoprotamine complex as inactive component due to highly condensed state of chromatin and the absence of whatever DNA transcriptional activity. Therefore DNA damaging just for spermatozoon functioning is of little importance. On the other hand, the spermatozoon is highly dependent on the integrity of its plasma and mitochondrial membranes due to the necessity to maintain ion-fluxes on due level. That is why membranes may represent the other essential target for radiation impact. It is well known cell membranes are rich in unsaturated fatty acids [3, 8]. These compounds are very sensitive to irradiation, especially their double bonds which are key points in radiation assault through hydroperoxide or hydroxyl radicals.

Consequently, lipid peroxidation chain reaction is launched resulting in further destruction of membranes.

However spermatozoon similar to every living cell possesses an antioxidant system of enzymic defence which through a set of enzymes, namely catalase, glutathione reductase, glutathione peroxidase 1, glutathione peroxidase 4, superoxide dismutase may catch and inactivate radicals converting them to water and oxygen [7, 11]. Our results have demonstrated that in radiation polluted territories the growth of accumulated radiation dose is accompanied with launching of apoptosis mechanism, the latter dealing with mitochondrial caspase cycle activation and further plasma membrane disintegration. Consequently, in such conditions the membrane bound antioxidant enzymes may sharply diminish their activities. In addition to this, shifts in mitochondrial membrane semipermeability according to our data caused a drop in mitochondrial potential and consequent uncoupling between electron transport and phosphorylation. These conditions are very favourable for intercepting electrons from electron transporting chain by free oxygen molecules, the superoxide radicals being produced. This pool of superoxide radicals is associated exclusively with mitochondria. In addition, there is a second pathway for superoxide radicals generation that is accomplished through special enzyme-oxidase which is located in the heads of spermatozoa [1, 2, 18].

Thereby, radiation pollution of territories and subsequent penetration of radionuclides in living organism may affect rapidly proliferating tissues, in particular generative epithelium, causing the production of defective sperm with damaged membranes, pertinent to apoptosis and with suppressed antioxidant enzymes. This idea was fully validated by our data concerning apoptotic indices of different sperm samples which proved the existence of close to linear dependence between accumulated radiation dose and said index rise. On the early stages of apoptosis phosphatidylserine translocates from internal to external side of plasma membrane thus decreasing the membrane asymmetry and distorting its function.

Abatement of antioxidant enzymic activities in radiation damaged spermatozoa facilitate the propagation of free radicals, especially superoxide radicals, in mitochondria and spermatozoid heads. In turn, ROS penetrate into the nucleus and exert a direct effect on chromatin DNA causing overall disruption and formation of apoptotic bodies. In the last stage of apoptosis development cell death follows. Our results have established the gradual increase of apoptosis dependent cell deaths comparing to necrosis dependent cell death whose contribution to spermatozoid mortality significantly diminished for highly radiation polluted regions compared to low radiation polluted and non-polluted ones. This phenomenon is a hallmark of male-infertility progress. Thus, our investigations have shown that subjects living on radiation polluted territories may generate a great deal of damaged spermatozoa with the hidden molecular and cellular lesions. The latter would predispose inhabitants of radiation polluted regions to male-infertility. Therefore a special system of prophylactic arrangements needs to be elaborated to prevent further spreading of male infertility.

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CHAPTER 17 NERVOUS SYSTEM AND PSYCHOSOCIAL ASPECTS

17.1 ACUTE RADIATION SYNDROME: NEUROPSYCHIATRIC EFFECTS

There is a strong and traditionally present opinion in the medical radiology that radiation damage of the central nervous system (CNS) is possible only under local exposure to the doses exceeding 10–50 Gy. Brain radiation necrosis was observed upon the local irradiation in doses over 70 Gy, after which the radiogenic dementia could occur. Brain tolerant dose was considered as 55–65 Gy, and fractional tolerant dose was believed as 2 Gy [1]. Primary damage of CNS under the whole-body radiation exposure is orthodoxy diagnosed only if the doses exceed 100 Gy (cerebral ARS form), while secondary radiation damage of CNS is diagnosed in case of 50–100 Gy respectively (toxemic ARS form). The total-body dose of 2–4 Gy is considered to be a threshold for radiation-induced neuroanatomical abnormalities [2].

On the other hand, the neuronal morphology abnormalities were found just after exposure to 0.25–1 Gy of total-body irradiation, and dose value of 0.5 Gy has been called “the red bar” of CNS radiation damage with primary neuronal injuries [3]. Stable disorders of cerebral electrical activity were featured with 0.3–1 Gy threshold and with increment proportionally to the absorbed dose value. CNS dysfunction is possible under the impact of relatively low radiation doses [4]. The modification of neurotransmission occurs under the irradiation leading to multiple cerebral and behavioral effects depending at most on radiation dose [5]. The diagnostic entity of slowly progressing *radiation sickness of CNS* after 1–6 Gy total-body irradiation has been announced by A.L. Karpovskiy (1985) [6]. It was stated in the UNSCEAR 1982 report that progressing cortex degeneration occurs after exposure to 1–6 Gy. The chronic progressing CNS damage is described in remote term after relatively low-dose exposure.

The post-radiation encephalopathy with primary radiation neuronal damage within scope of “mild radiation sickness” was described in the clean-up workers [7]. However at that neither any dosimetry data nor any other evidence of radiation disease were provided. Later on they have stated that post-radiation encephalopathy appears after more than 0.5 Gy of radiation exposure. Yet this opinion has drawn serious and rather enough substantiated criticism.

Therefore scientific debates go on about the radiation sensitivity of nervous system, that certainly makes impact on estimates of neuropsychiatric Chernobyl heritage. At that we had many times insisted on inadequacy of available opinions about the “functional sensitivity” and “morphological resistance” of nervous system towards ionizing radiation. It is clear that there are no “functional” disorders without damage to the respective biological structures at molecular level. Instead in real there are some limitations of our knowledge and diagnostic capabilities [8].

It is believed now that CNS involvement was underestimated within classical ARS descriptions, while the nervous system is sensitive to radiation, and extent of its dysfunction has been quantified by means of electrophysiological, biochemical and/or behavioral parameters. CNS dysfunction was identified through the same parameters under the low-dose total-body irradiation too. They insist that evolving conception of radiation-induced syndrome of multi-organ injury in ARS provides a background for understanding of CNS damage [9]. A lot of newer neuroradiobiology data on molecular-biological pathways of radiation brain damage has recently become available. Thus even the apologists of CNS radiation resistance concept were forced to reconsider their opinion in favor of cerebral radiosensitivity.

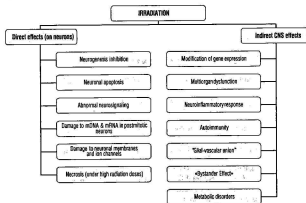


Fig. 17.1. Pathogenesis of radiation brain damage

Contemporary opinions about the radiation brain damage are no more focused only on the traditionally mentioned “glial-vascular union”, where damage of cerebral vascular endothelium and injury of neuroglia are the cause of all radiocerebral effects. Nowadays both primary-neuronal pathways (i.e. hippocampal neurogenesis inhibition [10], neuronal apoptosis, abnormal neurosignaling, damage of mitochondrial DNA and RNA

not subject to reparation, lesions of neuromembranes and ion channels along with necrosis under the high-dose radiation exposure) and modification of gene expression, "bystander effect", neuroinflammation, multiorgan dysfunction, autoimmune pathways, metabolic disorders and many others are in the focus of concern. It means that opinions on radiation neuropsychiatric effects and nervous system sensitivity do change dramatically [11].

It bears mentioning that opinions on nervous system radiation resistance remain persisting in the international practice of radiation protection and UNSCEAR reports 2006, 2008, and 2010. The recent data on pathogenesis of even the low-dose cerebral effects being summarized in the publication [11] and presented in Fig. 17.1. They are the disorders of hippocampal neurogenesis in adults, modification of gene expression, neuroinflammatory reactions, alteration of neurosignaling, apoptotic cell death, cell damage and death due to secondary injuries etc. Those disorders both with well-known "glial-vascular union" do probably explain the cerebral radiosensitivity pathways (Fig. 17.1).

17.2 EARLY NEUROPSYCHIATRIC MANIFESTATIONS

According to the UNSCEAR report 2000 (Annex J) the ARS was verified in 134 emergency workers in Chernobyl. Among them 41 persons had received the total-body external radiation doses under 2.1 Gy, while 93 patients were exposed to higher radiation doses resulting in more severe ARS, namely to 2.2–4.1 Gy in 50 cases, to 4.2–6.4 Gy in 22 men, and to 6.5–16 Gy in 21 of them.

The initial reactions and neurological disorders in ARS patients after the Chernobyl accident in wards of Moscow Clinical Hospital #6 have been described by F.S. Torubarov et al. (1989) [12]. In cases of mild ARS (grade 1, radiation doses 0.8–2.1 Gy) the disorders of autonomic nervous system (vegetative-vascular dystonia, VVD) and neurotic disorders were diagnosed, in moderately severe ARS (grade 2, 2–4 Gy) the VVD was prevalent, in severe ARS (grade 3, 4.2–6.3 Gy) the acute radiation and radiation-toxic encephalopathy, acute psychosis with visual and auditory hallucinations and cerebral edema were present, while in an extremely severe ARS (grade 4, 6–16 Gy) the acute radiation and radiation-toxic encephalopathy, subarachnoidal/parenchymal hemorrhages both with pronounced cerebral edema were found.

A few years upon ARS convalescence the so-called "functional" disorders of nervous system were revealed featuring neurocirculatory dystonia with asthenoneurotic syndrome and VVD with diencephalic paroxysms. No signs of organic brain damage were observed thereat. The decline in mental performance and the severity of asthenia were proportional to the survived ARS grade. In case of doses over 2 Gy the signs of brain tissue microdestruction were revealed by means of neuroimmune tests [13]. The disorders of cerebral hemodynamics were considered as one among other reasons of neurological manifestations after the ARS. The operator performance and cerebral electric activity abnormalities depended on severity of survived ARS. The electroencephalogram pattern slowdown due to theta-activity amplification was observed. Extent of the evoked cerebral activity disorders was also proportional to ARS grade. The brainstem, thalamocortical and intracortical disturbances were prevailing there. The signs of brainstem structures irritation in low-grade ARS evolved to their exacerbating depression of functional activity in ARS of intermediate and severe grade [14].

In the nearest terms of ARS recovery period i.e. 4–6 months upon irradiation the mental state and intellectual performance were in close link to both ARS severity and premorbid personality traits, awareness level and extent of social issues resolving. Professional affiliation, adequate job placement and personality features of survivors had become of especial importance in more late terms of recovery period i.e. in 12–18 months. Later on in the proximal consequences period i.e. in 2.5–3 years the mental disorders with pronounced fatigability were found in survivors of more severe ARS and in older persons. Further the serious anxiety, depression, decline in mental working capacity both with specific alterations in personality semantic structure were revealed [15].

Disorders of mental state and mental productivity were proportional to the severity grade of survived ARS. The fatigability and anxiety-depressive disorders had exacerbated along with time, and formation of personality disorders occurred. At that if a tendency towards some amelioration of mental state was observed in 3 years upon exposure, and then the recurrence of negative trend has become obvious 5–6 years later [15].

17.3 REMOTE NEUROPSYCHIATRIC EFFECTS

Prospective survey of ARS survivors provides data on origination and genesis of the progressing structural/functional postradiation brain damage (encephalopathy) [16]. Abnormalities in the cortical-limbic system and of its communications with mainly dominant (left) hemisphere accompanied by the pronounced alterations of central afferent pathways are the cerebral basis of postradiation organic mental consequences [17].

The neuropsychiatric disorders in remote ARS period are peculiar with a progressing course with sequential change of autonomous/vascular and autonomous/visceral phase (3–5 years after exposure) by the phase of cerebroorganic disturbances and somatogenic lesions (5–10 years and more since the irradiation) (Fig. 17.2).

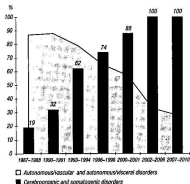


Fig. 17.2. Course of neuro-mental disorders in ARS survivors

The autonomous/vascular and autonomous/visceral disorders in patients with diagnosed ARS were distinct in the supra-segmental type, combination with pronounced abnormalities of the central afferent pathways, intensity, resistance and permanent-paroxysmal course with accompanying diencephalic crises, which we classified here as symptomatic partial epileptiform limbic attacks. These abnormalities combined with exacerbation and multiplication of somatic diseases including cerebrovascular lesions have

stipulated the progressive shaping of cerebroorganic and somatogenic mental disorders in remote period of ARS.

Along with years since ARS the psychopathological presentation has evolved to the most extent i.e. the "affective foreshore" (neurosis-like and sthenic psychopathy-like symptomatology) decreased gradually in parallel with exacerbation of the negative psychopathological symptomatology. The mentioned psychopathological symptomatology in remote period upon ARS consists mainly from 1) negative psychopathological symptomatology i.e. energy potential decrease, personality disharmony and regression, affective flattening (flattening of affect), apathy, anhedonia, hypobulia, impoverished (scanty) speech, social withdrawal; 2) adynamic depressive symptomatology; 3) cognitive deficit and 4) characteristic psychopathy-like personality changes (personality disorders) with the general functioning scores under 70. As a whole the described above psychopathology can be classified as an apathetic variant of psychoorganic syndrome. According to ICD-10 criteria it should be diagnosed as an apathetic variant of organic personality disorder (F07.0 code), corresponding to the apathetic type of personality disorder due to general medical condition (310.1 code according to DSM-IV). This disorder is of progressing type of the course and its incidence depends on severity grade of survived ARS and radiation dose respectively (Fig. 17.3). Therewith some other organic mental disorders (F06, F07), both with neurotic, related to stress and somatoform disorders are found in ARS survivors. The dose-effect relationship was fixed for mental working capacity decrease surveyed upon exposure to radiation doses exceeding 1 Gy.

Neurological symptomatology in remote ARS period is of microfocal and "tracery" type. The sensory-algic, vestibulo-ataxic, musculotonic and autonomous (vegetative) disorders with soft pyramidal and extrapyramidal symptoms, and also paroxysmal states mainly as diencephalic crises (autonomous-visceral attacks), and sometimes temporal epilepsy and amygdalohippocampal (mediobasal limbic) attacks are characteristic here. Neurological disorders are mainly observed within structure of cerebrovascular diseases (ICD-10: cerebral atherosclerosis — I67.2, hypertensive encephalopathy — I67.4, discirculatory encephalopathy within scope of other specified cerebrovascular diseases — I67.8), demyelinating diseases of encephalomyelosis type (other demyelinating diseases of central nervous system — G37) and paroxysmal disorders (G40, G43, G44, G45).

In 3–5 years after irradiation the EEG abnormalities of irritative type with paroxysmal activity lateralized to the left frontotemporal zone were registered at that being estimated as hyperactivation of corticolimbic system. Along with time in

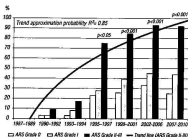


Fig. 17.3. Course of apathetic variant of organic personality disorder in persons with diagnosed ARS

5–10 years these abnormalities have evolved to the low-amplitude EEG with an excess of fast (beta-) and slow (delta-) activity under the depression of alpha- and theta-activity indicating to the organic brain damage with corticolimbic system inhibition [18].

The neurophysiology markers of ionizing radiation (1–5 Gy dose range) were identified by means of quantitative electroencephalography (qEEG) application in patients with verified ARS diagnosis (Fig. 17.4). They are the dominant frequency decrease in the left frontotemporal zone, the absolute delta-activity lateralization to the left hemisphere, relative delta-power increase in frontotemporal zones with lateralization to the dominant (left) hemisphere and diffuse decrease of absolute and relative alpha-power with lateralization of its depression to the left hemisphere. It can indicate that cortico-limbic dysfunction is present being lateralized to the dominant hemisphere with cortical frontotemporal and hippocampal damage [19].

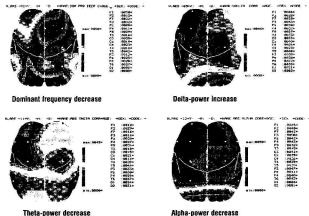


Fig. 17.4. Neurophysiology markers of ionizing radiation impact in 1–5 Gy dose range (adapted from [19])

Neurophysiological studies (qEEG and evoked brain potentials registration) indicate to the damage of corticolimbic system mainly in the dominant i.e. left brain hemisphere and to the disorders of central afferent pathways of all sensory modularities with the prevalence of sensory information processing by non-specific afferent systems against the background of depression of specific ones in remote period upon ARS [19].

Neuropsychology study has proved the pathological involvement of the frontal and temporal cortex of dominant hemisphere both with median cerebral structures and their cortical-subcortical links in remote period after the ARS. The left (dominant) hemi-

sphere inhibition and compensatory hyperactivation of the right one were revealed. It means that damage of structures of the left brain hemisphere anterior parts responsible for verbal information reception and processing, planning, regulation and control is the cerebral basis of cognitive disorders after the ARS. On the opposite of those patients the diffuse brain damage with anterior (regulatory) and posterior (gnostical) parts of both hemispheres involvement and predominant injury of non-specific median structures are observed in the compare group i.e. patients suffering the discirculatory encephalopathy (DEP). Functional indices in associative brain zones responsible for the control and regulation of motor programs execution and speech-mediated mnestic (verbal) and complex intellectual activities were found being dose-dependent [20].

Cognitive deficit is a base of brain damage clinical pattern in remote period of ARS. The specific dose-dependent abnormalities of cognitive functions were found being considered as a domain of post-radiation cognitive disorders. They include verbal Intellectual quotient (IQ) decrease compared to the premorbid verbal IQ, decline in cognition, memory and attention, deterioration of the short-term verbal memory and of the proactive and retroactive interference of verbal information. Lowering of the relative theta-range power in the left frontal zone and decrease of the cEEG theta-range total relative power ratio to the alpha-range one accompany these cognitive disorders. The theta-range is known being a hippocampal rhythm. Therefore the revealed dose-dependent neurophysiological and neuromental disorders can indicate to the injury of cortic limbic system, especially of hippocamp in the left (dominant) hemisphere. Therefore the revealed dose-related neurophysiological and neuropsychological disorders can be the proof of cortic limbic system abnormalities especially of the left (dominant) hemisphere hippocampus. The post-radiation cognitive disorders are of mild and moderate severity here and feature memory, attention, and verbal learning abnormalities that can be attributed to the post-radiation alteration of hippocampal neurogenesis (especially in the dominant hemisphere) [21].

The neuroimaging study in remote ARS period has provided the verification of mental disorders cerebroorganic type by the presence of cortical atrophy, ventricular dilatation, and focal lesions. According to magnetic-resonance imaging morphometry the neuroimaging markers of 1–5 Gy dose range ionizing radiation were identified featuring the contrast ratio decrease of the left internal capsule anterior limb (i.e. crus anterior) and genu, left parietal lobe in the radiate crown (i.e. corona radiata) zone, and right parietal lobe white matter. Brain hemisphere cortical atrophy and dominant hemisphere pathways alterations are the quantitative neuroimaging particularities of ARS remote period [22].

Cerebrovascular Doppler ultrasound visualization (non-invasive angiography) have found the acceleration of vascular wall involution, diffuse decrease in hemodynamic reactivity with “rigid” circulation pattern appearing, and restriction of blood flow in the extracranial cerebral vessels. As a result the reactive compensatory vasospasm occurs in all cerebral arteries. The excessive arterial tortuosity with diffuse stenosis are the characteristic features of cerebral vessels. Therewith the asymmetry of blood flow was found in the internal carotid arteries (ICA) due to more pronounced left ICA systolic velocity decrease. The left ICA systolic velocity decrease is regarded as a marker of cerebral arteries’ radiation damage, and just the left ICA as the target of deleterious action of ionizing radiation [23].

The specific and/or dose-related neurophysiological, neuropsychological and neurovisualizational effects have occurred in remote period upon ARS. At that the mentioned effects are lateralized to the left (dominant) hemisphere indicating the increased vulnerability of dominant hemisphere to ionizing radiation. Similar data for the left-handed persons unfortunately are not available. Thus the post-radiation brain damage in the right-handed individuals is localized primarily in anterior zones of left brain hemisphere. Upon ARS survivors the post-radiation abnormalities are found either in white and gray brain matter. The structure-functional markers of radiation brain damage have been identified, namely the abnormalities of big hemisphere cortex (frontoparietal zones atrophy), subcortical structures and dominant hemisphere pathways both with corticolimbic system mainly in the dominant (left) hemisphere. Therefore existence of the organic (i.e. structure-functional) cerebral damage in remote period upon ARS have been verified on the ground of neuropsychiatric, neuropsychological, neurophysiological, and neuroimaging study results.

Despite the data presented above and study results from other authors proving the existence of an organic radiation brain damage in remote ARS period some researchers consider that "response of the nervous system to less than 10 Gy of ionizing radiation occurs without a component of neuronal structures irreversible damage and is realized through the biochemical reparation of a neuron and functional reorganization of neural activity". They insist on high regenerative capacities of human body under the radiation impact and psychoneurological disorders at that are regarded as "reactive neurotic changes" [24]. Thus, more that centenary debates about the radiation sensitivity of nervous system are still going on.

The organic brain damage upon ARS is etiologically heterogeneous. In our opinion it is inappropriately to absolutize the radiation factor especially in case of low-dose radiation but we at that do not share the standpoint of ignoring the exogenous-organic impact of ionizing radiation on the human brain even at high radiation doses. Our research data conclusively prove the existence of brain structure-functional alterations in remote period of ARS after the Chernobyl catastrophe. As it was outlined above the cerebral damage had occurred as a result of combined impact of exogenous and endogenous factors. Undoubtedly the traditional risk factors and, in particular, age, cerebrovascular disease, somatic disorders, stress, psychoactive substance abuse, lifestyle and others do contribute to the organic brain damage after the ARS. However data are available on crucial importance of ionizing radiation in case of doses exceeding 1 Gy.

Therefore brain is sensitive to radiation, at that its vulnerability to ionizing radiation is different i.e. the radiation damage occurs ante omnia in neocortex, cortico-limbic system and dominant hemisphere. The elaborated differential-diagnostic algorithm for postradiation organic mental disorders is shown on Fig. 17.5.

The diagnostics of postradiation organic mental disorders (postradiation encephalopathy) is to be performed according to the following criteria:

- 1) radiation dose exceeding 1 Gy
- 2) progressive type of mental disorders course with transformation of neurosis-like states with pronounced asthenic, autonomic/vascular and autonomic/visceral abnormalities to the organic brain damage

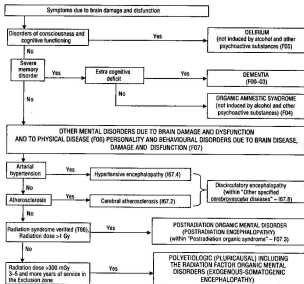


Fig. 17.5. Differential-diagnostic algorithm for postradiation organic mental disorders

3) signs of postradiation organic mental disorders:

- microfocal organic neurological symptomatology with sensory-algic, vestibulo-ataxic, and musculotonic syndromes, soft pyramidal and extrapyramidal symptoms, and progressing autonomous failure;
- paroxysmal states as diencephalic crises (autonomous/visceral attacks), temporal epilepsy and amygdalohippocampal (mediobasal limbic) attacks;
- cognitive deficit;
- senesthypochochondriac symptomatology;
- negative psychopathological symptomatology i.e. energy potential decrease (lack of energy), personality disharmony and regression, affective flattening, apathy, anhedonia, hypobulia, impoverished (scanty) speech, social withdrawal;
- depressive symptomatology;
- general functioning score <70;
- plane or disorganized EEG pattern with abnormal activity lateralization to frontotemporal zone of the dominant (left) hemisphere;

- dominant cEEG frequency decrease < 8 Hz in occipital zone; delta-power excess with theta-power reduction in frontotemporal zones especially of the dominant (left) hemisphere; alpha-power reduction;
- deformation and shortening of the latent periods (LP) and amplitude increase of the main components of visual evoked potentials (EP) to chess (chessboard) pattern reversion (VEPCP);
- LP deformation and prolongation with amplitude decrease of somatosensory EP (SSEP) main components, evoked response maximum (highest amplitude and shortest LP) not in projection zone but in associative ones under the right-sided stimulation;
- deformation of brain stem auditory EP (BSAEP) late components (i.e. III, IV, and V), elongation of component V latent period and III-V interpeak interval (IPI), component V amplitude decrease;
- structural-functional brain damage with frontal zones, left temporal lobe with cortical-subcortical links and deep brain structures involvement according to the neuropsychological tests;
- cerebral hemodynamics abnormalities due to atherosclerotic lesions, hypertonic type of vascular tone, interhemispherical asymmetry in brain perfusion with blood flow velocity decrease on the left, and obliterating processes;
- cerebral-organic nature is proved by the brain structure abnormalities according to MRI data (mainly as atrophy and brain ventricles dilatation, both with brain focal lesions)

4) no mental disorders of other origin.

The diagnosis of organic mental disorders is considered substantiated if criteria #1 and #2 are available both with at least 5 positions from criterion #3. In case of cerebrovascular disorders (hypertensive encephalopathy, cerebral atherosclerosis) with stable cerebral hemodynamic abnormalities the organic brain damage can be classified as a postradiation discirculatory encephalopathy.

In correspondence with multiaxial variant of ICD-10 mental disorders classification the typical complete diagnosis of postradiation organic mental disorders can be as follows:

Axis I — Clinical diagnosis. 1) Apathetic variant of the organic personality disorder F07.0. 2) Unspecified effects of radiation: Radiation sickness T66. 3) Other specified disorders of brain: Postradiation encephalopathy G93.8.

Axis II — Disability. Loss of earning capacity $> 40\%$.

Axis III — Situational factors: experience (affection) of the Chernobyl catastrophe.

Verification of organic brain damage after the irradiation is to be made on the basis of identification of structural-functional abnormalities through the clinical neuropsychiatric, neuropsychological, neurophysiological and neuroimaging application.

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17.4 MENTAL HEALTH AND NEUROPSYCHIATRIC EFFECTS IN THE CLEAN-UP WORKERS

Mental health disorders in survivors is an internationally recognized top-priority medico-social issue of the Chernobyl catastrophe not yet resolved till now. The UN Chernobyl Forum (2006) experts have outlined the four principal neuropsychiatric catastrophe consequences i.e. 1) the stress-related disorders; 2) effects on a developing brain; 3) organic brain damage in the Chernobyl accident clean-up workers (clean-up workers), and 4) suicide. The cardiovascular morbidity increase is recognized now in clean-up workers exposed to high radiation doses.

Long-term unfavorable impact of the Chernobyl catastrophe on mental health is recognized worldwide [1], whereas the causes of neuropsychiatric effects still remain debatable [2]. Contemporary experimental, clinical and epidemiological evidence disproves the canonical statement about the CNS radioresistance. Precise analysis of the ionizing radiation neuropsychiatric effects, new data on the molecular-biologic pathways of radiation impact on a brain, review of contemporary evidence of the "low-dose" radiation effects and the debatable issues perscrutation regarding radiation and stress role in the genesis of neuropsychiatric effects of the Chernobyl catastrophe are outlined in a number of publications [3].

Epidemiological studies

There is a substantial lack of the evidence-based epidemiological studies in the field of neuropsychic disorders in survivors and clean-up workers in particular. Most of such the studies are based either on the governmental statistical accounting data or on the arrays of state registries with incomplete and unverified data content on the neuropsychic disorders. So, for instance, according to official figures from the Ministry of Public Health of Ukraine the prevalence of mental disorders in Ukraine in 1994 was

2,236 per 100,000 of population (2.24%), in 1999 — 2,406 (2.41%), in 2000 — 2,432 (2.43%), in 2001 — 2,449 (2.45%), 2002 — 2,468 (2.47%), and in 2003 — 2,472 (2.47%) respectively. In the meantime as a result of the adequately designed epidemiological psychiatric study within WHO Initiative "World Mental Health Survey — 2000" with application of the Composite International Diagnostic (psychiatric) Interview (WHO-CIDI) it was revealed that in real the prevalence of only the "psychological" (non-psychotic) disorders in Ukraine was 20.5% (confidence interval: 17.7–23.3%) [4]. Obviously the official (registry) figures regarding mental disorders provide an about of one-order underestimation of the real state of things. Therefore a quite careful approach is required in epidemiological estimates making for mental disorders in the Chernobyl NPP accident survivors using the registry data.

Despite enough criticism about imperfection of the existing registries the SRU and the CER of BCRM remain the unique sources of epidemiological data acquisition on survivors in the country. Undoubtedly they require the state support and further updating. Mortality rate in survivors is a most unbiased reflection of the Chernobyl NPP accident consequence burden. Cardiovascular diseases which incidence and prevalence are dramatically raising along with time since the catastrophe are a leading cause of clean-up workers mortality [5]. Myocardial infarction and brain stroke are the principal sources of cardiovascular mortality here. However no respective systematic data are available for the survivors. That's why the launch of subregistries for such cardiovascular morbidity "endpoints" is urgent to control the risks of so fatal events.

Mortality rate from cardiovascular diseases in the clean-up workers is higher under the radiation doses exceeding 0.25 Gy, especially in younger persons (Fig. 17.6). It corresponds to our clinical data on higher radiation vulnerability of young adults up to 35 years old at exposure. This fact dictates the necessity of state control of admission to work with ionizing radiation sources (both under routine and emergency circumstances) of only the persons older than 35 years.

The official (registry) data on mental disorders provide an about of one-order underestimation of the real state of things because of a passive observation approach

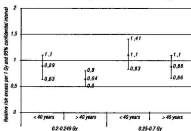


Fig. 17.6. Relative risk excess for cardiovascular mortality per 1 Gy in the clean-up workers of 1986-1987 depending on age at examination in 2003 (adapted from [5])

and the reluctance of mentally ill persons to apply for health aid. Active epidemiological studies are therefore the only way of an unbiased data obtaining on the mental health indices. The subproject #3.8.1 "Data base on psychological disorders in the Ukrainian clean-up workers of the Chernobyl accident" within The Franco-German Initiative for Chernobyl included the cross-sectional study of a randomized clean-up workers cohort (n=295) with WHO-

CIDI application. Further an international analysis of the obtained data was carried out owing to the US National Institute of Mental Health Grant (NIMH Grant MH51947). Data were compared to the Ukrainian population control from the WHO Project "World Mental Health Survey — 2000" [4]. According to the "healthy liquidator effect" (i.e. selection of mentally healthy men for the emergency works at the Chornobyl NPP) the prevalence rate of anxiety disorders and alcohol abuse in the clean-up workers before the accident was significantly lower (Table 17.1) [6].

Table 17.1. Mental health before the Chornobyl disaster: the "healthy liquidator effect" (adapted from [12])

	Clean-up workers (liquidators) n=295	Control n=387
Mood disorders	11 (3.7%)	27 (6.8%)
Anxiety disorder (except the posttraumatic stress disorder — PTSD)	5 (1.7%)	23 (5.8%)
PTSD	4 (1.4%)	1 (0.8%)
Alcohol abuse	25 (8.6%)	62 (15.6%)
Intermittent explosive disorder	11 (3.8%)	14 (3.5%)
Suicidal ideation	5 (1.7%)	8 (2.0%)

Upon correction by age in 1986 the liquidators and control groups were different only with anxiety disorder (adjusted odds ratio — AOR=0.3; 95% confidence interval 0.1; 0.9; $p=0.03$) and alcohol abuse (AOR=0.6; 95% confidence interval 0.3; 0.9; $p=0.02$).

Significant increase of the prevalence of depression (18.0% vs. 13.1% in Control) and suicidal ideation (9.2% vs. 4.1% respectively) was revealed in the clean-up workers after the accident. It is not however relevant to alcohol abuse and intermittent explosive disorder. There was an increase of the prevalence of depression (14.9% vs. 7.1%), post-traumatic stress disorder (PTSD) (4.1% vs. 1.0%), and headache (69.2% vs. 12.4%) in the clean-up workers within a year preceding the interview [6] (Fig. 17.7 and 17.8).

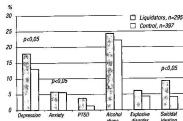


Fig. 17.7. Prevalence of mental disorders in the clean-up workers (altogether since 1986) (adapted from [6])

Difference significance defined through the adjusted odds ratio given the age in 1986 and the disorder onset before the Chornobyl catastrophe

The clean-up workers suffering depression and PTSD had more days lost from work (i.e. temporary disablement) yearly compared to the same patients in control. Extent of the catastrophe effect is associated with the severity of somatic symptoms and PTSD. Therefore the long-term unfavorable consequences of the Chornobyl catastrophe regarding the mental health are found in the clean-up workers. Further research is required to receive more reliable data on radiation and somatic health, to review the cognitive

disorders and psychotic symptoms, and to apply more precise psychiatric examinations [6].

The clinical-epidemiological evidence of incidence rise of mental disorders (organic, depressive etc.) and cerebrovascular disease in the clean-up workers with radiation risks in dose range $>0.25\text{--}0.5\text{ Gy}$ was received through the analysis of the CER data BCRM.

As against the widespread false opinion of an excessive use of “vegetative-vascular dystonia” diagnosis as a marker of radiation exposure the mentioned diagnosis was established in proximate years upon the Chernobyl catastrophe only in about a quarter of the clean-up workers (CER data). It is clear from Fig. 17.9 that diagnostics of vegetative-vascular dystonia has reduced substantially within a post-accident years reaching nowadays just about 5% of the representative clean-up workers sample.

Gradual increase of cerebrovascular disease prevalence occurred in clean-up workers after the accident featuring the most pronounced rise of the Cerebral Ischaemia (chronic) (ICD-10: I67.8), Cerebral atherosclerosis (I67.2) and to the less extent of the Hypertensive encephalopathy (I67.4). These disorders are traditionally classified as a “discirculatory encephalopathy” also not being perfect from the view of absolutization of only the vascular component of the organic brain damage pathogenesis, and also because of not enough strict diagnostic criteria. An international consensus on the optimal classification of such a kind of neuropsychiatric disorders will be certainly reached in the future.

Current (2008–2010) mental health assessment within randomized samples from representative clean-up workers and the Chernobyl exclusion zone evacuees cohorts (are on file in the CER) compared to the applicants/candidates for employment at the “Shelter” Object transformation into environmentally safe system has testified the long-term mental consequences of the Chernobyl catastrophe. As it appears from the Table 17.2



Fig. 17.8. Prevalence of mental disorders in the clean-up workers (for the last 12 months) (adapted from [6])

Difference significance defined through the adjusted odd ratio given the age in 1986 and the disorder onset before the Chernobyl catastrophe

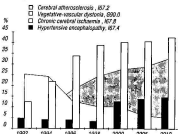


Fig. 17.9. Cerebrovascular pathology time pattern in the clean-up workers being on file in the clinical-epidemiological registry of BCRM

there are much more cases of mental and behavioral disorders in general, of vascular dementia, mental and behavioral disorders due to alcohol consumption, dysthymia, and PTSD among the clean-up workers and evacuees. Additionally the incidence of the organic depressive disorder, organic emotionally labile (asthenic) disorder and organic personality disorder had increased in the clean-up workers. Received data correspond to the results of international epidemiological psychiatric studies.

Table 17.2. Mental health in the liquidators and exclusion zone evacuees being on file in the CER RCRM

Mental and behavioral disorder according to the ICD-10	Comparison group (n=3444)	clean-up workers (n=410)	Evacuees (n=150)
No mental and behavioral disorder	2180 (63.3%)	78 (19.0%)*	53 (40.8%)*
Vascular dementia, F01	0	6 (1.5%)*	1 (0.8%)*
Organic delusional [schizophrenia-like] disorder, F06.2	2 (0.06%)	2 (0.5%)	0
Organic depressive disorder, F06.32	7 (0.2%)	27 (6.6%)*	2 (1.5%)
Organic anxiety disorder, F06.4	0	2 (0.5%)*	0
Organic emotionally labile [asthenic] disorder, F06.6	479 (13.9%)	152 (37.1%)*	26 (20%)
Mild cognitive disorder, F06.7	284 (8.2%)	34 (8.3%)	19 (14.6%)
Organic personality disorder, F07.0	106 (3.1%)	88 (21.7%)*	7 (5.4%)
Postconcussional syndrome, F07.2	45 (1.3%)	2 (0.5%)	3 (2.3%)
Mental and behavioural disorders due to use of alcohol, F10	83 (2.4%)	57 (13.9%)*	13 (10%)*
Mental and behavioural disorders due to use of opioids, F11	1 (0.03%)	0	0
Residual schizophrenia, F20.5	0	1 (0.24%)	0
Schizotypal disorder, F21	0	0	1 (0.8%)
Moderate depressive episode, F32.1	1 (0.03%)	1 (0.24%)	0
Dysthymia, F34.1	1 (0.03%)	6 (1.5%)*	1 (0.8%)*
Specific (isolated) phobias, F40.2	1 (0.03%)	1 (0.24%)	0
Panic disorder [episodic paroxysmal anxiety], F41.0	0	1 (0.24%)	0
Generalized anxiety disorder, F41.1	0	0	1 (0.8%)
Post-traumatic stress disorder, F43.1	0	6 (1.5%)*	3 (2.3%)*
Adjustment disorders, F43.2	31 (0.9%)	0	0
Somatiform autonomic dysfunction, F45.3	8 (0.2%)	0	1 (0.8%)
Persistent somatoform pain disorder, F45.4	0	1 (0.24%)	0
Neurasthenia, F48.0	164 (4.8%)	5 (1.2%)*	9 (6.9%)
Nonorganic sleep disorder, unspecified, F51.9	2 (0.06%)	-	-
Specific personality disorders, F60	36 (1.05%)	1 (0.24%)	1 (0.8%)
Enduring personality change after catastrophic experience, F62.0	0	1 (0.24%)	0
Mild mental retardation, F70	6 (0.2%)	0	0
Moderate mental retardation, F71	1 (0.03%)	0	0
Nonorganic enuresis, F98.0	1 (0.03%)	0	0
Stuttering [stammering], F98.5	13 (0.4%)	2 (0.5%)	1 (0.8%)
Accentuation of personality traits, Z73.1	31 (0.9%)	4 (1.0%)	2 (1.5%)

*significant differences vs. applicants/candidates for employment at the "Shcherb" unit by the χ^2 test or the double-sided Fisher's exact test. Differences were considered reliable according to Bonferroni correction if $p < 0.001$.

Neuropsychiatric consequences of the Chernobyl catastrophe undoubtedly are etiologically heterogeneous due to the joint effect of catastrophe radiation and non-radiation factors with stress above all, and also because of social changes and traditional risk factors (age in particular). Radiation and age make a synergic impact resulted in an accelerated aging. General level of the neuropsychiatric disorders morbidity is higher in older survivors. However the rate of morbidity rise in younger persons is more aggressive that is in details reviewed in epidemiology part of the book.

Dose-dependent increase of cerebrovascular morbidity in the clean-up workers of 1986–1987 period, specifically for cerebral atherosclerosis and especially for hypertensive encephalopathy was revealed [7] (Fig. 17.10).

Incidence growth of other neuropsychiatric diseases with a dose-related rise of dizziness and other vestibular organ disorders in particular was revealed in the clean-up workers of 1986–1987 [7] (Fig. 17.11).

According to SRU data the dose-related growth of nervous system and sense organs diseases, vegetative-vascular dystonia, arterial hypertension and cerebrovascular disease was found in clean-up workers of 1986–1987 [8] as shown at Fig. 17.12.

According to SRU and CER data the excess relative risk (ERR) per 1 Gy of radiation exposure was found out regarding the neuropsychiatric diseases in the clean-up workers of 1986–1987 (Fig. 17.13).

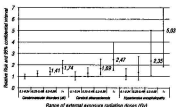


Fig. 17.10. Relative risk of cerebrovascular diseases in the male clean-up workers of 1986–1987 exposed to different external radiation doses. Control — the clean-up workers with doses <0.05 Gy (CER data, 1992–2004). Values of reliable relative risk provided (adapted from [7]).

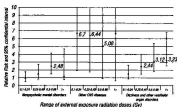


Fig. 17.11. Relative risk of some neuropsychiatric diseases in the male clean-up workers of 1986–1987 exposed to different external radiation doses. Control — the clean-up workers with doses <0.05 Gy (CER data, 1992–2004). Values of reliable relative risk provided (adapted from [7]).

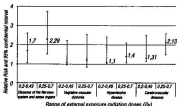


Fig. 17.12. Relative risk of neuropsychiatric diseases in the male clean-up workers of 1986–1987 exposed to different external radiation doses. Control — the clean-up workers with doses <0.05 Gy (SRU data, 1988–2001). Values of reliable relative risk provided (adapted from [8]).

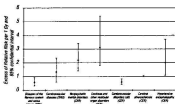


Fig. 17.13. Excess relative risk of neuropsychiatric diseases per 1 Gy in the male clean-up workers of 1986-1987 exposed to different external radiation doses (SRU 1986-2001 and CER 1992-2004 data) (adapted from [8]).

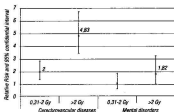


Fig. 17.14. Relative risk of neuropsychiatric diseases in adult evacuees exposed to different thyroid radiation doses. Control — thyroid dose <0.3 Gy cases (SRU data, 1989-2003). Values of reliable relative risk provided (adapted from [8]).

0.80). The clean-up workers exposed to more than 150 mGy of external radiation within less than 6 weeks of work in the exclusion zone are the group of highest risk of cerebrovascular disease (RR=1.18, 95% confidence interval 1.00; 1.40). The dose-related effect regarding cerebrovascular morbidity was revealed in 1986-2000 period studies featuring the ERR = 0.39 per 1 Gy (95% confidence interval 0.004; 0.77). There was a statistically meaningful cerebrovascular disease risk as an average radiation dose function under the exposure to 0.15 Gy and more (ERR per 0.1 Gy daily was 2.17 at the 95% confidence interval 0.64; 3.69) [10].

The WHO Chernobyl forum has recognized the "Chernobyl" data on cerebrovascular mortality excess in the clean-up workers as corresponding to the published study results on A-bombing survivors. The latest publication on cardiovascular risk after the A-bombing states that radiation doses over 0.5 Gy are associated with an excessive risk of both stroke and "heart disease" (i.e. coronary artery disease, arterial hypertension etc.). At that the risk values under lower radiation doses are not defined yet. Stroke

Thyroid radiation doses over 0.3 Gy in adult evacuees are followed by the risk increase of cerebrovascular disease, whereas in case of thyroid doses exceeding 2 Gy the mental disorder risk excess takes place [8] (Fig. 17.14).

The conciliatory data have been received for the clean-up workers on the radiation risks of non-cancer diseases (of cardiovascular ones especially and cerebrovascular ones in particular) [9]. Mental disorders excess is 0.4 per 1 Gy (95% confidence interval 0.17; 0.64), excess for the nervous system and sense organ diseases is 0.35 (0.19; 0.52), and for endocrine system disorders accounts 0.58 (0.3; 0.87). The cerebrovascular disease are of highest excess here — 1.17 per 1 Gy (0.45; 1.88), where the radiation risk values are available for respective mortality. Coronary heart disease excess is 0.41 per 1 Gy (95% confidence interval 0.05; 0.78), arterial hypertension reaches 0.36 per 1 Gy (0.005; 0.71), and excess relative risk (ERR) of cerebrovascular disease is 0.45 per 1 Gy (0.11;

and "heart disease" cause about one-third of radiation-associated excessive deaths in A-bombing survivors [11].

The systematic reviews have not yet provided the undoubted evidence of cardiovascular risk dependence on radiation within dose range 0–5 Sv [12, 13]. In the meantime the recently published data of an English epidemiological survey over the nuclear industry male workers testify the dose-related increase of cardiovascular mortality (ERR per 1 Sv is 0.65 with 90% confidence interval 0.36; 0.98) where the excess is meaningful in cumulative doses over 300 mSv [14]. It bears mentioning that these data correspond to our research results regarding the neuropsychiatric, neurophysiological, neuropsychological and neuroimaging effects in the clean-up workers just in radiation doses exceeding 0.3 Gy. Data analysis from studies in 15 countries among the nuclear industry personnel (average cumulative dose 20.7 mSv) revealed the ERR per 1 Sv of radiation for all non-cancer mortality as 0.24 (95% confidence interval –0.23; 0.78) and for cardiovascular disease as 0.09 (–0.43; 0.70). The highest risks were found in younger workers [15] that is also corresponding to our data on the clean-up workers.

A progressive course of neuropsychiatric and somatic disease was surveyed in clean-up workers of 1986–1987 especially in persons who had worked in the exclusion zone for the 3–5 years and more [16]. The neuropsychiatric disorders prevalence in the Chernobyl exclusion zone personnel exposed to radiation doses over 0.25 Gy was 80.5% whereas under less than 0.25 Gy exposure the respective value appeared being much lower (21.4%) [17] actually corresponding the real population indices of mental disorders prevalence in Ukraine [4].

The WHO Chernobyl Forum (2006) has defined the suicides in clean-up workers as one of important issues within post-accident period. Suicide was a leading cause of death in the Baltic clean-up workers [18]. Whereas low radiation doses are the risk factor of exacerbated suicidal behavior or there is a purely socio-psychological phenomenon here — it is to be clarified under the forthcoming epidemiological studies [19].

Since 1990 the substantial increase in schizophrenia incidence was found in the Chernobyl exclusion zone personnel (5.4 per 10,000 vs. 1.1 per 10,000 in Ukraine) [20]. Further epidemiological psychiatric research is required to identify the role of ionizing radiation in the origin of schizophrenia spectrum disorders [21].

One may state that there is an international consensus about the Chernobyl catastrophe impact on mental health as a top-priority healthcare problem in survivors. Given the prevalence and persisting resistance of their mental health disorders the educational, psychosocial and healthcare interventions both with further research are required.

The cerebrovascular disease and some neuropsychiatric disorders in the clean-up workers where radiation risks have been revealed can be considered in our opinion as the stochastic effects of a low-dose radiation (as also some other non-cancer diseases, especially cardiovascular ones with identified radiation risks). I.e., effects that occur by chance and which may occur without a threshold level of dose, whose probability is proportional to the dose and whose severity is independent of the dose (Health Physic Society definition <http://hps.org/publicinformation/radterms/radfact142.html>).

Clinical neuropsychiatric effects

The first formal estimates of mental health in the Chernobyl catastrophe survivors were extrapolated from an experience of previous radiation accidents and of the

Three Mile Island Nuclear Generating Station (a civilian nuclear power plant, near Middletown, Pa., USA) accident on March 28, 1979 in particular. Such an experience was coming down to the psychological stress and radiophobia ascertainment [22]. We have never shared and do not share the opinion of the "radiophobia" epithet adequacy or appropriateness towards even just psychological consequences of the Chernobyl catastrophe when there was a real danger under insufficient and contradictory information. The IAEA International Chernobyl Project (1992) experts have underlined that "if the stress factor is a real danger it would be dishonest to say or pretend that feeling of disquietude appearing in a response to that danger is something abnormal". The term "radiophobia" is applied in the Western Europe and USA only in diagnostics of a fear towards an event usually considered as absolutely safe. However as the experts note, "only few people will regard the Chernobyl catastrophe to this category". "Radioanxiety" term was proposed [23] then being more accurate in characterization of psychological reactions on the radiation danger.

For the years immediately ahead the Chernobyl catastrophe an opinion was preponderated of only the psychogenic effect of the Chernobyl accident and of stress concept, neurotic and psychosomatic disorders. Vegetative-vascular dystonia and asthenoneurotic syndrome were the most prevalent neuropsychiatric diagnoses among the clean-up workers in that period, that was and remains being subject to criticism especially from the side of international experts who qualify such diagnostics as a discrepancy "between the East and West". Vegetative-vascular dystonia was considered being purely a "soviet" diagnosis reflecting the pathophysiological background of the disorders, whereas a pragmatic approach applied in the West features those conditions syndromologically as anxious with panic attacks or somatoform ones. In very deed the radiation disorders of nervous system were first described by the German researcher Riecker (1915) and the "vegetative dystonia" or "neurovegetative syndrome" terms were proposed by the German psychiatrist Wichmann (1934) who later (1963) have transformed them to the "psychovegetative syndrome". Nowadays the vegetative-vascular dystonia is attributed to the competence of psychiatrists and according to ICD-10 is diagnosed as the "F45.3 — Somatoform autonomic dysfunction".

As it was mentioned above the vegetative-vascular dystonia for the years immediately ahead the accident was diagnosed in about every one in four clean-up workers been in file of the CER. Later as against the reducing prevalence of vegetative-vascular dystonia in the clean-up workers the substantial growth of cerebrovascular disease occurred i.e. the cerebral ischaemia (chronic) (I67.8), Cerebral atherosclerosis (I67.2) and hypertensive encephalopathy (I67.4) traditionally diagnosed as a "discirculatory encephalopathy" (see Fig. 17.9).

Neuropsychiatric disorders in the clean-up workers are peculiar by a progressive course with vegetovascular and vegetovisceral stage (up to 10 years upon the accident) consecutive exchange by the cerebroorganic and somatogenic disease stage (more than 10 years after). Vegetovascular and vegetovisceral disorders in the clean-up workers were suprasegmental ones and combined with abnormalities of the central afferent pathways, peculiar with permanent-paroxysmal course type and diencephalic crises including the hysteriform ones (in 1990–1995 period) that we consider being the symptomatic partial mediobasal limbic attacks. These disorders in combination with an increasing somatic

disease stipulated the progressing origination of the cerebroorganic and somatogenic mental disorders in remote period upon the catastrophe. It is especially relevant to the persons who had worked in the Chernobyl exclusion zone for a long time (more than 5 years) [24] (Fig. 17.15).

Neurological symptoms in the clean-up workers in remote post-accident period (except severe organic diseases of the nervous system i.e. sequelae of stroke, neuroinfection, trauma, toxipathy, tumor etc.) are of microfocal and "open-work" type. The sensory-athetic, vestibulo-atactic, musculotonic and autonomous disorders with mild pyramid and extrapyramid symptoms, and also the paroxysmal states mainly as diencephalic crises (autonomous-visceral attacks) and sometimes temporal epilepsy and amygdalohippocampal (medialbasal limbic) attacks are peculiar to the clean-up workers. Neurological disorders are mainly observed within cerebrovascular disease structure (cerebral atherosclerosis — 167.2, hypertensive encephalopathy — 167.4, cerebral ischaemia (chronic) i.e. discirculatory encephalopathy — 167.8), other demyelinating diseases of the central nervous system of encephalomyelosis type (G37), and episodic and paroxysmal disorders (G40, G43, G44, G45).

Incidence and intensity of hypochondriac symptomatology, "affective face" with neurosis-like and asthenic psychopathic-like manifestations had decreased in the clean-up workers within post-accident years, however the tendency to the negative psychopathologic symptomatology was surveyed especially in radiation doses exceeding 0.3 Gy. Time pattern of psychopathology in the clean-up workers lies in transformation of asthenicautonomic and hysterio-hypochondriac manifestations, anxiotic and obsessive hypochondria into the senesto- and paranoic-hypochondriac formations, in exacerbating personality disharmony and regression, explosiveness and affective-volitional instability into the depressive and negative symptomatology with cognitive deficiency, autization and social dysadaptation. Clinical presentation of these disorders to the most extent corresponds to variants of psychoorganic syndrome (labile, apathetic, paranoid, and combined ones) [24].

The negative psychopathic symptomatology is growing proportionally to radiation dose in doses exceeding 0.3 Gy and service record in the Chernobyl exclusion zone more than 5 years. PTSD manifestations on the contrary are reversibly related to radiation dose with a leading role of not the real danger of situation but its perception and realization. Personality defect in remote period upon the accident is featured by disintegration of intellectual and emotional spheres, shaping of psychopathic-like disorders with social dysadaptation and asthenic type of response. The Global Assessment of

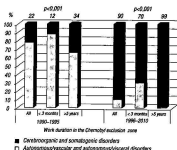


Fig. 17.15. Time pattern of neuropsychiatric disorders in the clean-up workers

Functioning score (GAF Scale) here is not more than 70 points. Verbal memory deficit, mental working capacity decrease and cognitive decline compared both to normal range and to the premorbid IQ values testify the psychoorganic nature of mental disorders. Variants of the organic personality disorder (F07.0 according to the ICD-10 and 310.1 according to the DSM-IV), the organic emotionally labile (asthenic) disorder F06.6 and organic depressive disorder (F06.32 according to the ICD-10 and 293.83 according to the DSM-IV) are the specific mental disorders in the clean-up workers in remote period after the accident. Aside from that the neurotic, related to the stress somatoform disorders (F40–F48), the enduring personality change after catastrophic experience (F62.0) or the PTSD (309.81 in the DSM-IV) are surveyed in the clean-up workers [24].

It is recognized that mental disorders in the Chernobyl catastrophe survivors have originated not only at the expense of direct or indirect impact of physical factors but also as a result of somatopsychic or psychosomatic pathways involvement straightforwardly related to the impact of the Chernobyl NPP accident [2]. We have proposed the systematization (classification) of mental disorders according to which the *pathological personality development* (posttraumatic, psychosomatic, schizotypal, and organic one) is considered as a principal nosological (clinical) entity in remote period after the Chernobyl accident. At that the equivalent ICD-10 and DSM-IV diagnostic categories are available [25] shown in the Table 17.3.

The *Posttraumatic stress personality development* entirely corresponds to ICD-10 F62.0 diagnostic category “enduring personality change after catastrophic experience” and DSM-IV 309.81 entity “posttraumatic stress disorder”. By lapse of time the post-traumatic stress personality development has gone complicated by psychosomatic, cerebroorganic and somatogenic disorders. And its gradual transformation occurred to the psychosomatic and organic pathologic personality development (mainly due to the secondary cerebrovascular disease).

Psychosomatic pathologic personality development preponderated in 1990–1995 and declined after 1996–2000 due to its transformation into the organic pathologic personality development and schizotypal one in remote period after the accident. Pathologic personality development appears gradually against the background of neuro-endocrine dysfunction as a result of an impact of unfavorable radiation and non-radiation factors. Such a dysfunction results in morphofunctional brain and visceral disorders. Three stages are surveyed within a psychosomatic pathologic personality development: 1) neurosis-like (asthenovegetative), 2) psychosomatic, 3) patho-characteristic. At that the pathological complex of a “vicious circle” type occurs where the cause becomes a consequence and vice versa. This complex includes psychological, somatic and psychosocial disorders. Psychosomatic pathologic personality development approaches the mosaic “marginal” psychopathy with a social and more often than not pronounced dysadaptation. Psychosomatic pathologic personality development can through the exacerbation of cerebro-organic and somatogenic psychopathologic symptomatology gradually transform into the organic pathologic personality development, and also due to the cortical-limbic system damage mainly in the dominant (left) hemisphere into the schizotypal one.

Schizotypal pathologic personality development corresponds to the ICD-10 F21 diagnostic category “schizotypal personality disorder” and the DSM-IV 301.20 or 301.22 entities “schizoid personality disorder”/“schizotypal personality disorder”.

Table 17.3. Systematization of mental disorders associated with the Chernobyl catastrophe (adapted from [37, 38])

Mental disorders within periods after the ChNPP		
Immediate (1985–1989)	Intermediate (1990–1995)	Remote (since 1996)
Neurotic, stress-related and somatoform disorders (ICD-10: F4; DSM-IV: 300)	Neurotic, stress-related and somatoform disorders (ICD-10: F4; DSM-IV: 300)	Enduring personality change after catastrophic experience (ICD-10: F62.0; DSM-IV: 309.81)
Reaction to severe stress, and adjustment disorders (ICD-10: F43; DSM-IV: 308.3)	Posttraumatic stress disorder, PTSD (DSM-IV: 309.81)	Psychosomatic pathologic personality development (ICD-10: F45, F54; F34.1, F60–F69, DSM-IV: 293.83, 293.89, 301, 301.22, 300.4, 316...)
Posttraumatic stress disorder, PTSD (ICD-10: F43.1; DSM-IV: 309.81)	Enduring personality change after catastrophic experience (ICD-10: F62.0; DSM-IV: 309.81)	Disorders of adult personality and behaviour (ICD-10: F60–F69 DSM-IV: 301)
Acute and transient psychotic disorders (ICD-10: F23, F28; DSM-IV: 293, 297, 298)	«Psychosomatic disorders» ICD-10: F54 + ...; DSM-IV: 316 ...;	Schizotypal disorder (ICD-10: F21 DSM-IV: 301.20, 301.22)
Behavioural syndromes associated with physiological disturbances and physical factors (ICD-10: F50–F52, F54, F59; DSM-IV: 307, 309, 302, 300)	Other mental disorders due to brain damage and dysfunction and to physical disease (ICD-10: F06; DSM-IV: 293, 310.1)	Other mental disorders due to brain damage and dysfunction and to physical disease (ICD-10: F06; DSM-IV: 293, 310.1)
Other mental disorders due to brain damage and dysfunction and to physical disease (ICD-10: F06; DSM-IV: 293, 310.1)	Personality and behavioural disorders due to brain disease, damage and dysfunction (ICD-10: F07 DSM-IV: 310.1)	Personality and behavioural disorders due to brain disease, damage and dysfunction (ICD-10: F07 DSM-IV: 310.1)
		Dementia (ICD-10: F01–F03 DSM-IV: 290, 294.1)
		Organic amnesic syndrome, not induced by alcohol and other psychoactive substances (ICD-10: F04 DSM-IV: 294)

Organic pathologic personality development entirely corresponds to the ICD-10 F07 diagnostic category "personality and behavioural disorders due to brain disease, damage and dysfunction" and DSM-IV 310.1 entity "personality disorder due to general medical condition" (subtypes: labile, apathetic, paranoid, disinhibited, other). Substantial increase of this kind of pathologic personality development due to psychosomatic pathologic personality development transformation had occurred within the post-accident years. Hereby the systematization of mental disorders after the Chernobyl catastrophe is a vivid/illustrative example of the consensus availability between the national psychiatric classification and ICD-10/DSM-IV [24, 25].

PTSD is a key mental disorder as a result of a purely psychological impact of the Chernobyl catastrophe. The mentioned disorder is a postponed response on the negative impact of extreme events and situations been outside the scope of human daily experience. According to ICD-10 the PTSD (F43.1 diagnostic category) is diagnosed within

6 months of a stress situation otherwise at the end of a stress period. The enduring personality change after catastrophic experience (F62.0) can be the sequelae of PTSD upon 2 years of its course. However according to the American psychiatric classification DSM-IV the PTSD (309.81 diagnostic code) is diagnosed irrespectively to the time since an experienced stress. The UN Chernobyl Forum (2006) experts have recognized the PTSD as a first among other main mental health problems in the Chernobyl NPP accident survivors. Psychological effect of ionizing radiation is the most powerful so-called "invisible" stress factor with an ahead-directed action. *Id est*, in radiation emergency the "flashback" symptom ("cutback", "back shot", "back frame" or "backward look") as a typical psychopathologic PTSD manifestation undergoes transformation to a "flashforward" i.e. "forelock" or "prospension". PTSD transformation has took place in the clean-up workers already 1.5–2 years upon the accident. Extreme agitation and irritability in response to elementary stress situations were noted at that, and the "flash-back" symptoms featured a lack of brightness and had appeared indirectly [26].

Psychotraumatic impact of the Chernobyl catastrophe is the result of not only the catastrophe itself but rather of a possible future effect perception. PTSD in the Chernobyl NPP accident survivors unlike the Afghan war veterans featured fear and danger projection ahead that corresponds the concept of an "anticipatory stress" i.e. the stress of expectation or prediction. The PTSD in the Chernobyl NPP accident survivors comprise different semantic components. The radiation-associated PTSD component includes agitation with hypochondriac fixation elements related to the future "probable" own health disorders, cancer genesis, health deviations in children, congenital malformations in future generations etc. The socio-psychological PTSD block in survivors comprises the unwarranted (unguaranteed) future syndrome (fear to lose own social status, fear of socio-economic problems appearing and family disruption, the delicacy of perception of an unjust attitude from society to the Chernobyl accident survivor), a subjective time space narrowing (lack of time to solve the daily living problems, permanent time handicap feeling, life perspective absence), the "runaway" or "flight" into illness (bad well-being and low working capacity, interest area narrowing, disability due to the illness, search of a serious disease in the own organism) and the acquired helplessness (helplessness feeling because of the Chernobyl accident, activity decrease, shifting of some own duties and responsibility on others, mood deterioration with own life worthlessness sensation). PTSD as a result of radioecological catastrophe is characterized by the prediction stress, neuropsychiatric comorbidity (mostly cerebrovascular), neurocognitive deficit, personality change, affective disorders, atherosclerotic lesions of the right common carotid artery and deterioration of cerebral venotribasilar circulation, and limbic system disfunction with possible hippocampal involvement [27].

There are a lot of published consistent data (we quote the most impressive ones only) about the characteristic neurophysiological [28, 29], neuropsychological [30] and neuroimaging [31] abnormalities in the clean-up workers. The organic brain damage in the clean-up workers was verified in such and some other studies, however with no dosimetry support and no dose-response estimations. At that the accelerated CNS aging was justified [32].

Spontaneous and evoked brain bioelectrical activity and its topographic distribution in the clean-up workers, especially in a long service in the Chernobyl exclusion zone is

substantially different from that in a population norm and the compare (control) groups. Our first "blind" brain mapping studies just 20–22 years ago had revealed the rigorous difference of exposed persons from the Afghanistan war veterans by excess and lateralization of delta-power to the left frontotemporal zone both with beta-power excess.

There was a diphasic cerebral electric activity pattern upon the exposure [33]. The dose-dependent (in doses exceeding 0.3 Gy) cortico-limbic irritation with EEG spectral power amplification was surveyed in the clean-up workers 10 years immediately ahead the accident. The dose-related (again in doses over 0.3 Gy) signs of an organic brain damage with cortico-limbic inhibition and deficiency psychopathologic symptoms and cognitive deficit were observed in remote period (more than 10 years upon exposure). Irradiation to more than 0.3 Gy is followed mainly by the fronto-temporal zone involvement of the dominant (left) hemisphere, EEG's slowdown, progressing total decrease of an absolute spectral power ("plain" polymorphous EEG) with delta- and beta-activity amplification along with theta- and alpha-activity decrease. The dose-dependence of neurophysiology effect is surveyed only in dose range over 0.3 Gy. Notably the dependence becomes significantly stronger after the long-term work (5 years and more) under the conditions of Chernobyl exclusion zone that can testify the amplifying effect of a prolonged impact of zone factors including the chronic radiation. Dose contribution to the EEG pattern changes (dose range 0.3 Gy and more) is 28%, cerebrovascular disease contributes here 29%, somatic disease — 11%, and age — 19% respectively [24].

Study of the cerebral information processes through an evoked cerebral electric activity registration showed the following dose-related (under the radiation doses exceeding 0.3 Gy) neuro- and psychophysiological effects: alterations of the central afferent pathways of all sensory modalities, overweight of sensory information processing by non-specific afferent systems over the depression of specialized ones, damage of the dominant hemisphere, cortico-limbic-reticular dysfunction with suppression of brainstem reticular formation resulted in a "sensory gate" dysfunction with a sharp increase of sensory afflux to the cerebral cortex that can lead to an origination of senestopathy and its hypochondriac experience [24, 34].

Cerebral circulation in the clean-up workers is peculiar with decreased vascular wall elasticity that is inconsistent with physiological age criteria especially under radiation doses exceeding 0.25 Gy and indicates the acceleration of a vascular wall involution, circulation reactivity decrease with formation of a "rigid" circulation, perfusion decrease in the extracranial parts of cerebral vessels, circulation asymmetry in the internal carotid artery due to more decrease of systolic velocity in the left internal carotid artery after exposure to more than 0.3 Gy, compensatory vasospasm of all cerebral arteries, no hemodynamically significant (over 65%) stenosis. Cerebral vessels are peculiar with an excessive arterial tortuosity and diffuse obliterating lesions [35].

Unlike a "classical" chronic cerebrovascular disease the brain atrophy prevail in the clean-up workers, whereas lacunar lesions are on the contrary less prevalent. The characteristic dose-related neuroimaging features of an organic brain damage reflecting cortical atrophy and the left (dominant) hemisphere pathways involvement were found through the magnetic resonance imaging morphometry. These effects are observed upon exposure to the doses exceeding 0.3 Gy, whereas in case of more than 1 Gy irradiation the dependence on dose becomes stronger that can indicate to a radiation factor role

in origination of the described characteristic neuroimaging pattern of a radiation brain damage [36].

The specific peculiarities of neuropsychiatric disorders in the clean-up workers reside in a tendency of the left hand predominance in right-handers, speech disorders, torpidity of the fine motor acts, sensory-motor memory abnormalities, direct memorization, short-term memory and interference decrease, memory function dissociation i.e. disorder of audioverbal memory along with preserved visual one, altered calculating operations, and specific abnormalities of an associative process. Neuropsychological study results in the clean-up workers highlight the localization of a pathological process in the frontal parts of the left brain hemisphere especially upon irradiation with doses exceeding 0.3 Gy [37].

Cognitive disorders in the clean-up workers (except gross organic CNS lesions e.g. stroke, neuroinfection, trauma, intoxication, tumor etc.) do not reach the dementia level and correspond to the mild cognitive disorder (ICD-10: F06.7). The specific neuropsychological peculiarities of the cognitive disorders are found in the clean-up workers exposed to more than 0.3 Gy radiation doses. These cognitive disorders unlike such ones in vascular and demyelinating CNS disease reside in a verbal memory, speech, and intellectual abnormalities with regulation disorders of a voluntary activity along with preserved orientation ability and gnostical functions. The non-specific abnormalities of memory, dynamic praxis, visuospatial synthesis, thinking/brainwork and ability to plan were found in the clean-up workers exposed by doses under 0.3 Gy. Those abnormalities occurred being similar to cognitive disorders under the organic brain damage due to cardiovascular disease (hypertensive, atherosclerotic encephalopathy) and CNS demyelinating lesions [38].

The dose-related cognitive disorders lying in the decrease of a general intelligence quotient and verbal learning score proportionally to the radiation dose have been identified after exposure by doses exceeding 0.3 Gy. Specific features of cognitive disorders in the dextrmanual clean-up workers exposed by 0.3 Gy doses and more indicate to a predominant injury of the left temporal cortex zone, medial structures and frontal zone both with cortical-subcortical pathways mainly in the dominant (left) hemisphere. The young age (up to 35 years at exposure), low educational level, low occupational skills, and radiation dose over 0.3 Gy are the key factors of cognitive disorders in the clean-up workers given the excluded severe somato-neurological and mental disease. The "Diagnostic tool for the post-radiation cognitive disorders" has been developed for the probability estimation of a cognitive disorder radiation origin in remote terms after the total-body human irradiation under radiation accidents. The post-exposure cognitive disorders (radiation dose 0.3 Gy and more) are featured by a mild and moderate cognitive deficit, deterioration of memory and attention, both with verbal learning disorder that in a whole can be attributed to the post-radiation injury of hippocampal neurogenesis especially in a dominant hemisphere [39].

The post-radiation brain damage in the clean-up workers under 0.3 Gy and more radiation doses involves both white and gray brain matter. Revealed dependence of a cerebral electric activity on the state of its structures points at the each functional disorder of any brain structure correspondence to its structural disorders. Structure-functional pattern of the radiation brain damage features the abnormalities of the cerebral cortex

(fronto-temporal zone atrophy), subcortical structures and pathways to the dominant hemisphere both with cortico-limbic system primarily in the dominant (left) hemisphere [39]. Our data about the left hemisphere damage in the right-handed clean-up workers are confirmed by an independent research with an auditory evoked potentials application [40], however other authors notify that a left-hemisphere dysfunction is only the initial phase of a psychoorganic syndrome genesis [41].

Just due to the left-hemisphere fronto-temporal dysfunction and schizotypal traits we have introduced the diathetic-stress hypothesis of the ionizing radiation as a risk factor of schizophrenia spectrum disorders [20].

Among the major part of the clean-up workers exposed by low radiation doses (under 0.3 Gy), in the Chernobyl exclusion zone personnel, and in the “Shelter” unit staff members got employed in a recent few years the diseases are found corresponding to the chronic fatigue syndrome (CFS) criteria. The hypothesis has been introduced on the CFS origination and genesis under the synergic impact of low/very low radiation doses and stress. The left (dominant) hemisphere cortico-limbic dysfunction is a psychophysiological background of the CFS following a radioecological catastrophe [42]. At that the CFS incidence in the clean-up workers had decreased substantially (65.5% vs. 10.5% since 1990–1995 till 1996–2001), whereas the incidence of metabolic syndrome X (MSX) has increased as the years go by (15% vs. 48.2%). The CFS and MSX are considered as the neuropsychiatric and somatic pathology developmental stages where the CFS undergoes transformation to the MSX. The radiation-induced injury to the mitochondrial DNA (mtDNA) in postmitotic tissues peculiar with a low proliferation can be one of the key pathways of a low-dose radiation effects towards a non-cancer morbidity elevation in the Chernobyl NPP accident survivors [43]. That is to say the CFS can be considered within the Chernobyl catastrophe model as an induced by the multiple environmental factors (acute and chronic external and internal irradiation, chemicals, viruses, psychological stress etc.) predisposition to neurodegeneration, cognitive injury and neuropsychic disorders [44].

Ionizing radiation impact is a risk factor of the age-associated diseases with neurodegeneration in particular, and the aging and neurodegenerative processes can be exacerbated through the synergic effects of heterogeneous disturbing factors such as immune ones, oxidative stress and molecular-genetic abnormalities. Thus the radiation can accelerate aging and cause neurodegeneration [45].

Undoubtedly the neuropsychiatric effects in the clean-up workers are etiologically heterogeneous however the clinical and paraclinical research provides data on the probable role of ionizing radiation in their onset and genesis under total radiation doses exceeding 0.3 Gy.

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CHAPTER 18 SENSORY ORGANS

In contemporary conditions the human working capacity is largely determined exactly by functional possibility of these sensory organs. Unfortunately, without regard to the several attempts of researcher collaboration in this field, this report is the first complex analysis of the state of sensory organs exposed to radiation as a result of the Chernobyl catastrophe.

Functional disorders of sensory analyzers, first of all, visile, vestibular and auditory, occupy the leading places in the structure of pathology among those exposed to radiation [1].

Presently there is practically a consensus that the functional state of sensory analyzers is one of reliable criteria of estimating human health, and their changes often result in the loss of occupational suitability, a decrease in working capacity and disability [1-5].

Visual analyzer (VA), auditory analyzer (AA) and vestibular analyzer (VeA) anatomically, physiological and functionally are closely connected not only between themselves but also with the central nervous system, the circulatory system and with other body systems that cause their high sensitiveness to the changes in external and internal environments.

For estimating possible changes caused by radiation in VA, VeA and AA of radiation-exposed persons, and first of all in healthy and capable of Chernobyl clean-up workers we and other researchers went the mainly difficult way of realizing large-scale epidemiology researches on the basis of the long-term monitoring and careful analysis of clinical and electro-physiology indices of sense-organs state.

The purpose of researches was to estimate the state of VA, VeA and AA in radiation-exposed persons, correlation between the IR dose and the effects caused by it in clean-up workers, the risk of visual, vestibular and auditory disorders, their character, a degree of manifestations and features of clinical course in the dynamics of postaccidental period.

Latterly the interesting clinical immunological researches were conducted (see subsection 18.1.3).

A problem for assessing state of the sensory organs in inhabitants of the territories contaminated with radiation remains not solved to the significant degree. But the last researches of refractogenesis features in children-inhabitants of the territories contaminated with radiation (subsection 18.1.4) considerably extended our conceptions of eye development in children who were permanently exposed to ionizing radiation as a result of soil contamination with radiation substances.

And, in conclusion, last years some aspects of a state of the sight organ in children, born to the radiation-exposed persons, — clean-up workers were first studied (subsection 18.1.5).

(R. Fedirko, N. Mishanchuk)

18.1 EYE: CLINIC, DIAGNOSTICS, REGULARITIES AND RISKS FOR DEVELOPMENT OF EYE PATHOLOGY IN CHORNOBYL CATASTROPHE SUFFERERS

Researches conducted for 20 years since the Chornobyl catastrophe testify that just the eye is one of basic targets of radiation influence [1–5, 6].

We studied the state of the sight organ in 25,344 persons among them 11,123 Chornobyl clean-up workers, 5,270 evacuee from alienation zone and 5,297 inhabitants of the controlled territories, as well as 3,454 persons of other categories who were examined in RCRM. Mainly examinations were carried out within the limits of CER.

As a result of the conducted researches, it was established, that eye diseases related to radiation are divided into two groups.

Radiation cataract [7, 8] which is examined as a deterministic effect (results of studying radiation cataract in radiation-exposed person in connection with the Chornobyl catastrophe are presented in part 2) was traditionally considered as a basic consequence of radiation effect on the eyes. The other known consequence of radiation influence is radiation retinopathy including two its new forms such as "syndrome of chestnut" (early and late) and "syndrome of diffraction grate" are described in victims as a result of the Chornobyl catastrophe.

At the same time the new radiogenic effects with deterministic signs were also found:

- functioning of the eye as a complex of receptors is accompanied by the generation of permanent retinal potential. An exposure to radiation disorders the generation of this potential with threshold dose 20 cGy.
- radiation causes a dose dependent decrease in capacity for accommodation. A threshold dose for this effect is equal to 15 cGy.

It was also proved, that radiation influence substantially modified the morbidity due to some other eye diseases. In addition, the functional changes in the sight of radiation-exposed persons were revealed.

SPECIFIC RADIATION DAMAGES OF THE EYE

Radiation cataracts

Examinations performed after Chornobyl catastrophe proved that radiation cataract appeared at the doses significantly less than it was early considered. The first results of UACOS studies evidenced of possible existence of threshold at the level nearly 0.1 Gy for some age groups, i.e. significantly lower than 2 Gy; the threshold was dependent on cataract form and could be higher than 0.7 Gy [6]. A dose threshold for radiation cataract was not fixed in other study, a latent period could exceed 24 years. Data of studies and mathematic modelling proved in favour of the view that radiation cataract was a stochastic effect of radiation exposure.

It has been shown that an appearance of non typical changes in the children and adolescent lens was possible as partial opacities and changes in optic infiltration without decreasing visual acuity within certain time and were only diagnosed in purposeful examination under chronic radiation effect. A dose dependency was proved for this pathology.

Radiation retinopathies. Hemorrhages on fundus of eye, retinal edema, exudates focuses were noted soon after the catastrophe in persons who had worked since the first days in the most radiation contaminated areas. Two forms of retinopathy, caused by the action of IR, with enough clear clinical picture were detected: "syndrome of chestnut" and "syndrome of diffraction grate" [3].

In the fall of 1986 we have observed certain changes in the retinal vessels — loss of tonus of venules and arterioles with appearance of huge number of aneurisms in the perimacular zone, and also sacculations having a stretched form which contacted with the extended veins. The picture resembled a crown or rather a leaf of chestnut tree. Macula was surrounded by such "leaves". Such specific clinical picture was called the "early chestnut syndrome" [3]. It could be observed till the end of 1986 — first months of 1987.

Since 1993 in the locations of micro aneurisms and extended venules under "early chestnut syndrome" loss of pigment was beginning, then sclerotic foci appeared in clean-up workers of acute period and evacuees from the city of Prypiat. Macula was closely surrounded by a circle of ellipsoid foci of sclerotic character representing imprint of microaneurisms and sacculations. We called this clinical picture the "late chestnut syndrome" [3].

In separate individuals exposed under special condition during their stay within the direct vision of the bared active zone of the 4th reactor we observed a different clinical picture. Small round foci of white color appeared around the macula located behind the retinal vessels. As compared with the clusters of a vitreous plate a distinct order of location of those foci was observed: distances from each focus to neighboring ones was equal, it seemed that they appeared in foci of a diffraction grating. Near the macula foci were located closer to each other, and were bigger; beyond the fundus of the eye they looked rather like points on the periphery of diffraction picture. This picture was called the "syndrome of diffraction grating" [3]. Disruption of color perception, weakly expressed metamorphopsias were observed under this syndrome. More often we found the weakly expressed form with few foci.

Now it is impossible to find out the frequency of acute radiation retinopathies observed because the systematic examinations of victims in an early postaccidental period was not conducted and only those, who applied to ophthalmologic help were examined [3].

Generation disorder of permanent potential in the retina

The pigmental epithelium of retina, the photoceptors and choroid take part in forming a permanent potential of the eye. As a disorder of the functional state of pigmental epithelium is one of main pathogenetic links in the development of retinal dystrophic processes, the method of electrooculography (EOG) — registration of permanent potential of the eye — was used by us during examination of persons exposed to radiation at the Chornobyl catastrophe [9].

An experimental group (44 eyes) was formed by the matching method. Matched clean-up workers were aged 40–60 years (middle age was 49.8 ± 1.3 years). The officially registered doses of whole body irradiation were presented in 34 cases and ranged from 4.6 cGy to 94.5 cGy. Middle dose of irradiation consisted of 27.24 ± 4.1 cGy. EOG examination was also carried out on 22 clinically healthy eyes of control group persons (middle age — 49.1 ± 2.3 years), the inhabitants of Kyiv, without a contact with ionizing radiation. Visual acuity 0.7 — 1.0 was in the persons of studied and control groups without a correction and 1.0 — with a correction, an ametropia did not exceed 3.0 D. Electrooculograms were registered by the method [9].

The eyes of persons in control group were examined for clarifying the normal values of EOG indices. An Arden coefficient ranged from 200% to 299.5% in the control group with a mean value of $245.99 \pm 7.63\%$. No significant changes in the Arden coefficient were noted in observed person depending on age.

The average Arden coefficient was $199.49 \pm 8.20\%$ in the group of the radiation-exposed persons.

EOG for one of examined clean-up workers and a person of control group is shown in Fig. 18.1.

A dependence of the Arden coefficient on a dose loading was found. There was a direct middle feed-back between the documented dose and the value of Arden coefficient ($r=0.41 \pm 0.14$, $p<0.05$), i. e. with increasing dose of irradiation a light dark coefficient is decreased.

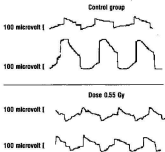


Fig. 18.1. Electrooculograms for one of examined clean-up workers and a person of control group

No significant differences were revealed in EOG values of the majority of people with the dose loading up to 20 cGy. The values of the Arden coefficient ($173.36 \pm 15.22\%$) were significantly ($p<0.05$) lesser in persons with the dose loading over 20 cGy, than in persons with a dose up to 20 cGy and significantly ($p<0.05$) lesser than in control. The amplitude of dark minimum was insignificantly changed in radiation-exposed persons, while the amplitude of light maximum was significantly decreased in persons with the greater dose load comparing to control as well as dose group of patients irradiated up to 20 cGy.

A decrease in EOG indices characterizing a state of the pigmental epithelium, the photoceptors and the choroid, testifies the presence of pathological changes in these structures. The initial functional disorders can be detected by EOG before a clinical manifestation of pathology [8].

In spite of the absence of clinical symptoms in fundus of the eye pathology, premed disorders in generation of retinal permanent potential were connected with

metabolic disorders in its external layers of radiation-exposed persons with the dose loading over 20 cGy. Changes found in EOG indices and their dose dependence was an objective argument of negative influence of ionizing radiation on the retina. A dose of 20 cGy was a threshold for arising this effect.

A dose dependent decrease of accommodation capacity.

To study the accommodation ability of eyes as one of symptoms of age changes in individuals exposed to irradiation [10–12] 456 randomly selected clean-up workers were examined. They were divided into groups according to levels of absorbed doses and age.

Control group consisted of 136 industrial workers (aged 20–64 years; mean 43.9 ± 0.72) living in area with normal radiation conditions (more than 800 km from Chernobyl). Individuals with ametropia more than 2.0D were excluded.

The examination was conducted in period of 1992–1998. Volume of accommodation was measured by accomodometer AKA-01. Instrument AKA-01 is intended for monocular measuring of volume of accommodation.

Analysis of results of examination and statistical processing was implemented with the help of special programmes (CER 3.00 and CER 3.02); analysis was conducted separately for each group, each age group and for age groups with intervals of 5 years; in all the cases average value of volume of accommodation, error of average value, signal deviation, trust interval and variation coefficient were determined.

Both in group with various doses of absorbed external radiation statistically reliable decrease of volume of accommodation was noticed in comparison with control in number of age groups (Fig. 18.2). Volume of accommodation was reliably higher in group of individuals with absorbed external irradiation doses below 0.25 Gy in comparison with individuals with absorbed doses of 0.25 Gy and more in age groups of 28, 29, 34, 37, 38, 40, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58 years, $p < 0.05$ (in age groups 40 and 56 years $p < 0.001$).

During correlation analysis it has been noted that volume of accommodation depended first of all from age ($r = 0.83 \pm 0.01$; $p < 0.001$; relation was reliable reverse high). Refraction in examined group did not influence on volume of accommodation. Reliable ($p < 0.001$) reverse connection ($r = 0.25 \pm 0.03$) was noticed for volume of accommodation with absorbed dose.

However compared to controls it was clear that the ranges of accommodation in exposed group suffered greater decrements than did the control. To describe the changes of the accommodation ability in the entire irradiated persons group, beside the age of its members, the adequate ($p < 0.05$) linear model

$$RA = b_0 + b_1 \cdot \text{age} + b_2 \cdot \text{dose} \quad (18.1)$$

where b_0 , b_1 , b_2 — coefficients; age — age, years; dose — dose, Gy.

and logarithms model ($p < 0.05$) took account the dose loading (Fig. 18.2). Excess of ranges of accommodation was -0.78 D/Gy, and -0.19 D/year (linear model).

A highly significant inverse correlation (Pearson) was found between the absorbed dose and the range of accommodation in the groups of patients of the same age. Correlation coefficient r between the absorbed dose and the volume of accommodation in age groups 26, 28, 30, 33, 34, 37, 41, 42, 44, 45, 46, 48 yrs. varied from -0.28 to -0.49

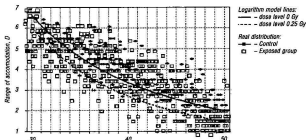


Fig. 18.2. Range of accommodation, D , in control group and in the exposed group.

Lines — logarithmic model lines, symbols — real distribution

($p < 0.05$, Pearson). In all age groups the increase of the dose loading was accompanied by a decrease in the accommodative ability.

It has been established that model doses are thresholds for the factor, and threshold for this effect is a dose of 15 cGy.

(P. Fedirko)

OTHER EYE PATHOLOGY

Involucional cataract.

An age cataract, a disease of elderly, is the most prevailed pathology of lens that causes a special actuality and social influence of researches regarding regularities of its development [1].

Data mentioned below are based on the results of examination within the limits of CER. A prevalence of involucional cataract (per 1000 persons) was increased for examination period from 294.3 ± 32.0 in 1993 to 766.7 ± 35.9 in 2004.

Its frequency was greater in elder age groups, in a group of clean-up workers as well as in a control group. But, as is shown below, an age prevalence of involucional cataract in liquidators is considerably exceeded a control level, a change in beginning of illness development into the side of more young age groups is observed. So, a relative risk made up 15.89 (CI — 2.22; 110.82) in clean-up workers under 40 years; in a group of 40–49 year old — RR=8.7, CI — 4.02; 18.85; in a group of 50–59 years — RR=3.86 and CI — 2.17; 6.88 comparing to the corresponding age group of control. A difference was also remained significant in an age group of 60 years and more (RR=2.98, CI — 1.57; 5.5), however a tendency to relative risk decrease caused by radiation has remained with an age: it can be an after-effect of greater sensitiveness of lens to radiation influence in relatively younger persons.

Thus, the frequency of involucional type cataract was under influence of age, time of stay under the risk, and a dose.

A frequency of involutional cataract was studied in the groups of patients of different age and with the different dose loadings (Table 18.2). The results are evidence of the presence of dependence between the prevalence of this disease and the dose loading.

The model of absolute risk of involutional cataract is shown below:

$$R = \frac{\exp(\beta_0 + \beta_1 \cdot t + \beta_2 \cdot v + \beta_3 d)}{1 + \exp(\beta_0 + \beta_1 \cdot t + \beta_2 \cdot v + \beta_3 d)}, \quad (18.2)$$

where R — absolute risk; $\beta_0, \beta_1, \beta_2, \beta_3$ — coefficients; t — time of stay under the risk; v — age at the moment of examination; d — radiation dose.

A model of relative risk of involutional cataract is as follows:

$$\lambda = \frac{e^{\beta_0 + \beta_1 v + \beta_2 d + \beta_3 t}}{1 + e^{\beta_0 + \beta_1 v + \beta_2 d + \beta_3 t}}, \quad (18.3)$$

where v — age, years; d — $\sqrt{(d \cdot \bar{d})}$; t — time of stay under a risk, years; d — radiation dose, Gy.

Relative risks calculated by the model (18.1) are shown in Table 18.1. An influence of age factor was the greatest, $RR = 2.89$ (2.53; 3.31).

Table 18.1. Relative risks by the model of relative risk of involutional cataract

A coefficient at:	RR	CL
β_0	0.0057	0.0027; 0.012
β_1 — age, years	2.895	2.529; 3.313
β_2 — $\sqrt{(d \cdot \bar{d})}$, where d — radiation dose, Gy; t — time of stay under a risk, years	1.681	1.033; 2.735
β_3 — time of stay under a risk, years.	1.139	1.057; 1.228

Relative risk of involutional cataract depending on the dose loading at time of stay under the risk 5 year is shown in Fig. 18. 3.

The conducted modelling revealed that a statistically significant increase in a risk of involutional cataract is the most significant at fifth year stay under a risk. Thus, an absence of the significant difference of involutional cataract frequency has been found explanation in clean-up workers and non-irradiated persons before 1991.

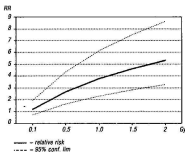


Fig. 18.3. Relative risk of involutional cataract depending on the dose loading at time of stay under a risk 5 year

The results of our studies do not contradict the data of other researches. During an international study UACOS [6, 13], in addition to typical radiation, cortical, i.e. involutonal cataract was studied. An extract from the results of researches within the limits of UACOS is shown in Table 18.2.

Table 18.2. Adjusted Odds Ratios (OR) and 95% Confidence Intervals by dose group and the estimated OR at 1 Gy for outcome variables observed. Reference — 0–99 mGy dose range (UACOS, cit Worgul B.V., Kundiyaev Y.I., Sergiyenko N.M. et al, 2007 [13]).

Variable	OR at 1Gy	OR for dose range, mGy				
		100–249	250–399	400–599	600–799	800+
Early pre-cataract changes	1.15 (0.79–1.66)	1.19 (1.1–1.3)	1.12 (0.9–1.4)	1.16 (0.8–1.5)	0.85 (0.5–1.3)	1.07 (0.6–1.8)
Stage 1–5 cataract	1.70 (1.22–2.38)	0.94 (0.8–1.3)	1.02 (0.8–1.3)	1.07 (0.8–1.5)	1.55 (1.1–2.3)	1.77 (1.1–2.8)
Stage 1 cataract	1.49 (1.08–2.06)	0.92 (0.8–1.1)	0.97 (0.8–1.2)	0.89 (0.7–1.2)	1.46 (1.0–2.1)	1.65 (1.1–2.6)
Stage 2–5 cataract	1.57 (0.79–3.11)	1.04 (0.7–1.7)	1.56 (0.9–2.8)	2.37 (1.2–4.6)	1.29 (0.5–3.1)	1.28 (0.5–3.4)
Mean dose (mGy)		149	298	490	689	1095

Expressed cataracts were more frequently observed in the group with a dose over 400 mGy (40 cGy). The initial changes in lens were detected already in a group with a dose exceeding 100–249 mGy; it was obvious that a risk of cataract in this dose group would be also confirmed by following observation [6].

Thus, our data and researches of other authors conducted after the Chernobyl catastrophe demonstrated, that ionizing radiation is a risk factor of the involutonal cataract development.

(B. Fedirko)

Retinal angiopathy and angiosclerosis.

An expressed growth of retinal angiopathy prevalence (314.8 ± 14.5 per 1000 persons in 1993 and 911.9 ± 19.7 per 1000 persons in 2004), and also an increase in the frequency of angiosclerosis were found in the cohort of clean-up workers based on the CER. The similar results were obtained by other authors [5].

Risk of retinal angiopathy and angiosclerosis in groups with the different dose loading is shown comparing to control in Table 18.3

Table 18.3. Relative risk of vascular pathology of retina in radiation-exposed persons comparing to control

Indices	Dose range, Gy		
	<0.1	0.1–0.249	0.25 and over
Relative risk	2.51	2.89	2.65
Confidence intervals	2.03; 3.1	2.17; 3.32	2.15; 3.26
χ^2	121.61	126.13	161.24
p	<0.001	<0.001	<0.001

The model of absolute risk of retinal angiopathy resembled a formula (18.4) of relative and excess risks of angiopathies, risks were also described by dependences of logistic regression, for a relative risk:

$$\lambda = \frac{e^{\beta_0 + \beta_1 \cdot \log(d)}}{1 + e^{\beta_0 + \beta_1 \cdot \log(d)}} \quad (18.4)$$

and excess risks:

$$\pi > e^{\beta_0 + \beta_1 \cdot \log(d)} \quad (18.5)$$

where d — dose, Gy.

Relative risks calculated by a model (18.4) are shown in Table 18.4 (Likelihood Ratio Statistic on 2 DF = 297.78, $p < 0.001$)

Table 18.4. Relative risks of retinal angiopathy, counted by the model of relative risk

Coefficient	Relative risks	Confidence interval
β_0	5.925	2.68; 12.34
β_1 — $\log(d)$, where d — dose, Gy	1.29	1.069; 1.557

Thus, basic factors that cause the risk of retinal angiopathy are the age and the time of stay under the risk: a contribution of irradiation dose is lesser.

Subsequent researches in this direction could be based on involving other contingents. For involuntal effects where radiation effect is a risk factor, it is important to conduct an examination at a certain period after radiation influence (so-called "window of risk analysis") that was turned out by a research on the basis of CER. Therefore a large cohort study of retinal angiopathy morbidity including prolonged monitoring of Dnipropetrovsk region inhabitants, the Chernobyl clean-up workers with the known dose loadings (2,892 radiation-exposed persons) was conducted. 15 annual stages of examination were conducted with a middle duration of examination 12.83 ± 0.08 years. On the whole, there were 26605.24 men-years examinations.

A middle ages of patients consisted of 35.46 ± 0.13 years at a moment of the examination; a distribution according to the age was normal. An official dose loadings (dose of external whole-body irradiation) were known for all examined patients — from 0.01 to 0.7 Gy, middle dose loading — 0.21 ± 0.01 Gy, a distribution in accordance with the age groups was normal.

Morbidity of retinal angiopathy was increased with the time increasing of stay under the risk, and a maximum was reached in 9 years after radiation influence. Then there was an insignificant

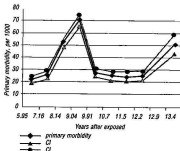


Fig. 18.4. Dynamics of primary morbidity of retinal angiopathy in the cohort of clean-up workers

decrease in morbidity, with the repeated increase in 13 years after radiation influence (Fig. 18.4).

Such character of curve is evidence of significant effect of two factors as minimum on primary morbidity of retinal angopathy. It is most likely, if a cohort, with above shown normality by the age, was only under effect of the age factor, we would look only one increase of morbidity.

An analysis of morbidity was conducted depending on a dose loading. Relative risk to fall ill was 1.65 (1.02; 2.67) at $\chi^2 = 4.15$; $p = 0.041$ in all group of radiation-exposed patients in a dose of 30–70 cGy comparing to radiation-exposed patients in a dose up to 0.3 Gy.

Thus, a of cohort analysis resulted in a statistically significant higher level of primary morbidity of retinal angiopathy was found in the group with greater radiation-exposed dose.

(P. Fedirko, N. Gorkava)

Macular chorioretinal degeneration.

The retina was considered to be relatively resistant to impact of ionizing radiation. Still before the Chornobyl catastrophe a number of authors expressed opinion based on their studies about extreme sensitivity of the retina to irradiation and showed that the retina can be damaged both under considerable and "low" doses of ionizing radiation. Experimental studies conducted after the Chornobyl catastrophe on rats constantly kept in the alienation zone around the Chornobyl NPP proved direct impact of ionizing irradiation on the retina. Photoreceptors appeared to be the most vulnerable ones; changes were also noticed in bipolar and ganglion cells and pigmental epithelium. Response of the vascular membrane of the eye on chronic impact of radiation factors of the Chornobyl NPP accident was studied in the similar series of experiments. Affection of collagen structures and basic substance of vascular membrane, dystrophic changes on endothelium of the vessels, increase of weight of connective tissue were detected [1–3, 5].

According to our data, the central chorioretinal dystrophy became a frequent type of pathology, well after the catastrophe. After the development of sclerosis of the retinal vessels, changes in the macular area appeared: foveated reflex was smoothed out; foci, blocks of pigment appeared.

In a few years after the catastrophe the frequent and premature development of macular chorioretinal degeneration in irradiated persons was noted. The pathological changes of retina mainly were represented by drusen, dots of chorioretinal atrophy, pigment redistribution. Cases of "wet" form of this disease in our cohort were rare. Predominance of initial stage of disease in our patients was accounted by fact, that clean-up workers cohort consisted mainly of young and middle-aged men, who were healthy at the moment of their participation in clean-up operation. Later on the pathological process was progressing slowly, mainly as chorioretinal atrophy. The eyesight was failing slowly, but irrevocable. It is necessary to take into account that repeated examinations were conducted under conditions of permanent medical treatment of the patients. It is possible, that this circumstance had an influence on course of disease [3].

Macular degeneration prevalence and primary incidence rate in our cohort exceeded populational ones. A stable trend for the increase of this disease frequency was

observed. The macular chorioretinal degeneration frequency increased from 83.1 ± 5.7 per 1000 persons in 1992 to 585.7 ± 23.8 per 1000 persons in 2004. Prevalence of this disease in cohort was 224.83 ± 5.8 per 1000 persons, that considerably exceeds control level (55.56 ± 13.5 per 1000). The cohort research allowed to establish, that many new cases of macular degeneration appeared in groups which had been examined before. A considerable rising of macular degeneration morbidity was noted in 6.5–7.0 years after irradiation, its maximum level was observed in 10 year after catastrophe. Detailed analysis in dose-age groups was performed. Some results are shown in Table 18.7. The frequency of macular degeneration increased along with age both in basic and the control groups, but in the same age group the risk of developing this pathology was significantly greater in the basic one (Table 18.7).

The increasing of macular degeneration incidence when increasing of dose loads were noted in every age group. For example, chorioretinal degeneration morbidity for the age group under 40 years was 120.17 ± 31.13 per 1000 men-years in dose-group of 0.25–0.499 Gy, and 47.29 ± 17.94 per 1000 men-years in dose-group below 0.05 Gy (difference statistically significant, $p < 0.05$). Thus, the morbidity level dependence on radiation dose was observed. The analysis of macular degeneration morbidity in different age-dose groups confirmed its level dependence on both the age and the dose load.

The computation of relative risk of chorioretinal degeneration in comparison with the control for various age group makes us to believe that risk of development of this disease is greater for persons irradiated in more young age (Table 18.5).

Table 18.5. Relative risks of macular chorioretinal degeneration in the examined clean-up workers of different age groups with different doses of external irradiation (Gy) in comparison with a control groups

Age, years	Index	Doses of external irradiation (Gy)	
		<0.25, n=1138	0.25≤, n=856
under 40	Relative risk (Confidence Limits)	5.81 (1.42; 23.79)*	7.89 (1.91; 32.57)*
40–49	Relative risk (Confidence Limits)	3.34 (1.25; 8.91)*	5.70 (2.15; 15.08)*
50–59	Relative risk (Confidence Limits)	3.60 (1.64; 7.89)*	3.88 (1.77; 8.44)*
60 >	Relative risk (Confidence Limits)	3.60 (0.85; 5.63)	3.88 (1.77; 8.44)*
All	Mantel-Haenszel Relative Risk of Disease, given Exposure (Greenland/Robins Confidence Limits)	3.44 (2.12; 5.58)*	4.46 (2.76; 7.23)*

*difference significant, $p < 0.05$ between corresponding age control groups

The high level of morbidity in relatively young age groups of clean-up workers is indicative of an early beginning of this heavy pathology development.

The study and analysis of influence of some non-radiation factors on risk of macular chorioretinal degeneration development were conducted. Except age such factors as chemical, physical and biological professional harmfulnesses, diet peculiarities, smoking, alcohol abuse, stresses etc.) did not influence considerably on evaluated risk.

An adequate non-linear multifactor model of macular chorioretinal degeneration prevalence was obtained, which follows:

$$R = \frac{\exp(\beta_0 + \beta_1 t + \beta_2 v + \beta_3 \log(d))}{1 + \exp(\beta_0 + \beta_1 t + \beta_2 v + \beta_3 \log(d))} \quad (18.6)$$

where R — absolute risk; $\beta_0, \beta_1, \beta_2, \beta_3$ — coefficients; t — time after exposure, years;
 v — age, years; d — dose, Gy

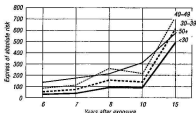


Fig.18.5 Excess of absolute risk of maculodystrophy by a trifactorial model in radiation-exposed patients at the doses of 50–200 cGy in the different age groups depending on time of stay under the risk (per 1000 persons)

frequency of maculodystrophy in the different age groups of radiation-exposed patients (Fig. 18.5).

The nonlinear model of relative risk of maculodystrophy is as follows:

$$\lambda = \frac{e^{\beta_0 + \beta_1 \text{doz} + \beta_2 v}}{1 + e^{\beta_0 + \beta_1 \text{doz} + \beta_2 v}} \quad (18.7)$$

and the model of risk excess:

$$\pi > e^{\beta_0 + \beta_1 v + \beta_2 \text{doz}} \quad (18.8)$$

where $\beta_0, \beta_1, \beta_2$ — coefficients; $\text{doz} = \sqrt{(d \cdot t)}$, where d — dose, Gy, t — time after exposure, years; a — age, years.

Model coefficients (18.9) are shown in Table 18.6

Table 18.6 The relative risks of maculodystrophy counted by a model (18.7)

Coefficients	Relative risk	95% confidence interval
β_0	0.019	0.011; 0.034
$\beta_1 = \sqrt{(d \cdot t)}$, where d — radiation dose, Gy; t — time of stay under a risk, years	6.453	3.115; 13.37
β_2 — age, years	1.727	1.498; 1.990

The relative risk of maculodystrophy counted by a model depending on the dose loading at time of stay under risk 5 year is shown in Fig. 18.6

Summarizing the results of modelling relative risk and excess of relative risk of maculodystrophy, one can state that the greatest is an influence of such factors, as an age

(relative risk — 1.727 (1.498; 1.727) per 1 year, an excess of relative risk — 1.076 (1.056; 1.095) per 1 year), a time of stay under the risk and a dose (relative risk 6.453 (3.115; 13.37), an excess of relative risk — 1.253 (1.137; 1.37) per $1 \sqrt{(d \cdot t)}$, where d is a dose of whole-body irradiation, Gy, t is a time of a stay under a risk, years).

Thus, the results of cohort research and mathematical modeling allowed to establish that the risk of maculodystrophy to the greatest degree depends on the age of radiation-exposed patients at the period of examination, the time of stay under the risk, a dose of external irradiation. A significant increase in the prevalence of this ophthalmopathy was not expected during the first years of staying under the risk after irradiation in a dose up to 2 Gy, its latent period consisted of 6.5 years. These propositions coincide with presented in literature [1] data of examinations and allow to explain them.

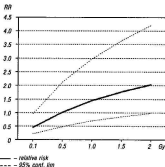


Fig.18.6. Relative risk of maculodystrophy counted by a model depending on the dose loading at time of stay under risk 5 year

(P. Fedirko)

CHANGES IN ELECTROPHYSIOLOGIC INDICES OF THE EYE IN RADIATION-EXPOSED PERSONS

It is known that preclinical functional changes are preceded to the development of clinical pathology. The analysis of literary data testifies that an influence of ionizing radiation (IP) on the functional state of visual analyzer is not enough studied. All ophthalmologists who were examined the consequences of the Chornobyl catastrophe applied visometry and perimetry, the reports of using other methods are not numerous [8].

The methods for determining a critical flicker frequency (CFF), an estimation of the limit of retinal electric sensitiveness (RES) by the phosphene phenomenon and critical flicker frequency by a phosphene (CFFP) were used for studying the functional state of macular zone, internal layers of retina and visual nerve. A group of 95 Chornobyl clean-up workers was matched among the patients of CER by the matching method. An age of examined patients was 30–60 years, a dose diapason of external irradiation consisted from 0.02 to 1.9 Gy. A control group included 42 persons at the age from 30 to 60 years who did not have a contact with ionizing radiation. Clinically healthy eyes, visual acuity not smaller than 0.8, emmetropia or ametropia, no more, than 3.0 D were revealed in all examined patients. CFF of the area in yellow spot was determined by perigraph. An electric stimulator was used for determinining RES and CFFP. An active electrode of this stimulator was placed on the upper eyelid, an indifferent ones — on

the forearm of examined patient. An examination was carried out in a dark-room after 5-minute adaptation to darkness.

The results of analysing findings demonstrate the presence of substantial changes in the functional state of the sight organ in Chernobyl clean-up workers comparing to control group. Middle levels of CFF to the stimuli of different color for the Chernobyl clean-up workers are presented comparing to control in Table 18.7.

Table 18.7. Levels of critical flicker frequency to the stimuli of different color for clean-up workers in comparison with control

Color	Critical flicker frequency (Hz) for:			
	right		left	
	clean-up workers	control	clean-up workers	control
white	32.7±3.9*	39.1±0.9	32.0±3.4*	38.6±1.2
blue	28.0±3.7*	35.0±0.3	28.2±3.2*	34.3±1.8
green	31.2±2.9*	35.1±0.3	31.4±2.9	34.9±0.5
red	27.9±3.2*	34.4±0.4	27.4±2.9*	33.8±1.4

*difference with control is significant, $p<0.05$.

The significant decrease in CFF was observed independently on the color of twinkling stimulus in a group of clean-up workers comparing to control. This was an evidence of a decrease in functional capacity of macular retina in the radiation-exposed persons, despite an absence of organic changes. CFF did not depend on different dose loading.

Electric sensitiveness of retina and CFF by a phosphene in the Chernobyl clean-up workers is shown comparing to the control group in Table 18.8.

The decrease in an electric sensitivity of the retina (increase in tension current causing appearance of the phosphene phenomenon) and declined ability of distinguishing fluctuations by a phosphene in the radiation-exposed persons are evidence of disorders in internal layers of the retina and the visual nerve.

Table 18.8. Limit of electric sensitiveness of the retina and critical flicker frequency by a phosphene in the Chernobyl clean-up workers comparing to the control group

Category of examined persons	Electrophysiologic index	
	retinal electric sensitiveness, mCA	critical flicker frequency by a phosphene, Hz
clean-up workers	66.20±0.73*	42.1±0.6*
control	53.73±0.89	46.7±0.89

*difference with control is significant, $p<0.05$.

Thus, the significant changes in electrophysiologic indices of the visual analyzer state, that the sign of worsening functional state in the retina and the visual nerve, their pathological changes in a subclinical level were found in the group of clean-up workers without clinical manifestations of eye diseases.

(R. Fedirko)

PATHOLOGICAL CHANGES OF THE EYE AND IMMUNOLOGICAL DISORDERS IN CLEAN-UP WORKERS

To study a possible correlation between immune disorders and eye pathology in the radiation-exposed persons the cytokine profile of tear was investigated from 1998 to 2004 in the groups of 387 clean-up workers at the age from 33 to 57 years with absorbed doses from 0.14 to 0.25 Gy with diseases of the sight organ and without such diseases.

The tear sampling was carried out by micropipettes from one eye. Immunological researches were conducted with the laboratory equipment Sanofi Diagnostic Pasteur (France) by tests systems "ProCon" certificated in the Ukraine.

Changes of tear cytokine profile were found even in a group of clean-up workers without an eye pathology comparing to control. A significant increase in the concentrations of proinflammatory cytokine TNF α up to 176 ± 2.5 pg/ml ($p < 0.01$), and proinflammatory cytokine IL-1 β — up to 154 ± 2.8 pg/ml ($p < 0.01$) were noted comparing to control. The level of anti-inflammatory cytokine IL-4 in tears remained at the low limit of norm — 32.6 ± 2.2 pg/ml ($p > 0.05$).

The presence of ophthalmopathology in clean-up workers increased the level of TNF α 1.8 times higher, IL-1 β 1.9 times higher comparing to control values ($p < 0.001$). The level of anti-inflammatory cytokine IL-4 in tears was moderately decreased and made 26 ± 1.5 pg/ml ($p < 0.01$) in the group of patients with maculodystrophy, 28 ± 2.1 pg/ml ($p < 0.01$) in patients with peripheral chorioretinal dystrophy; and 25 ± 1.9 pg/ml ($p < 0.01$) — in patients with an initial cataract.

Thus, a significant increase in the concentration of proinflammatory cytokines and a decrease in the level of anti-inflammatory cytokine were observed in clean-up workers with ophthalmopathology.

(A. Petrunia, V. Prolov)

REFRACTOGENESIS PECULIARITIES IN CHILDREN DWELLERS OF RADIATION CONTAMINATED TERRITORY

The features of refractogenesis in children with persistent irradiation influence were studied in long-term research [4]. During 11 years, 989 pupils (born in 1981–1992) of 1–11 classes living in one of climate-geographical zone of the Zhytomyr region, but in ecologically different districts were subjected to the repeated examinations.

The 1st group (main) consisted of 533 pupils from the radiation-contaminated settlements of Ovruch district included two sub-groups: the 1st sub-group — 485 pupils at the age of 6.5–16.5 years dwellers of urban village Pershotravneve and village Pryluky with ^{137}Cs of 5–10 Ci/km 2 ; the 2nd subgroup — 48 pupils at the age of 8–15 from villages Rudnia, Berezhest, Lyudvynivka, Vystupovychi by caesium-137 contaminated by 10–15 Ci/km 2 of ^{137}Cs). Children of both sub-groups were simultaneously examined and interpreted as children who are living in a radiation contaminated territory.

The 2nd group (control) was made of 334 pupils aged from 6.5 to 16.5 living in Baranivsk district (conditionally clean territory, CCT).

3rd group (additional) consisted of 122 pupils aged 16–17.5 years from settlement Olevsk (^{137}Cs contamination 5–15 Ci/km 2) and 42 school-leavers of secondary school at the age of 16–17.5 years from a urban village Pershotravneve.

It has been established that a refraction in children who were living in RCT ranged from -19.0 D to $+8.75$ D. Refraction variation from -14.0 D to $+8.0$ D was noted in children who were living in CCT. Hypermetropia of weak degree prevailed in both areas. A middle refraction in pupils from CCT statistically did not differ from a middle refraction in children from RCT. Emmetropia (29.6% and 34.3%, $p=0.04$) was significantly rarely observed in CCT comparing to RCT and myopathy of weak degree (5.9% and 2.3%, $p=0.0003$) — significantly frequently.

For the first time the features of forming refraction (the process of emmetropization) were diagnosed in children living in RCT and CCT: an emmetropia was diagnosed in 0.0–18.8% cases of pupils at the age of 7–12 years from an RCT, who were born in 1987–1992, and in 12.3–39.0% cases — in the children of contaminated zone. An emmetropia was more frequently diagnosed in pupils at the age of 13–16 years, who were born in 1981–1986 in RCT (60.0–81.8%) comparing to CCT (35.3–47.9%). From other side,

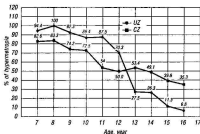


Fig. 18.7. Percent of diagnosed hypermetropia in children of different age category depending on the area of living

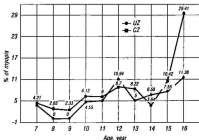


Fig. 18.8. Percent of myopia detection in children of different age category depending on a zone of living (18).

hypermetropia was diagnosed in 70.0–100.0% children at the age of 7–12 years from RCT, and in 50.0–83.3% cases from CCT; in 6.8–27.5% and 35.3–53.4% in children aged 13–16 years, respectively (Fig. 18.7).

Data obtained demonstrate the acceleration of emmetropization process in children aged 7–12 years, born in 1987–1992, and living in CCT comparing to children of analogous age from RCT, and at the same time the increased process of refractogenesis in children of 13–16 years from CCT, born in 1981–1986.

Risk of hypermetropia in the children of CCT, born before the Chernobyl accident was 22 times higher ($p=0.0006$) at the age of 12–14 years, and 2.3 times higher ($p=0.015$) — at the age of 15–16.5 years than in children of analogous age from RCT.

The percent of myopia (Fig. 18.8) among children of the young school age was

caused, as a rule, by the hereditary predisposition. With the increase of age the percent of diagnosed myopia was increased in the "clean" as well as contaminated zones. A frequency of diagnosed myopia was 29.4% in children of 16 years from CCT comparing to 11.4% from a "clean" zone ($p=0.049$). The risk of myopia development in children aged 15–16.5 years from CCT was 1.9 times higher, than in the children of identical age from RCT ($p=0.01$).

A frequency of mixed astigmatism (0.45% and 1.6%, $p=0.03$ respectively) was higher in a radiation contaminated region. The mixed astigmatism was almost noted in all age groups of children in CCT, while it was diagnosed only in 13-years-old pupil from RCT.

Thus, the conducted researches discovered that refraction forming i.e. a development of the eye differed between children living in "uncontaminated" and radiation contaminated zones: an emmetropia was marked in 9% children born after the Chernobyl at the age of 7–12 years from an RCT, and in 26% children of CCT.

Hypermetropia was more frequently diagnosed in children at the age of 13–16 years who were born before the Chernobyl accident in a CCT (44%), than in RCT (17%) that is the sign of refractogenesis deceleration.

Myopia frequency in the age interval 7–16 years was raised from 3.7% to 11.4% in the children of "uncontaminated" zone and from 4.2% to 29.4% in the children of zone contaminated with radiation. Risk of myopia development is 1.9 times as much in children aged 15–16 years who were born before the Chernobyl accident from the zone contaminated with radiation, than in the children from "clean" area.

It was first established that a myopia frequency was different in adolescents aged 16–17.5 years at a finishing phase of refractogenesis depending on birth time regarding the date of the Chernobyl accident: in those exposed to irradiation at an embryo period or at the first year of life — 11%; in radiation-exposed patients at the age of 1–3 — 29%; in children born in 4–6 years after Chernobyl NPP accident — 5%.

(*Y. Sirdyuchenko, O. Nostaghyrova*)

STATE OF SIGHT ORGAN AND ITS FUNCTION IN CHILDREN, BORN TO EXPOSED PERSONS (clean-up workers)

A study of the eye state in children, born at the postaccidental period to the Chernobyl clean-up workers, living in Kyiv comprised 2202 children taking part in cohort research within 1992–2006

The age of examined patients was from 0.74 to 17.2, mean age — 8.3 ± 0.1 years, A control group consisted of 252 inhabitants of Kyiv of same age, including persons whose parents were not additionally exposed to (comparing to other inhabitants of Kyiv) radiation influence. An age of control group persons was from 0.5 to 17.58, mean age — 8.42 ± 0.29 years.

The prevalence of eye pathological changes was 229.34 ± 10.62 per 1000 children of clean-up workers, 170.64 ± 23.69 per 1000 children of control group. The relative risk of pathological change presence for descendants of radiation-exposed parents was increased (due to the age group of 13 years and older) and consisted in 1.33 (CI — 1.0; 1.76, a difference was statistically significant, $\chi^2 = 4.12$, $p = 0.042$) comparing to the control group.

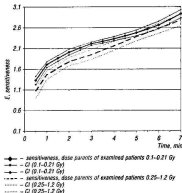


Fig. 18.9. A sensitivity and its confidence intervals (CI) for groups of patients examined depending on time of adaptation

The analysis of results witnessed that a sensitivity (E) for all examined persons was within the limits of permissible values. At the same time some functional indices were worse in the group of children born to parents exposed to the radiation doses over 0.24 Gy, than in children whose parents were exposed to radiation doses up to 0.21 Gy (Fig. 18.9).

Thus, a significant decrease in rate of adaptation to darkness was observed in the second group (children born to parents exposed in the doses of 25–120 cGy)

The mechanisms of these changes require a subsequent verification for better understanding of visual analyzer function.

(P. Fedirko, I. Kadoshnikova)

CONCLUSIONS

Except a radiation cataract, several effects with signs of deterministic ones were detected in radiation-exposed patients as a result of the Chornobyl catastrophe: radiation retinopathy — in the victims exposed as a result of the Chornobyl catastrophe — two its new forms such as “syndrome of the chestnut” and “syndrome of diffraction grate” and also disorder of generation in the eye permanent potential (threshold dose is 20 cGy) and dose dependent decrease in capacity to an accommodation (dose 15 cGy can be considered as threshold).

In addition, radiation influence substantially modifies a morbidity of other eye diseases; the functional changes in the sight of radiation-exposed patients were found.

It has been shown that a radiation exposure causes a significant dose dependent increase in the prevalence (369.9 ± 6.7 per 1000 clean-up workers versus 83.33 ± 16.3 per

Thus, the results of primary examination in groups of clean-up workers were indicative of the greater risk of eye pathological changes for descendants of radiation-exposed patients, but not due to the expressed congenital changes in the eye.

To study the functional possibility of the eye in children born to clean-up workers 32 patients, whose parents were informed of dose loading, were matched for investigation of dark adaptation by standard method using recording semi-automatic adaptometer. Mean age of examined children was 15.91 ± 0.31 , they were born in 2–8 years after exposition of their parents to the mean irradiation dose of 0.307 ± 0.06 Gy.

1000 in control) and premature development of involutional cataract. In the risk model of involutional cataract the basic parameters are the age at the period of examination, the time of stay under a risk, absorbed dose of external irradiation. The relative risk of involutional cataract caused by radiation consists of 1.681 (1.033; 2.735) per $1 \sqrt{(d \cdot t)}$, where d is a dose of irradiation, Gy, t is time under a risk, years.

Effect of ionizing radiations causes a significantly increased risk of pathology in the eye vessels (694.3 per 1000 clean-up workers and 246.53 per 1000 persons of control group). By mathematical modelling it was established that the risk of basic factors in retinal angiopathy of clean-up workers population is the age of radiation-exposed persons, and a dose of external irradiation to the lesser degree.

In other cohort study it was revealed that morbidity of retinal angiopathy was increased with the increase of time for staying under a risk, and attained a maximum in 9 years after radiation influence in clean-up workers. Relative risk to fall ill in all group of radiation-exposed patients in the dose of 30–70 cGy in comparing to radiation-exposed patients in a dose up to 0.3 cGy was 1.65 (1.02; 2.67) at $\chi^2=4.15$; $p=0.041$. Thus, the statistically significant higher level of primary morbidity of retinal angiopathy was found at the period after radiation influence in the group of radiation-exposed in a greater dose as a result cohort analysis.

Radiation influence is a risk factor of premature development of retinal dystrophy at a remote period after irradiation. As a result of radiation the absolute risk of central chorioretinal dystrophy is increased; a prevalence of maculodystrophy among clean-up workers is 224.83 ± 5.8 per 1000 persons, that exceeds significantly control numbers (55.56 ± 13.5 per 1000). The risk basic factors of maculodystrophy development is the age of radiation-exposed patients, the time of stay under a risk, the absorbed dose of external irradiation in population of clean-up workers, by the data of examinations and mathematical modeling. The relative risk of maculodystrophy consists of 6.453 (3.115; 13.37) per $1 \sqrt{(d \cdot t)}$, where d is a dose, t is time under a risk, years.

A significant decrease in critical flicker frequency was found in groups of clinically healthy clean-up workers comparing to control that is evidence of an increase in functional possibility of the macular retina in radiation-exposed persons, without regard to an absence of organic changes. There was also a decrease in electric sensitivity of the retina and a decrease in ability of distincting the fluctuations by a phosphene in the radiation-exposed persons that testify to disorders of functional capacity in the retinal internal layers and the visual nerve.

It has been detected that there was disorder of a correlation between proinflammation and antiinflammation cytokines in the tear of Chernobyl clean-up workers. This disorder was especially expressed at the presence of ophthalmopathology. It is possible that immune disorders, in particular, a correlation of cytokine level, play a substantial role in pathogenesis of diseases in the sight organ of clean-up workers.

The prolonged observation of the refractogenesis process in children who live in the territories contaminated with radiation revealed that they have a delayed development of the eye refraction system, and an increased frequency of myopia that is diagnosed in children who were exposed a maximal irradiation at an embryo period or at the first years of life.

It has been established that a greater frequency of pathological changes in the eye were found out in the children of clean-up workers, born after radiation influence, but not due to congenital changes of the eye; their capacity to dark adaptation depends on the dose loadings of parents of examined persons, although it remains within the limits of permissible values.

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(P Fedirko)

18.2 SENSOR NEURAL DIMINISHED HEARING IN PERSONS EXPOSED AS A RESULT OF THE CHORNOBYL CATASTROPHE, IN THE DYNAMICS OF POST-ACCIDENTAL PERIOD

The results of scientific analysis are based on the clinical, audiometric and electrophysiological examinations of auditory analyzer (AA) in the clean-up workers of Chernobyl NPP accident exposed to short-term, relatively short-term and long-term ionizing radiation (IR) with different doses, under long monitoring during different years of post-accidental period.

Data of other authors who examined changes in the parts of auditory analyzer were analyzed in the categories of persons who lived in clean or in three ionizing radiation contaminated areas of Ukraine (the obligatory resettlement zone, the voluntarily resettlement zone, and the zone of enhanced radioecological control) in the dynamics of different terms since the Chernobyl catastrophe.

There were 7,031 male patients among examined clean-up workers, who were sent to S.I. "O. S. Kolomiychenko Institute of Otolaryngology of Natl. Acad. Med. Sci. of Ukraine" from RCRM for clinical observation and expert estimation of the AA functional state. Individual doses of IR were used from the informative base of this institution as initial data.

The directed persons complained of auditory disorders, such as a noise in the ears of different spectrum of sounding and intensity (low-frequency, high-frequency or mixed), and also a decrease in keen ear and disorder of appeal speaking perception during examination. Hearing within the normal age limits was registered in 55.5 % cases among 1,200 clean-up workers at a primary audiometric examination (1986-1987). A decrease in keen ear that caused the manifestations of sensor neural diminished hearing (SNDH) of different degree was found in the other 44.5% cases. It was related to the diseases under the rubric H.90 by the ICD-10.

Four degrees of hearing-impairment were detected in them according to the V. Bazarov and A.Roskladka classification of 1995 [1]: the 1st degree — easy, the 2nd — middle in 37.0 % cases that are considered as a socially adequate ear, and the 3rd, the 4th degrees of diminished-hearing acuity in 7.5 % cases that were regarded as socially inadequate significant hearing impairments.

In particular, there were 26.3 % cases with the 1st degree of diminished hearing acuity, 10.7 % — with the 2nd degree, 6.1 % — with the 3rd degree and 1.4 % cases — with the 4th degree among them. These data are shown in Fig. 18.10.

For the prolonged examinations 325 clean-up workers were selected by us from the general amount of examined patients. The

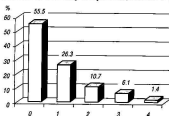


Fig 18.10. State of hearing acuity in accordance with data of audiometry in 1200 clean-up workers at a primary examination (1986-1987). Degree of impaired hearing: 0 — normal hearing; 1-4 degrees of hearing impairment

changes in an auditory analyzer of these persons were studied in a dynamics of 22–23 post-accidental years including 2009. They were divided into three groups depending on a dose and duration of IR effect.

The first group comprised 57 ARS convalescents, among them 31 with ARS grade 1 (I-A group) and 26 patients who had ARS grade 2 (I-B group).

The second group consisted of 120 clean-up workers who were exposed to relative short-term IR in the first 2 months of clean-up works. They were divided into the II-A and II-B groups at primary examination in 1986–1987. The II-A group included 64 clean-up workers whose dose of IR made 0.20–0.25 Gy, and the II-B group consisted of 56 persons who were exposed to IR in the doses of 0.26–0.50 Gy that were retrospectively established after dosimetric calculation (by an occupational route letter).

In the third group 148 clean-up workers exposed to long-term IR were examined before and after work during 6–8 years at the Chernobyl alienation zone in the dynamics of post-accidental period. Their IR dose was analogous to that of patients in the II group and made 0.20–0.50 Gy (by individual physical dosimetry). The III-A group included 79 clean-up workers whose IR dose was 0.20–0.25 Gy, and the III-B comprised 69 persons whose IR dose made 0.26–0.50 Gy.

For the study of the AA functional state a methodology of clinical examination including the complex of standard examinations of auditory function was used: audiometry, electro acoustic examinations — otoacoustic emission of distorted products (OEDP), dynamic impedancecymetry; electrophysiology examinations — the auditory induced potentials (AIP); short-latent (SL) or brain stem and long-latent (LL) or cortical ones.

Hearing acuity was within the limits of age norm in 33 from 57 clean-up workers who had ARS in the I group, and SNDH of the 1st and the 2nd degree was found in 24 persons at a primary examination in 1986–1987. Delayed legibility of speaking was noted in 19 from 33 persons with a normal tonal hearing and in all 24 clean-up workers with SNDH of the 1st and the 2nd degrees in the I group. In particular, their thresholds of 100% legibility of linguistic tests were registered at the level of 60.3 ± 0.5 dB (an expected 45.5 ± 0.7 dB).

Increases in the number of persons with SNDH and the degree of its manifestation were marked in the dynamics of post-accidental period among examined clean-up workers in the I group [2].

Thus, in 2008–2009 in the I-A group only 3 from 29 persons had hearing within the limits of age norm, 2nd as well as the 3rd degree of SNDH were revealed in 11 persons each and 4 persons had the 3rd degree of SNDH. In the I-B group only 1 from 21 persons had normal hearing, 4 had SNDH of the 1st degree, 10 — the 2nd degree, 4 — the 3rd degree and 2 — the 4th degree of SNDH. It should be noted that more considerable degree of auditory changes was fixed in patents of the I-B group who had ARS grade 2 and were accordingly exposed to greater IR doses.

Hearing within the limits of age norm in 34 clean-up workers of the II-A group and in 30 persons in the II-B group. Diminished hearing was noted in other 56 clean-up workers of II group, 33 persons from them had SNDH of the 1st degree, and in 23 — of the 2nd degree.

In addition, a delayed legibility of linguistic tests was noted in 13 from 64 clean-up workers with normal tonal hearing acuity and in 17 from 56 persons — with diminished hearing in the II group.

Hearing within the limits of age norm was registered in 32 persons from 106 clean-up workers in the II group, examined in 2008–2009, SNDH of different degree of manifestation was found in other 74 patients. In particular, SNDH of the 1st degree was registered in 22 clean-up workers from the II-A group, and in 16 persons of the II-B group; SNDH of the 2nd degree was fixed in 16 persons of the II-A group and 12 persons of the II-B group. SNDH of the 3rd degree was diagnosed in 3 persons of the II-A and II-B groups, and SNDH of the 4th degree — only in 1 person of the II-B group.

In addition, a delayed legibility of linguistic tests was fixed in 16 from 32 clean-up workers with the normal hearing acuity and in 53 from 74 — with SNDH of different degree manifestation in the II group.

Hearing within the limits of age norm was observed in all 148 persons of the III group before the work at the Chernobyl alienation zone.

A number of persons with an ear within the limits of age norm were decreased up to 59 persons, and in 89 patients SNDH of the 1st and the 2nd degrees were registered after stopping clean-up works at the alienation area. In 2008–2009 36 persons of the III group had normal hearing among 141 examined patients; SNDH of different degrees was noted in 105 clean-up workers.

Thus, an increase in diminished hearing both by frequency and by the degree of manifestation was noted in clean-up workers of the III group at the remote period after the accident comparing to the II group with analogous small doses, but absorbed in considerably shorter time.

TEMPORAL INDICES OF DYNAMIC IMPEDANSMETRY IN CLEAN-UP WORKERS AT THE EARLY POST-ACCIDENTAL PERIOD.

In published works of 1990 and 1992 [3] at a primary examination the indices of acoustic reflex of entotic muscles (AREM) of clean-up workers aged 23–50 years with normal hearing as well as with SNDH of the 1st and the 2nd degrees who received different doses of IR (from 0.23 to 2.0 Gy) have been presented. Especially interesting were AREM temporal indices of clean-up workers, whose hearing acuity was within the limits of norm by an audiometry in the two groups.

Convalescents of the ARS grade 1 with absorbed doses 1.0–1.9 Gy — 13 persons (26 ears), were included in the I group. The II group consisted of 10 clean-up workers (20 ears) exposed to short-time IR in the small doses (0.23–0.25 Gy). For comparison, the III (control) group was made from 10 practically healthy non-irradiated persons (20 ears) with normal hearing

Temporal indices of AREM under ipsi- and contralateral stimulations are shown in the Tables 18.9 and 18.10. Revealed changes in a time increase (Ti) of AREM are less in the I and II groups than the Ti size in the persons of control group.

Particularly, at the sizes of 136.8 ± 8.1 ; 176.0 ± 28.5 ; 220.0 ± 31.6 ms in control group a time increase was equivalent to 112.4 ± 12.1 ; 157.1 ± 19.1 ; 197.1 ± 16.7 ms respectively under an ipsilateral stimulation at the studied frequencies 0.5, 1.0 and 4.0 kHz in the persons of the II group. The analogous decrease in size of AREM time increase was marked at contralateral stimulation, and also in I group under both types of stimulation.

Table 18.9. Temporal indices of acoustic reflex of entotic muscles under ipsilateral stimulation in clean-up workers with normal and diminished hearing at the different ionizing radiation doses comparing to the control group

Groups	stimul. kH	Temporal characteristics of acoustic reflex of entotic muscles, ms						
		Latent period	time increase	time of active action	time lengthening	time stamp	total time	
III control	0.5	126.2±12.9	136.8±8.1	1070.0±41.6	118.0±20.0	175.0±22.7	1312.0±66.0	
	1.0	149.0±6.1	176.0±28.5	948.0±84.4	131.0±21.6	215.0±28.0	1320.0±91.0	
	4.0	224.4±9.3	220.0±31.6	789.0±45.6	121.4±13.1	205.0±22.5	1249.0±84.1	
	0.5	122.9±9.5	112.4±12.1	1088.2±20.4	110.0±15.7	132.3±18.7	1225.8±30.4	
II clean-up workers (0.23-0.25)	1.0	130.0±7.1	157.1±19.1	1058.0±33.4	119.4±18.4	191.3±24.7	1258.0±53.5	
	4.0	220.0±20.5	197.1±16.7	888.6±84.2	142.4±22.1	177.8±24.0	1160.0±58.0	
I APS grade 1 (1.0-1.9)	0.5	117.8±12.6	86.8±8.8*	1091.7±20.7	143.9±20.9	118.7±19.9	1270.8±46.4	
	1.0	118.26±12.7	141.8±11.1	1051.7±47.5	137.8±14.1	143.5±19.8	1303.0±76.1	
	4.0	232.2±22.2	181.1±16.9	873.9±42.0	154.5±16.1	132.8±12.2	1235.0±87.5	

*Significant differences comparing to control, $p < 0.05$

Table 18.10. Temporal indices of acoustic reflex of entotic muscles under contralateral stimulation in clean-up workers with normal and diminished hearing and different radiation absorbed doses comparing to the control group

Groups (dose, Gy)	stimul. kH	Temporal characteristics of acoustic reflex of entotic muscles, ms						
		Latent period	time increase	time of active action	time lengthening	time stamp	total time	
III control	0.5	128.0±13.6	144.4±14.1	1044.0±67.0	112.2±16.0	245.0±32.7	1442.0±107.4	
	1.0	171.0±31.6	166.7±18.1	856.0±91.0	114.0±9.5	251.0±50.0	1380.0±84.8	
	4.0	247.5±22.6	162.5±24.7	744.4±91.0	111.4±9.5	247.5±30.2	1211.1±105.1	
	0.5	149.3±26.3	136.3±17.0	1019.4±58.7	131.3±11.0	130.8±13.7	1281.5±64.0	
II clean-up workers (0.23-0.25)	1.0	135.6±18.5	106.9±28.3	1047.5±59.1	152.5±13.6	148.0±17.0	1131.9±99.1	
	4.0	242.9±26.1	170.7±26.1	894.3±78.6	180.7±22.8	136.3±20.1	1177.0±100.1	
I APS grade 1 (1.0-1.9)	0.5	132.3±19.6	121.9±12.1	1055.5±49.3	150.0±19.6	188.6±15.2	1330.5±66.5	
	1.0	113.5±15.3	122.6±24.6	1016.7±62.6	166.0±23.9	220.9±26.8	1336.9±84.1	
	4.0	190.6±32.6	146.9±25.5	966.2±22.5	189.4±22.9	157.1±27.1	1334.4±132.4	

No statistically significant changes in time of active action (T_a) of AREM at ipsi- and contralateral stimulations were fixed in the two groups.

However, a tendency of time lengthening (T_l) of active after-effect was marked in both under ipsilateral and contralateral stimulations in persons of the I and the II groups comparing to control (Table 18.9 and 18.10).

A time slump (T_s) of AREM was noted in clean-up workers comparing to this index of control group at the studied frequencies under ipsi- and contralateral stimulations. These changes were more manifested in ARS convalescents of I group. No differences of total time (T_E index) of AREM under ipsi- and contralateral stimulations between the three groups were noted.

Thus, T_l , time slump (T_s) of AREM and time lengthening of active after-effect (T_a) of AREM could specify the disorders of correlation between the stimulation and inhibition processes at the nuclear level of reflex arch of AREM in clean-up workers in the I and the II groups comparing to the persons of control group under ipsilateral stimulation.

Shortening of T_l , T_s and lengthening of active T_a of AREM under contralateral stimulation, testify to disorders of correlation between stimulation and inhibition processes at the nuclear level of reflex arch of brain stem structures of AA, that at the same time demonstrate early preclinical disorders in these structures comparing to control.

They were registered in clean-up workers, who received the small IR doses and were more manifested in persons with ARS, i.e. a dependence on the dose was marked ($p < 0.05$).

INDICES OF THE FUNCTIONAL STATE OF EXTERNAL CORTI'S CELLS IN INTERNAL EAR IN CLEAN-UP WORKERS BY THE DATA OF OTOACUSTIC EMISSION OF DISTORTED PRODUCTS (OEDP) AT THE REMOTE POST-ACCIDENTAL PERIOD.

Registration of OEDP was conducted in 93 clean-up workers (186 ears) divided into the three groups in 15–20 years after the accident, and 10 non-irradiated persons (20 ears) with normal hearing acuity of control group among them.

Ten clean-up workers (20 ears) with hearing within the limits of age norm registered by audiometry were included in the I group.

The II group consisted of 31 clean-up workers (62 ears) with presbycusis praecox.

The III group included 52 clean-up workers, 27 from them (44 ears; III-A group) with acute bilateral SNDH of the 2nd degree and 25 (50 ears; III-B group) — with acute unilateral SNDH of the 2nd degree as a result of hypertensive crisis. OEDP examination was not conducted in clean-up workers with the 3rd and the 4th degree of SNDH, because its registration was impossible under considerable losses of ear.

The values of acoustic range (AR) of OEDP in clean-up workers of the three groups and persons of control group are presented in Table 18.11.

Data shown in Table 18.11 and in Fig. 18.11 specify that AR value of OEDP at the studied frequencies was greater than 10 dB in clean-up workers with hearing acuity within the limits of norm and diminished hearing with presbycusis praecox in the I and the II groups as well as in patients of control group i.e. external Corti's cells (ECC) have normal function.

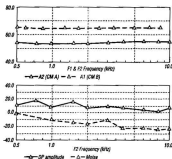


Fig.18.11. OEDP of patient T, aged 54 years, who was clean-up workers, and had ARS grade 2 with diminished hearing according to presbycusis praecox of corresponding age, AR between measuring noise level curve and that of OEDP exceeds 10 dB

In the III-A and the III-B groups of clean-up workers with acute bilateral and unilateral SNDH, that aroused against a background of hypertensive crises, the value of OEDP AR at the studied frequencies less than 10 dB demonstrated an absence of ECC response of internal ear, i.e. the functional state of ECC in the internal ear was impaired.

Thus, it was first established that the functional state of internal ear ECC in the hearing acuity within the limits of age norm and in SNDH by the type of presbycusis praecox in clean-up workers was not impaired at a remote period, but it was impaired in auditory disorders aroused against a background of hypertensive crises.

CHANGES IN THE FUNCTIONAL STATE OF BRAIN STEM STRUCTURES OF AUDITORY ANALYZER IN CLEAN-UP WORKERS IN THE DYNAMICS OF POST-ACCIDENTAL PERIOD.

In 1990 the results of primary examinations of changes in indices of the brain stem structures of auditory analyzer, that were manifested by the significant increase in a size of LP in peaks of the third, fifth components of short-time latent hearing induced potentials (SHIP) and intervals between peaks of the I-V SHIP were analyzed not only in clean-up workers with revealed diminished hearing but also in patients with hearing acuity within the limits of norm by T.V. Shidlovskaya, N. S. Mishchanchuk, O. I. Kotov. These data testify the early changes in the brain stem structures of auditory analyzer before the clinical signs of auditory impairments.

The changes in temporal parameters of brain stem or short-latent auditory induced potential (SHIP) components were analyzed in clean-up workers depending on state of hearing acuity comparing to the control group of the non-irradiated persons with normal hearing.

The qualitative and quantitative changes in basic components of SHIP were established in clean-up workers with the normal hearing of the I group, with the 1st degree of SNDH in the II group, as well as with the 2nd degree of SNDH in the III group comparing to control.

Qualitative changes were manifested with SHIP smoothing (by low amplitude) components. Quantitative sizes of IBP from I-III, III-V and I-V SHIP components in clean-up workers with normal hearing in the I group, and also with the 1st degree of SNDH in the II group and with the 2nd degree SNDH in the III group, comparing to control — under ipsilateral sound stimulation with frequency of 1.0 kHz, are shown in Table 18.12.

Table 18.12. Indices of intra spikes interval of short-latent auditory induced potential components in persons of control group and clean-up workers of the three groups with the different state of ear under ipsilateral sound stimulation with frequency of 1.0 kHz

Groups:	Intra spikes interval, ms		
	I-III	III-V	I-V
control	2.15±0.03	1.87±0.01	4.02±0.01
I	2.16±0.01	2.08±0.02*	4.22±0.02*
II	2.18±0.01	2.11±0.02*	4.29±0.02*
III	2.20±0.01	2.16±0.03*	4.36±0.01*

*a difference between the indices of clean-up workers of the three groups and control group is significant, ($p<0.05$).

No significant differences between the values of IBP from components I–III in clean-up workers of the three groups were marked comparing to control group by the data of Fig. 18.12. However, lengthening of IBP indices in the III–V and the I–V components of SHIP were fixed in clean-up workers of the three groups, comparing to control ($p<0.05$). For example, lengthening of IBP in the III–V and I–V components of SHIP was registered in the I group of clean-up workers with normal hearing, that was equal to 2.06 ± 0.02 and 4.22 ± 0.02 ms respectively comparing to control group where the first index was 1.87 ± 0.01 , and the second one — 4.02 ± 0.01 ms, $p<0.05$.

The more prolonged were indices of IBP in the III–V and I–V components of SHIP comparing to control and between itself in clean-up workers with the 1st and the 2nd degrees of SNDH in the groups II and III: the above-named indices were 2.11 ± 0.02 and 4.29 ± 0.02 ms in the persons of the group II and 2.16 ± 0.03 and 4.36 ± 0.01 ms in patients of the group III ($p<0.05$), respectively.

The increased values of IBP in the III–V and I–V components of SHIP in clean-up workers of the three groups testify the delayed impulse conducting by the auditory ways of the brain stem and at the same time underline early preclinical sensibility of the brain stem structures of AA under IR effect in clean-up workers with the normal hearing acuity.

The changes in IBP value of the III–V components of SHIP under ipsilateral sound stimulation with frequency of 1.0 kHz in clean-up workers exposed to short-term IR in the large doses (I-A and I-B groups), in relation to short-term IR (II-A and II-B groups), long-term IR (III-A and III-B groups) in small doses under the long-term monitoring studied by us [9] in the dynamics of post-accidental period comparing to the persons of the corresponding age in control groups are shown in Fig. 18.12.

The value of IBP in III–V components of SHIP made 2.12 ± 0.03 ms, at a control index — 1.86 ± 0.03 ms in the persons of the group I-A at a primary examination. This size was decreased up to 2.08 ± 0.01 ms in 5 years, and up to 2.06 ± 0.02 ms in 10 years.

A gradual increase of this index that made 2.11 ± 0.02 ms in 2004 have been noted at the second decade. Analogous changes were revealed in the persons of the I-B group in the dynamics of post-accidental period, but an above-named index was greater in them, i. e. it was dependent on IR dose.

A peculiarity of IBP index in the III–V components of SHIP in clean-up workers of the I group was its considerable increase at a primary examination, and following

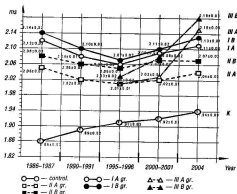


Fig.18.12. Indices of IBP in III-V components of SHIP under isothermal sound stimulation with frequency of 1.0 kHz in clean-up workers of the I-A and I-B groups, the II-A and II-B groups, and the III-A and III-B groups, and also persons of corresponding age in control groups within 18 year dynamics after the accident.

decrease during the first decade of post-accidental period. In the second decade, on the contrary, the progressive increase of this index sizes was marked, that in 18 years almost reached values fixed at the primary examination.

A feature of changes in sizes of IBP in the III-V components of SHIP in clean-up workers of the II group at the primary inspection was the fact that they were lesser than sizes of IBP in clean-up workers of the I group. So, it made 2.05 ± 0.02 ms in the persons of the II-A group, and 2.08 ± 0.03 ms in the persons of the II-B group, they were gradually decreased during the first decade, and were gradually increased in the second. The changes in SHIP parameters were fixed at short-term IR at 0.2 Gy that under this variety of exposure were also dependent on IR dose.

Other conformity of changes in index of IBP in the III-V components of SHIP was established in clean-up workers of the III group in the dynamics of post-accidental period. Its accelerated value was noted in them after stopping works at the Chernobyl alienation zone (taking into account age changes). So, in 2004 this index made 2.15 ± 0.03 ms in the persons of the III-A group and 2.18 ± 0.03 ms in the persons of the III-B group, and it consisted of 2.04 ± 0.02 ms and 2.07 ± 0.02 ms in clean-up workers of the II-A and the II-B groups who were received analogous doses, only during considerably more short time, respectively.

An accelerated increase in sizes of IBP in the III-V components of SHIP at the identical IR doses was a feature of long-term IR in clean-up workers of the III group comparing to the persons of the II group at a remote period. Long-term IR in small doses caused the more substantial manifestation of impairments in the AA brain stem structures,

Thus, as a result of the conducted examinations, the significance of dose size and duration of IR effect was established in clean-up workers of the three groups, that was manifested by the changes in SHIP parameters, proving for early and progressive disorders in the brain stem structures of auditory analyzer in the dynamics of post-accidental period.

(N. Mischchenchuk, Yu. Sushko)

**CHANGES IN THE FUNCTIONAL STATE OF AUDITORY
ANALYZER CORTICAL STRUCTURES IN CLEAN-UP WORKERS
IN THE DYNAMICS OF POST-ACCIDENTAL PERIOD.**

The sizes of LP in peaks of the P_2 and N_2 components of LHIP were studied by us [4] under sound stimulation with a frequency of 1.0 kHz in clean-up workers of the three groups with the different doses and duration of IR in the dynamics of post-accidental period, which are shown in Table 18.13.

Table 18.13. Sizes of latent periods in peaks of the P_2 and N_2 components of long-latent auditory induced potential fixed in clean-up workers of the three groups under long-term monitoring during different years after an accident

Groups:	Years of investigation				
	1986–1987	1990–1991	1995–1998	2001–2002	2003–2004
Sizes of latent periods of P_2 component peak, ms					
control	175.0±3.1	178.0±3.3	183.1±2.6	186.5±2.5	189.1±2.1
I-A	223.1±2.1	196.7±3.3	197.5±3.2	199.6±2.1	208.6±2.6
I-B	228.1±3.5	206.5±2.8	199.6±3.9	203.7±3.7	215.5±3.6
II-A	188.2±2.8	195.5±2.7	197.5±2.9	198.9±2.1	199.1±2.5
II-B	195.5±2.6	199.5±2.5	200.1±2.7	201.5±2.7	205.6±3.1
III-A	175.2±3.1	—	187.2±2.1	190.0±2.0	198.5±2.4
III-B	179.0±3.1	—	189.6±2.5	194.0±2.9	206.6±2.7
Sizes of latent periods of N_2 component peak, ms					
control	269.0±4.8	272.2±3.4	275.1±3.0	278.6±3.1	279.9±3.2
I-A	319.5±3.5	291.1±3.1	287.3±3.7	295.6±3.6	310.6±3.1
I-B	326.1±4.2	299.7±3.6	289.2±4.1	306.1±3.3	318.6±2.9
II-A	288.5±3.2	289.0±2.9	290.0±2.6	290.5±2.1	294.7±2.1
II-B	290.7±3.9	291.1±3.0	293.0±2.7	296.0±2.6	301.5±2.4
III-A	268.0±4.5	—	289.0±2.6	291.0±2.5	296.4±2.7
III-B	269.0±4.6	—	292.3±3.1	296.5±2.4	306.6±2.9

In particular, the feature of LP parameters of peaks of P_2 and N_2 components of LHIP was their considerable increase in clean-up workers of the I group at the primary examination, with following gradual decrease during the first decade after an accident and a new increase — in the second decade.

The sizes of above-named indices of LHIP were greater in the persons of the I-B group than those in persons of the I-A group, i.e. they demonstrate a dependence on the size of IR dose.

The peculiarities of LP indices in peaks of the P_2 and N_2 components of LHIP were in their increase in clean-up workers of the II group at the primary examination comparing to control, however they were lesser than that in clean-up workers of the I group who were exposed to short-term radiation in large doses (see Table 18.5).

It is necessary to accent, that the changes in size of LP in peaks of the P_2 and N_2 components of LHIP in the persons of the II-A group were marked in a dose of 0.2 Gy, but in the persons of II-B groups who received a small, but two times greater dose; the sizes of peak LP of the P_2 and N_2 components of LHIP were grater than those in the persons of II-A group, i.e. at this exposure variety the LP size in peaks of P_2 and N_2 components of LHIP were also dose dependent.

The feature of changes in LP peaks of the P_2 and N_2 components of LHIP in clean-up workers of the II group was their gradual lengthening in the 1st as well as in the 2nd decades of post-accidental period.

Other data have been obtained in clean-up workers of the III group with similar to the II group small doses, but absorbed within prolonged stay in the Chernobyl alienation zone. Before clean-up works sizes of their LP of peaks in the P_2 and N_2 components of LHIP did not differ from indices in the persons of the corresponding age categories in control group.

An increase in LP sizes of peaks in the P_2 and N_2 components of LHIP was marked after the prolonged clean-up works within the different years of the long-term monitoring. In 2003–2004 they were equal to 296.4 ± 2.7 ms and 306.6 ± 2.9 ms in the persons of the III-A and the III-B groups and above-named indices were 294.7 ± 2.1 and 301.5 ± 2.4 ms in the persons of the II-A and the II-B groups in those years, respectively.

These data give basis to assert that prolonged IR is as harmful and causes considerable disorders in the subcortical and cortical structures of auditory analyzer at the remote period, as relatively short-term exposure in similar small doses.

A direct correlation ($r=0.71$) between the size of LP peak in the N_2 component of LHIP and thresholds of 100% legibility of linguistic tests has been established by us [4]. That is the manifestation of inhibitory processes in the cortical structures of AA is synchronously demonstrated by electrophysiologic examinations as well as by speaking audiometry.

Thus, according to data of objective audiometry (by the indices of registration of AREM, SHIP and LHIP), disorders of brain stem and cortical structures of auditory analyzer were found in all the liquidators (in 100% cases) with normal as well as diminished hearing.

These data specify that the physical methods of examination allowed to find early changes in the central departments of auditory analyzer under radiation effect, before the development of clinical audiometrical signs and deviations in the indices of tonal and speaking audiometry and at the same time prove for the early disorders in brain stem and cortical structures.

Such changes in the indices of objective audiometry are deepening with the development of impairments in hearing acuity. The analogous changes in the same brain structures in radiation-exposed persons with progressive development of psychoneurologic pathology as a result of the Chernobyl catastrophe were noted, by other authors [5, 6].

The value of dose commitment and duration of IR influence were established in clean-up workers of the three groups that was manifested by the changes in parameters

of SHIP and LHIP evidencing an early and progressive inhibitory disorders in brain stem and cortical structures of auditory analyzer in the dynamics of post-accidental period.

Certain conformity of the obtained results was confirmed by different research groups with other actual clinical material of examinations in special contingents of persons exposed or were being exposed to ionizing radiation as a result of the Chernobyl catastrophe, including A.N. Golod [7], I. Kardash [8], V.V. Rymar [2], M.M. Kozak [8] and others.

In particular, the data of deviations number by psychoacoustic audiometry and objective audiometry (APEM, SHIP and LHIP) in clean-up workers and dwellers of Narodychi and Ivankiv districts were presented by T.V. Shidlovska et al. [9]. Analogous to our results deviations of APEM indices from a norm, were revealed in 100% clean-up workers, in 43% dwellers of Narodychi and 21% of Ivankiv districts.

According to the data of SHIP and LHIP registration, the deviations from a norm were fixed in the brain stem and cortical structures of auditory analyzer in 100; 85.5; 19.7% and 100; 78.0 and 33.7% cases, respectively. The authors accent an attention that above-named changes were found in all clean-up workers exposed to radiation over 0.25 Gy without an exception. Especially significant changes were noted in acute radiation syndrome convalescents.

The study of APEM, SHIP and LHIP characteristics are of significance for the objective determination of initial preclinical impairments in the central departments of auditory analyzer, that it is important for resolving some expert questions, and also for the development of treatment-and-prophylactic measures.

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(N. Mishchenchuk)

18.3. VESTIBULAR DYSFUNCTION IN THE CHORNOBYL CLEAN-UP WORKERS IN THE DYNAMICS OF POST-ACCIDENTAL PERIOD. MORBIDITY AND RISKS FOR DEVELOPMENT OF VESTIBULAR DYSFUNCTION: RESULTS OF MATHEMATICAL MODELING

It is known that various pathological reactions of three kinds are occurred in the cases of impairing functional state of VeA: vestibule-sensory, vestibule-somatic and vestibule-vegetative with different degree of manifestations [1, 2].

Vestibulo-sensory reactions cause the processes that take a place at the level of cerebral cortex. They are manifested by feeling of permanent or periodic dizziness and different manifestations of disorders connected with the orientation in space.

Vestibulo-somatic reactions are manifested by the reflexes on body muscular structures, that are accompanied by spontaneous disorders of static kinetic equilibrium, resulting in disorder of walking and coordination of movements, and also pathological vestibule-oculomotor reactions, as a spontaneous or positional nystagmus of different orientation and intensity.

Vestibulo-vegetative reactions are occurred under a decrease or an increase in sensitiveness of vestibular analyzer, and also under increased vestibular loadings. They are characterized by epidermosis, the changes in skin staining — pallor or reddening, breathing disorders, disorders from the side of the cardiovascular system (the changes in pulse rhythm, arterial pressure) and gastrointestinal tract (nausea, vomiting), that cause not infrequent development of severe symptom-complex — disease of movement.

The mentioned reactions have different value in human life. The vestibule-vegetative and vestibule-sensory reactions are the most significant for occupational activity. The first ones predetermine the state of vegetative discomfort disordering self-feeling, decreasing a working capacity; the others disorientate a man in space and can cause a serious danger in implementation of the works, connected with exact and coordinated movements.

From 10 to 20 % of specialists, whose professions are related to the effect of the prolonged or increased irritants on vestibular dysfunction (VeD), can lose a working capacity or occupational adaptability as a result of VeD, according to published data [3]. Different disorders of occupational activity promoting occurrence of technological accidents or even catastrophes can be provoked as a result of the un-coordinated movements or disorientation in space of those specialists.

For studying the functional state of vestibular analyzer (VeA) a methodology of clinical examination was used: the complex of standard vestibulometric methods including determination of the state of staticokinetic equilibrium, electronystagmography (ENG) of spontaneous, positional nystagmus, and also experimental nystagmus, caused by caloric and rotary stimulations, taking into account the indices of electrooculography (EOG).

In sum 7031 clean-up workers were examined within post-accidental period (1986–2009).

One of the basic were complaints to permanent dizziness of different intensity with equilibrium imbalance and pathological vestibulo-vegetative disorders in 91.3 % cases simultaneously with complaint to headache in 1200 clean-up workers at the primary examination (1986–1987).

VeD of different degree was found by primary vestibulometry in 1200 clean-up workers, including 325 patients selected for the long-term monitoring.

VeD was manifested by the type of central vestibular syndrome (H-81.4 by ICD-10) with prevailed affection of those or other structures of central department of VA in the greater number of patients (97.0% cases) among them and by the type of mixed vestibular syndrome in accordance with L.B. Soldatov et al. classification (1980) [4] in considerably smaller number of patients (3.0% cases).

It was clinically manifested by progressive (85.7% cases) or relapsing course (14.3 %) with the various changes in vestibulo-somatic, vestibule-sensory and vestibulo-vegetative pathological reactions during the different years of post-accidental period.

The 1st degree VeD was registered in 252 persons (21.1% cases), the 2nd degree VeD — in 668 persons (55.7%), the 3rd degree VeD — in 246 persons (20.1%), the 4th degree VeD — in 34 persons (3.1% cases) from 1200 clean-up workers in 1986-1987 at a primary vestibulometric examination.

Indices of staticokinetic equilibrium in clean-up workers with the 1st degree of VeD were as follows: a static equilibrium made 3.7 ± 0.6 a.u. by the data of cephalography index (CGI), and a kinetic equilibrium (at an implementation of Fukuda stepping test — a body rotary around own axis) consisted of 40.5 ± 1.9 deg. No spontaneous and positional nystagmuses were found.

The insignificant symmetric, rare asymmetric hyperreflexia or hyporeflexia of experimental nystagmus with the signs of dysrhythmia, the presence of different duration of the "mute" fields, and also by the phenomena of its reversion were revealed under the caloric (circulating) and rotary stimulations.

The hyperreflexia of experimental nystagmus was accompanied by an inadequacy of vestibule-sensory reactions, often more prolonged, than experimental nystagmus duration, and also vestibule-vegetative reflexes of the I and II grade according to K.L. Khilov [5] (prevailed intermediate subcortical and cortical levels of central vestibular syndrome).

The moderate changes in staticokinetic equilibrium were diagnosed in clean-up workers with VeD of the 2nd degree. So, their CGI was equal to 5.4 ± 0.5 a.u. — a body rotation around own axis using a stepping test — 64.3 ± 5.5 deg., a deviation of writing test — 12.7 ± 1.3 deg.

Positional nystagmus was noted in a half of examined clean-up workers with VeD of the 2nd degree by background ENG that was low-amplitude horizontal nystagmus changing its direction depending on the head position (type I by Nylen) in 25.0 % cases. In other 25.0% the intensity and direction of positional nystagmus were inconstant in different head positions (type III by Nylen).

The considerable disorders (degree III) of staticokinetic equilibrium were registered in clean-up workers with diagnosed VeD of the 3rd degree: CGI made 6.1 ± 0.3 a.u., a stepping test — 82.0 ± 5.1 deg., a writing test — 15.5 ± 1.1 deg. (those patients required an outside assistance).

The significant deviations were registered under performing static and kinetic tests in clean-up workers with diagnosed VeD of the 4th degree. Thus, the cephalography index exceeded 6.5 a.u., a body rotation around an own axis in stepping test was 115.5 ± 2.3 deg., the deviations in writing test — 20.6 ± 1.4 deg. (tests were difficult for performance and such patients could not neither stand nor move without assistance).

The indices of staticokinetic equilibrium in clean-up workers with VeD of the four degrees and those of control healthy persons are shown in Table 18.14.

Table 18.14. Indices of static kinetic equilibrium in vestibular dysfunction of the four degrees in clean-up workers and healthy persons (control) without radiation influence

Degree of vestibular dysfunction	Indices of the studied tests		
	static equilibrium, cephalography index	kinetic equilibrium, deg.	
		stepping (body rotation)	writing (deviation)
control	3.2±0.4	27.0±2.1	5.3±0.4
I	3.7±0.6	40.5±2.1	8.5±0.3
II	5.4±0.5	64.3±3.5	12.7±1.3
III	6.1±0.5	82.1±5.1	15.5±1.1
IV	6.5±0.5	115.5±2.3	20.6±1.4

In the background ENG the middle-amplitude nystagmus was diagnosed in the majority of patients with VeD of the 3rd degree (76.4% cases) and in the others was low-amplitude, horizontal or diagonal spontaneous nystagmus of the II and III grade of manifestation.

More significant manifestation of symmetric or asymmetric hyperreflexia or hyporeflexia, and also dissociated experimental reactions with the prolonged vestibulo-sensory and vestibulo-vegetative reflexes of the II and III grades — vomituration, increased sweating, changes in pulse rhythm, change in arterial pressure that proved for the disordered correlative processes in brain stem, intermediate cortical and cortical structures in the central vestibular syndrome have been fixed under experimental (caloric) stimulations in VeD of the 3rd degree (rotary stimulations were selectively conducted after that of caloric depending on a general condition).

Spontaneous middle- or large-amplitude horizontal or diagonal nystagmus of the III grade was registered on phone ENG recording in VeD of the 4th degree in all cases.

Significant hyperreflexia of experimental nystagmus with its considerable dysrhythmia, prolonged reversion, vestibulo-vegetative reactions of the III grade (vomiting, ephidrosis, hypersalivation, disorders of pulse rhythm, a change in arterial pressure) was fixed under experimental (caloric) stimulation in VeD of the 4th degree. The rotary stimulation was not conducted in connection with patient feel worsening.

All structures of central part of VD in the brain were significantly involved in pathological process in VeD of the 4th degree.

An analysis of results of vestibulometric researches in 325 clean-up workers revealed that the degree of VeD signs was changed to growing spontaneous symptomatology in the dynamics of post-accident years: by increasing disorders of staticokinetic equilibrium, an appearance and increased intensity of positional or spontaneous nystagmus, the qualitative and quantitative indices of experimental nystagmus in caloric as well as in rotary stimulations.

The qualitative changes were manifested by progressive increase in dysrhythmia of experimental nystagmus, an appearance of toothed or bifurcated top of nystagmus, more frequent and more prolonged "mute" fields on ENG, the phenomena of nystagmus reversion.

The quantitative changes in experimental nystagmus were manifested by diminished frequency of nystagmus, increased middle angular rate of slowly phase of nystagmus.

The changes in these indices objectively indicate that experimental nystagmus had a clear tendency to transformation from clonic into clonicotonic, and then — into tonic in the dynamics of post-accidental period

both. An increase in tonicity both spontaneous and experimental nystagmus was observed as quantitative expression in clean-up workers with the different degree of VeD manifestation in the dynamics of post-accidental period. They were fixed in all clean-up workers, especially in patients with VeD of the 3rd and 4th degrees to the small or greater grades that testified a prevailed progressive disorder of deep brain stem structures of VeA [2, 4].

The average statistic values of basic quantitative parameters of experimental nystagmus at the different degrees of VD manifestation are shown in Table 18.15.

Table 18.15. Indices of experimental post-caloric nystagmus in clean-up workers with vestibular dysfunction at the different years of post-accidental period

Parameters of nystagmus	Grade of vestibular dysfunction	Years of investigation			
		1986–1987	1994–1995	2008–2009	p*
Frequency of nystagmus, Hz	I	2.2±0.2	1.5±0.1	1.3±0.1	<0.01
	II	2.0±0.3	1.3±0.2	1.2±0.1	<0.01
	III	1.9±0.2	1.2±0.1	0.9±0.2	<0.01
	IV	1.8±0.1	1.1±0.2	0.8±0.2	<0.01
Middle angular rate of slowly phase of nystagmus, deg./s	I	37.4±2.5	45.9±1.7	56.3±1.2	<0.01
	II	43.3±1.8	52.4±1.7	59.5±1.2	<0.01
	III	48.3±2.3	56.3±1.9	66.7±1.5	<0.01
	IV	54.8±3.1	60.3±1.6	76.8±1.3	<0.01

*difference between the basic indices of experimental nystagmus in vestibular dysfunction of the 1st – 4th degrees is significant, $p < 0.01$.

The parameters of experimental nystagmus were significantly changed into the side of tonicity development in clean-up workers with the increasing degree of VeD manifestations in the dynamics of 19–23-year examination: nystagmus frequency was decreased and the size of middle angular rate of SFN was increased in the culmination phase of post-caloric nystagmus.

The change in qualitative and quantitative indices of spontaneous and experimental nystagmus into the side of tonicity was proving for development of general cerebral phenomena as hypertensive syndrome [2, 6]. These phenomena arised in clean-up workers at the remote post-accidental period of the first, second and third decades. They progressed against a background of progressing organic cerebrovascular pathology of the brain — dyscirculatory encephalopathy that was conformed to other neurophysiologic and MRT examinations [3].

Data obtained were confirmed with other actual clinical material by other researchers.

We [7–9] established that the greatest VeD manifestations were observed in 57 persons of the group I with ARS of grade 1 and grade 2 at a primary examination in 1986–1987. Thus, in the group I-A VeD of the II grade was fixed in 26.7% cases, and the III grade — in 74.3%, in the group I-B it was in 15.3% and 84.7% cases respectively. It is necessary to note that considerable spontaneous disorders such as staticokinetic disorders and spontaneous nystagmus of the II and III grades were found in clean-up workers ARS convalescents. Under functional stimulations the hyperreflexia of experimental nystagmus with its dysrhythmia accompanied by the prolonged vestibulo-sensory reactions and vestibulo-vegetative reflexes of the II and III grade of manifestation, including vestibulo-vascular ones were noted. At early period the VeD signs were at the decompensation stage in clean-up workers of the I group.

A predominance of the irritation phenomena in brain stem (nuclear, under- and overnuclear), and also in the intermediate subcortical and cortical structures of VA central department was the feature of clinical course of VeD in an early post-accidental period.

In 1.5–3 years after the accident the degree of VeD manifestation was decreased in clean-up workers of the I group.

A progress of VeD renewed since 1994–1996 and during 1997–2009. Thus, the percent of persons with VeD of the 2nd and the 3rd degrees was increased among 52 examined clean-up workers of the I group at the end of the second decade (2005–2006). In particular, there were 27.3% with VeD of the 2nd degree in the I-A group, and 25.1% — in the I-B group, 72.7% — with VeD of the 3rd degree in the I-A group, and 74.9% cases — in the I-B group. They were manifested by an increase in disorders of equilibrium up to 83.9% cases, spontaneous nystagmus — up to 77.9%, changes in qualitative and quantitative parameters of experimental nystagmus — up to 71.8%. VeD of the 3rd degree was registered in 79.9% cases in 2008–2009 among 50 examined.

An increase in spontaneous vestibular symptomatology, the qualitative and quantitative changes in experimental nystagmus were indicative of the progressing disorders in the brain stem structures of VA and a growth of hypertensive syndrome in the brain. At the same time a decrease in vestibulo-vegetative reactions up to 35.1% cases was fixed that can be obviously explained by a decrease in activity (by the increase of inhibition processes) of the brain cortical structures, including the cortical structures of VA.

Among primary examined 120 clean-up workers of the II group the VeD manifestations have been already registered at a dose of 0.2 Gy.

In particular, VeD of the 1st degree was in 18.3% cases, VeD of the 2nd degree — in 21.6%, VeD of the 3rd degree — in 7.5%, VeD of the 4th degree — in 5.0% cases in the patients of the II-A group. At that time VeD of the 1st degree was in 11.6% cases, VeD of the 2nd degree — in 17.5%, VeD of the 3rd degree — in 10.8%, VeD of the 4th degree — 7.5% cases in the persons of the II-B group. The degree of VeD manifestation was greater in the patients of II-B group who had absorbed the greater dose of IR.

An increase of VeD manifestations was marked in the dynamics of post-accidental period. At the end of second decade (2005–2006) VeD of the 1st degree was observed in 1.8% cases of the II-A group and in 0.9% — of the II-B group, VeD of the 2nd degree — in 35.5% of the II-A group and in 24.4% — of the II-B group, VeD of the 3rd degree — in 10.9% of the II-A group and in 11.9% of the II-B group, VeD of the 4th degree — in

7.5% of the II-A and in 8.3% cases of the II-B group. The same dependence of VeD manifestations on the IR doses was noted in the dynamics of post-accidental period. At the beginning of the third decade after an accident (2008–2009) 43.8% cases with VeD of the 3rd and 4th degrees were registered.

The feature of VeD clinical course in clean-up workers of the II group was characterized by two phases of development. At the first phase VeD was mainly manifested by dissociated reactions, indicating disorders of correlation at brain stem, subcortical and cortical levels to the end of the first decade after IR.

In the second decade and at the beginning of the third decade after an accident (the second phase) a progressive growth of spontaneous symptomatology: disorders of static and kinetic equilibrium, appearance of positional, and then spontaneous nystagmus, a change in their character and intensity, and also qualitative and quantitative changes in experimental nystagmus were revealed.

It is necessary to mark that VeD of different degree was registered in clean-up workers of the II group at the stage of subcompensation during 22–23 years of the long-term monitoring. This fact can be explained by slow growth of VeD degrees from insignificant in 1986–1987 to considerable — in 2008–2009, that gave the possibility for active inclusion of VA compensatory mechanisms developed in the process of phylogenesis.

In 148 persons of the III group before the work at the Chernobyl alienation zone the vestibular function corresponded to age limit variations that were fixed in the persons of control group. After leaving 30 km zone a VeD frequency and degree were grater in clean-up workers of the III group, than those in the II group at identical IR doses.

In particular, VeD of the 3rd and the 4th degrees with severe manifestations were registered in 58.5% cases in 2008–2009 among 141 clean-up workers of the III group in comparison with the II group of clean-up workers where they were fixed only in 43.8% cases.

Thus, the obtained results of vestibulometry in clean-up workers in the dynamics of post-accidental period allowed concluding as follows.

- VeD of different degree of manifestation had character of central vestibular syndrome in the majority (in 97% cases) of clean-up workers exposed to different doses of irradiation.
- VeD as a result of IR exposure is an after-effect of CNS compensatory responses at the level of brain stem and intermediate subcortical and cortical structures of VA in the brain that have been already registered under the early clinical manifestations of brain disorders.
- The progress of VeD signs was manifested by growth of spontaneous symptoms, namely: by appearance or strengthening of positional or spontaneous nystagmus, strengthening of static kinetic disorders in clean-up workers in the dynamics of all the studied post-accidental period.
- The progressing qualitative changes were manifested as strengthening of dysrhythmia, toothed or bifurcated tops of nystagmus, increased frequency and duration of the “mute” fields, and quantitative ones were revealed as the change in parameters of spontaneous and experimental nystagmus.
- The development of nystagmus tonicities manifested by the quantitative decrease in the frequency and increase in the middle angular rate of SFN arised against a background

of forming the general brain phenomena in the progress of cerebrovascular diseases at remote post-accidental period.

- The feature of VeD clinical course was predominance of the irritation phenomena in brain stem and also in the intermediate subcortical and cortical structures of VA at an early post-accidental period, and a gradual progress of inhibitory processes in above-named structures at the remote period.
- The peculiarity of VeD clinical course in clean-up workers of the II group was characterized by two phases of development: at the first phase, to the end of the first decade after exposure VeD was manifested by dissociation reactions proving for impaired correlation at the intermediate subcortical and cortical levels. The development of inhibitory processes was marked in the above-named structures of VeA at the second phase (the second decade and beginning of the third decade).
- The peculiarity of VeD clinical course in clean-up workers of the III group at the remote period consisted in accelerating growth of pathological reactions both by the frequency and by the degree of manifestation comparing to the persons of the II group exposed to equal doses of IR.

Detected above-mentioned manifestations of VeD and peculiarities of their clinical course in clean-up workers exposed to different doses and duration of IR under long-term monitoring in the dynamics of post-accidental period were used at medico social examination for studying their occupational adaptability and working capacity, the decision of prognostic questions, as well as for realizing treatment-and-prophylactic and correcting measures.

VED MORBIDITY OF CLEAN-UP WORKERS

An analysis of VeD morbidity (per 1000) was conducted [9] according to the results of vestibulometrical examination of males' drivers-clean-up workers (420 persons) and the control group of 102 drivers-professionals without radiation exposure.

A distribution by experience and age was identical in both groups. The majority of examined patients (88 %) were the persons under 50.

The results of analysis are shown in Table 18.16.

Table 18.16. Vestibular dysfunction morbidity in drivers-clean-up workers - and drivers of control group, per 1000 persons

State of vestibular function	Drivers			
	control group		clean-up workers	
	absolute number	per 1000 persons	absolute number	per 1000 persons
normal	32	314	55	132*
dysfunction of I grade	50	490	121	288*
dysfunction of II grade	13	123	168	402*
dysfunction of III grade	7	69	75	178*
total		102		420

*difference with control are significant, $p < 0.05$.

VeD of the 1st degree was prevailed in the persons of control group, and more severe VeD of the 2nd and the 3rd degrees — in the main group. Relative risk of VeD manifestations was 1.9–3.0 times greater in the main group than in the persons of control group.

Thus, it was established that degree of VeD morbidity in the drivers-clean-up workers was significantly higher, than in non-irradiated drivers of control group, i.e. the radiation factor was the most substantial in VeD disease.

The risks of primary VeD morbidity by the type of central vestibular syndrome in Chernobyl NPP accident clean-up workers of 1986–1987 at the post-accidental period were studied by cohort analysis and risk-analysis.

A cohort of 4017 persons was formed from the general amount of 6412 clean-up workers of 1986–1987 registered in CER of RCRM submitted to vestibulometric examinations in SI "O.S. Kolomiychenko Institute of Otolaryngology of National Academy of Medical Sciences of Ukraine" in 1992–2001 [8]. The average dose of IR consisted of 0.220 ± 0.006 Gy. A distribution of examined patients by dose groups was realized by Poisson. Middle age at a time of exposition was 38.35 ± 0.22 .

The cohort study was focused on primary vestibular pathology incidence based on initial diagnosis identified at the first examination or at earlier stages of medical examination the diagnosis was absent.

The morbidity relative risks for all above-mentioned dose groups of recovery operation workers 1986–1987 were analyzed in comparison with those of the internal control group.

Fig. 18.13 illustrates the relationship between the relative risk for vestibular dysfunction and the radiation dose. Reliably higher risks for those exposed to doses 0.25–0.49 Gy ($RR=3.43$, $CI=1.43$; 4.13, $p<0.05$), 1.0 Gy and over ($RR=7.76$, $CI=4.18$; 14.39, $p<0.05$) were established.

Risk for some nosologies in relation to radiation dose and age at the time of exposure was analyzed; the risks for vestibular impairment was found to be much higher in senior age groups. Relative risk for dose groups of 1.0 Gy and over as compared to internal control was 5.39 (2.03; 14.3), $p<0.05$ (age at exposure 30–39), 7.63 (2.56; 22.7), $p<0.05$ (age at exposure 40–49) and 16.3 (4.42; 60.16), $p<0.05$ (age at exposure 50–59).

Central vestibular syndrome (H-81.4, ICD-10) was prevalent among vestibular analyzer diseases [10]. The relative risk here was reliably higher for those exposed to doses 0.25–0.49 Gy, 1.0 Gy and higher.

Analysis of some age groups revealed the reliable increase in risk for central vestibular syndrome relative to radiation dose. It was observed only for people aged 50–59 at the time of exposure (radiation dose 1.0 Gy and more) compared with those exposed to doses below 0.05 Gy ($RR=10.87$, $DI=2.62$; 45.1, $p<0.05$).

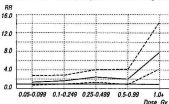


Fig. 18.13. Relationship between the relative risk for vestibular dysfunction and the radiation dose

The results of conducted researches prove for:

- a significant increase in of VeD primary morbidity risks in clean-up workers of 1986–1987;
- risks of VeD disease were dose dependent in persons exposed to 0.25–0.49 Gy and 1.0 Gy, and more;
- risk of vestibular impairment depended on age at exposure higher risk was observed in senior age groups, doses being equal.

VED MORBIDITY IN CLEAN-UP WORKERS: RESULTS OF MATHEMATICAL MODELING OF RISKS

The task of this research was to determine the dependence of VeD primary morbidity risk on the size of IR dose, age and other factors in clean-up workers of 1986–1987 by the method of mathematical modeling.

The distribution of examined clean-up workers cohort with the known dose loading by age groups was normal at a time of irradiation.

VeD primary morbidity was studied: a diagnosis, first established at the primary examination, or diagnosis that was absent at the previous stages of prophylactic medical examination were taken into account.

The results of studying risks of VeD disease appearance were presented by the method of mathematical modeling. The model of multiple logistic regression based on the method of maximal probability was used.

The model of multiple logistic regression was as follows:

$$Y = \frac{e^{\beta Z}}{1 + e^{\beta Z}} \quad (18.8)$$

where β – coefficients; Z – risk factors.

The analysis of VeD morbidity established that VeD primary morbidity began to grow substantially in 6–7 years of stay under the risk after irradiation, in 9.5 years it reached a maximum, with temporal decrease and following increase.

The second maximum was observed in 10–11 years of stay under the risk. Farther a primary morbidity began to decrease. The dynamics of morbidity depending on time of stay under the risk reminds a parabola and was described by a formula:

$$Y = -0.005 x^3 - 0.023 x^2 + 0.82x - 1.17 \quad (18.10)$$

where Y — frequency of morbidity; x — time under a risk, years.

It has been supposed, that the dynamics of relative risk can be described by an exponential model. After a mathematical modeling the exponential model of relative risk of VeD development was obtained.

A central vestibular syndrome was prevailed among VeD in 97.0% cases of clean-up workers by rubric H-81.4 (ICD-10). It was established that VeD morbidity was under effect of such factors as age at a time of exposure, dose of irradiation, a time of stay under the risk after irradiation. The formula of model for disease relative risk is below presented:

$$RR = \frac{\exp(\beta_0 + \beta_1 \cdot \text{dose} + \beta_2 \cdot \text{age} + \beta_3 / t)}{1 + \exp(\beta_0 + \beta_1 \cdot \text{dose} + \beta_2 \cdot \text{age} + \beta_3 / t)} \quad (18.11)$$

where RR — relative risk; $\beta_0, \beta_1, \beta_2, \beta_3$ — coefficients; dose (conditional designation) is a number of dose category, including this dose, Gy; age (conditional designation) is an age at the moment of work at the Chernobyl zone; t — is a number of category including this term of stay under a risk (1 — it is 6 years after an accident; 2–7 years and so on).

A direct dependence between the IR dose, age and relative risk of VeD appearance was observed. A relative risk of VeD occurrence calculated with a mathematical model at the IR dose (at the dose increase for every next dose group comparing to previous ones) was 1.24 (confidence interval 1.01; 1.39); at an age at the time of irradiation (at an age increase for every next age group comparing to previous ones) — 1.28 (1.04; 1.59). The risk was significant, $p < 0.05$.

The results of calculations indicated that with the increase of dose the risk of VeD development was increased. A greater risk was observed in the older persons exposed, a smaller risk is in the younger ones.

A found conformity is interesting not only from scientific but also from the practical point of view. It is necessary to underline that a reverse conformity was observed in the majority of diseases regarding data of the risks dynamics depending on a IR dose in different age groups, i.e. the risk was greater for young age groups.

Since, as it was established, β_3 coefficient has a negative value, so primary morbidity with increasing time of stay under a risk is at first increased with following decrease. A relative risk of VeD development (at 1/hour under a risk) is 0.099 (0.012; 0.82), $p < 0.05$.

Found conformities have a difficult nonlinear character. Obtained mathematical model of relative risk allowed to calculate the dynamics of VeD relative risk depending on a IR dose for different age groups and different time of stay under the risk after IR.

The corresponding graphs are shown in Fig. 18.14.

The graphs in Fig. 18.14 illustrate a dose dependence of VeD risk (with increasing dose loading of IR a relative risk is increased). A risk becomes significant at the doses over 0.2 Gy. The dynamics of relative risk shown in graphs depending on time of stay under the risk after an irradiation (10 and 15

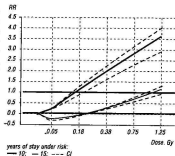


Fig. 18.14. Relative risks of VeD disease and their confidence intervals are calculated by a model depending on the dose of irradiation in persons aged 41–50 years at the moment of exposition in 10 and in 15 years of stay under the risk after IR

years) had significant differences. A pronounced tendency of a decrease in relative risk with increasing period of stay under a risk is noted at a remote post-accidental period. These results agree with those obtained by P. A. Fedirko in 2002: the presence of "window for analysing radiation risks", limited in time was established on the basis of risk dynamic analysis of other age-dependent diseases

It has been established that change in VeD risk dependent on a size doses can not be diagnosed in 15 years after IR as testifies the graph in Fig. 18.14. The calculations by model (3413), revealed that VeD primary morbidity will slowly decrease in 20 years after a stay under a risk, and VeD morbidity due to cumulation of cases will be grow, although in less measure.

Thus, as a result of researches one can make following conclusions:

- the relative risk of VeD primary morbidity for clean-up workers is described by an exponential model which takes into account the age at the time of irradiation, a dose of irradiation, a time of stay under the risk after irradiation;
- direct significant dependence between a dose of irradiation, an age and a relative risk of VeD appearance was shown;
- VeD primary morbidity after IR was increased during 11 years with following decrease;
- as a result of mathematical modeling it has been established that the prognosis of VeD primary morbidity will be slowly decreased at a period after 20 years of a stay under the risk.

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CHAPTER 19 SOCIO-PSYCHOLOGICAL STATUS OF THE POPULATION LIVING ON RADIATION CONTAMINATED TERRITORIES IN THE REMOTE PERIOD AFTER THE CHORNOBYL CATASTROPHE

As a result of the Chernobyl catastrophe, on the affected territories, an environment was formed with a set of factors causing adverse effects for the life of population. In particular, these are ecological, sanitary and hygienic, economic and social factors [1].

Despite the large volume of protective measures implemented at the radiation contaminated territories (RCT) and annual allocation of a portion of budget funds for overcoming the humanitarian consequences, the highest death rate on the territory of Ukraine is observed among the residents of the areas polluted with radionuclides. The rise of incidence in all classes of diseases was recorded. The state of health of newly-born children is deteriorating [2].

The processes of self-purification of natural biotic communities cause the reduction in radionuclide rate in the environment and agricultural products. The speed of natural rehabilitation of radionuclide decomposition surpasses the technical, agro-technical and chemical means of protection [3, 4]. As of today, the unresolved and the most important are the issues of restoration of adequate life activities of the population in the zones of guaranteed voluntary resettlement and ecologic control. The shortcomings of the legal framework concerning the issues of social protection, medical and psychological rehabilitation exacerbate the perception by the suffered population of the social and political changes taking place in the country; and influence the formation of the sense of social wellbeing, social activities, and health [5, 6].

The long-term monitoring of the social and psychological consequences launched in 1991-1992 pointed out the necessity of introduction of a new strategy at the RCT, based on well-grounded complex development of territories taking into account the state of health, value systems, attitudes, and behavioral motivation of the suffered population [7, 8].

The study of ecological and social problems of various regions allowed to determine that the adverse conditions of the natural environment, as the result of unfavorable tendencies in the economic development, affect the society itself, sharply worsening the medical and ecological indices, increasing the level of social tension, invoking moral and psychological discomfort among people [9, 10]. Such understanding of the essence of the social and ecological situation has in many respects redefined the distribution of structural elements of the socio-psychological study on the RCT. The main elements are as follows: socio-psychological, socio-political, socio-infrastructurel, socio-economic, and medical.

Taking into account the above-mentioned, the aim of long-term studies of the socio-psychological monitoring is optimization of life quality of the population permanently residing on the radiation polluted territories, based on determination of the cause-and-effect relations between the social, ecological and economic factors of the conditions of life and the socio-psychological status.

Materials and methods of study. The object of study was the able-bodied population residing in the zones of guaranteed voluntary resettlement (3rd zone), increased radio-ecological control (4th zone), and conditionally clean territories (CCT). The following methods were used in the process of study: standardized polls, test of Integral Index of social wellbeing [11], method of self-evaluation of the state of health [12]; "General Health Questionnaire (GHQ) — 28" [13]; statistical and mathematical methods with the use of software packs SPSS, EXCEL (comparison of generalized characteristic features: relative and average values; authenticity of paired variations according to the Student criterion (*t*); establishment of interaction of factors with the help of pair correlation (*r*), factorial and cluster analyses). Sampled aggregates were calculated in the dynamics of studies of the years 1993–2010 based on the total population size in each particular zone. The estimated error of a sample made up 0.035–0.060.

Results of the study. The conducted standardized sociological polling of the population residing in the radiation contaminated territories allowed to find out that even 23 years after the accident the population of the 3rd and 4th zones irrespectively of gender, age, and profession, believes that among the 31 factors of health risks the most dangerous remain those connected with the presence of radio-nuclides in the air, water, soil, food products and their effect on the organism. Such state of things is caused, first of all, as well as in the first years after the Chernobyl NPP catastrophe, by the absence of comprehensive information on the radiological situation at the places of residence. The mass media remain the main sources of information, however, their number is significantly smaller (Fig. 19.1). It is known that this information cannot be complete, adequate, and varied. Expert articles are almost absent, however the awareness of the problem at workplaces has improved. Thus, one of the factors of the socio-psychological tension at the RCT under consideration is, without doubt, the factor of information.

The impossibility of resolution of personal everyday problems contributes to the sustained socio-psychological tension in the suffered region. The main problems, which concern the population of the radiation contaminated territories are financial ones, connected with the state of health and caused by the ecological situation at the places of residence. For the major part of population in the regions under consideration,

in the current conditions, the top-priority problem is the financial issue, in particular, for Ovruch and Korosten districts. Nominal wage and pensions are gradually increasing, but in the dollar equivalent, during the 1990-ies the wage fell 3.6 times, and the pensions — 3.9 times (Fig. 19.2). The share of employees with the income lower than the subsistence wage has decreased significantly, however as much as 32.9% of the RCT population still remain poor. The growth of prices for services causes an increase in the number of families, who need subsidies, and, as a result, the growth in the volume of state subsidies.

For the population of Ivankiv, Vyshhorod, and Ripky districts, health remains the primary issue.

The third place is occupied by the ecological problem caused by the place of residence — the radiation contaminated territories. Among population's vitally important problems there is also the issue of political and economic situation in the country, the future of the region under consideration depends on. Worth noting is the fact that significant portion of the respondents of Ivankiv (25%), Vyshhorod (30%), Chernihiv (33%), and Dubrovysia (26%) districts do not believe in improvement of socio-economic conditions of life, which affects the psychological and physical wellbeing of the population.

The integral index of population's social wellbeing in all districts under consideration is within the regular range of the average level (from 45 to 55 points) (Table. 19.1). Among the districts under comparison we can point out Ivankiv and Dubrovysia districts with lower indices of social wellbeing and Vyshhorod district with the highest figures.

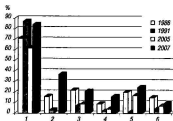


Fig. 19.1. Distribution of respondents according to the evaluation of information sources in dynamics by years (%). 1 — mass media; 4 — experts in radiation issues; 2 — work; 5 — friends, neighbours; 3 — medical workers; 6 — others

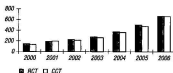


Fig. 19.2. Average monthly nominal wage of employees on the radiation contaminated territories (RCT) and conditionally clean territories (CCT) in UAH

Table. 19.1. Level of integral index of Social Wellbeing (ISW) depending on the district of residence

Districts		ISW level	
		M	sm
1	Ripky	51,00	2,08
2	Chernihiv	51,83	2,28
3	Ivankiv	45,29	1,62
4	Vyshhorod	53,98	1,18
5	Ovruch	52,29	4,27
6	Korosten	53,24	1,86
7	Rokytno	50,56	1,32
8	Dubrovysia	47,35	1,59
in general		50,46	0,67

In the most disadvantaged Ivankiv district, the reduction of the level of the social wellbeing was, first of all, influenced by dissatisfaction with the recreational-and-cultural and informational-and-cultural spheres. For the population of the three districts (Ripky, Vyshhorod, Ovruch) the hierarchies of the spheres of living, for the most part, are the same and the level of IISW was reduced due to the dissatisfaction with the social security sphere. In these districts the population was dissatisfied with the level of legal support in protection of their rights and interests. In Korosten, Rokytne, and Dubrovytsia districts the unfavorable sphere of living was socio-political. In Chernihiv district the IISW level was formed by equally low levels of sufficiency in most of the spheres of living.

The general level of social wellbeing among the population of the RCT is credibly lower compared to the population of the CCT (56.46 ± 0.67 and 63.78 ± 0.91 respectively). The share of the population with the high IISW level makes up 70.8% at the CCT and mere 54.3% at the RCT. In the hierarchy of spheres with the negative influence on the social wellbeing of the RCT population the first places are occupied by the socio-political, recreation-and-cultural, and social security spheres. Among the population of the CCT, we can also observe the negative evaluation of the socio-political sphere and the sphere of social security. The difference in the evaluation of the spheres of living between the population of RCT and CCT lies in the highest dissatisfaction with the recreation-and-cultural sphere among the RCT population, and with the professional and labor sphere among the CCT population (Tabl. 19.2).

The reduction of the social wellbeing level among women, compared to men, is characteristic of all population groups, however the authentic correlation with the respondents' gender was found among the population of the 4th zone of the RCT ($r = -0.217$ $p < 0.05$). In the 3rd zone of the RCT, there were two distinguished age groups with low social comfort level: the pensioners aged over 60 and those who retired before the pension age due to residence in the "Chornobyl-area" — aged 40–49. In villages of the CCT, the lowest IISW level was observed among young people aged 16–19 and able-bodied people (aged 30–39).

Table 19.2. The level of sufficiency of social life spheres among populations of radiation contaminated (RCT) and conditionally clean territories (CCT)

Social life spheres	Sufficiency index*	
	RCT	CCT
Recreation-and-cultural	1.63	1.63
Social security	1.64	1.59
Socio-political	1.44	1.54
Professional and labor	1.87	1.70
Material and utility (2 nd level)	1.80	1.76
Social relations	1.69	1.61
Interpersonal relations	1.99	1.77
Personal qualities	2.17	1.97
Informational and cultural	1.80	1.72
Material and utility (1 st level)	2.10	1.94

*In points, from 1 to 3; 3 points designate the highest comfort level

According to the data of previous studies, the low indices in the socio-political sphere have determined its first place in the hierarchy of the negative spheres. The socio-political sphere is the base one for resolution of many other vital issues; it is closely connected with the professional and labor sphere. The professional and labor sphere is determined by the needs for a "suitable job" and "possibilities to have a side job". The sufficiency of this social sphere depends on the gender ($r = -0.311$ $p < 0.01$), where the professional troubles are traditionally intrinsic to men; on the level of

satisfaction with job ($r = -0.291$ $p < 0.05$); on the desire to change job ($r = 0.424$ $p < 0.01$); on willingness to move to another location ($r = 0.329$ $p < 0.01$). In the evaluation of the given needs, the population residing on the RCT has neutral influence on the state of social wellbeing and has no direct correlation with the problems of economic and entrepreneur activities. At the same time, among the population of the OCT a chain sequence of social aims can be traced: low level of social wellbeing is significantly determined by the dissatisfaction with the professional-and-labor sphere of living, which, in its turn, is manifested in dissatisfaction with the job and desire to change it, and, as a result, move to another location.

According to poll data, around one quarter of population residing in inhabited localities of the RCTs were dissatisfied with current job, around 32.2–35.1% of the polled in all the inhabited localities wanted to change it. The level of wages or pensions on the RCT, which could be satisfactory for a person, was significantly lower, compared to the CCT. Thus 42.7% in the village of Hladkovychi and 34.4% in the village of Zarichchia would be satisfied with the remuneration within UAH 1000; and only 2% of the population dream of wages exceeding UAH 5000.

Around 80% of respondents at RCTs as well as at CCTs believe the economic situation in the country to be the main obstacle for their economic activities. 32.2% in the 4th zone and 27.1% of the population in the 3rd zone believe that the status of the territory that suffered from the consequences of the Chernobyl NPP catastrophe is an essential obstacle for development of the economy. Every fifth polled person pointed out the absence of specialists and producers with knowledge concerning work in the conditions of radiation contamination.

With the help of factorial analysis of the indices of social wellbeing, three groups of social needs were discovered, with the highest influence onto the formation of social wellbeing for separate population groups (Table 19.3). The 1st factor — the main shares in the general dispersion of indices are occupied by the material, recreation-and-cultural and professional and labor spheres, irrespective of gender and place of residence of the polled.

Table 19.3. Contribution of factors into general dispersion of the indices of social wellbeing of the polled depending on their place of residence (on the basis of factorial analysis), (ln%)

Factors	Contribution into general dispersion of the indices (%) on territories	
	radiation contaminated	conditionally clean
Material, recreational and professional-and-labor spheres	22.60	35.02
Countrywide (legal and political spheres), the sphere of personal qualities	21.61	26.32
Social security and interpersonal relations sphere	13.65	11.26

The 2nd factor contains the spheres of human living which are formed by the state socio-political system of the society and by the sphere of personal qualities. The 3rd factor is determined by the high level in the sphere of interpersonal relations.

In the study of social factors of the life conditions of the population living in the special conditions of radiation contamination, it is necessary to take into account the subjective evaluations of the radiation risk and changes in the living conditions after

the catastrophe. The level of anxiety in connection with the radiological situation on the territory of residence remains high among 30% of the respondents in the inhabited localities of the RCT (Table 19.4). At the first place, as it was in the previous studies, is the anxiety connected with their own state of health and children's state of health. At the second place the highest is the share of people worried with the possibility of radiological pollution of food stuffs (49%) and of drinking water (20%). The data provided indicates the high level of perception of the radiation risk by the population, which has remained in place since the first years after the accident and today it does not always correspond to the objective data of the radiological situation in the inhabited localities under consideration, especially concerning the quality of drinking water. Thus, according to the studies of the sanitary and epidemiological station, the level of radio nuclides in drinking water for the past 10 years not exceed the allowed norm of 2 Bq/L.

Table 19.4. Distribution of the population according to perception of the negative impact of the Chernobyl disaster

Negative consequences	Distribution of the population (%) on territories	
	radiation contaminated	conditionally clean
Anxiety concerning the radiological pollution of food products	49.0	59.5
Anxiety concerning drinking water	19.8	27.0
Problems connected with moving to another location	8.3	23.0
Anxiety in connection with the radiological situation on the territory of residence	31.3	28.4
Anxiety connected with their own health and children's state of health	78.9	64.9
Involuntary change of habitual way of life	6.3	8.1
Anxiety connected with the absence of clear information on the population's state of health	27.1	16.2
Anxiety connected with the absence of clear information on the state of environment	20.8	18.9

In general, the level of perception of radiation risk 24 years after the Chernobyl NPP catastrophe remains high for the population living on the RCT as well as on the CCT. From 50% to 75% of the population of all the inhabited localities believe it is possible that their health would be deteriorated due to environmental pollution, in particular, due to the consequences of the Chernobyl catastrophe — from 60% in the CCT and up to 85.6% — in the 4th zone of the RCT. The evaluations received are substantially caused by the events after the catastrophe. The deformation of the information flow in the field of the Chernobyl catastrophe has changed the classical peculiarities of information perception by the consumer. Even 24 years after the catastrophe, the population holds no trust in the means of communication of the official authorities. The trust is somewhat higher towards medical workers, foreign sources of information, and scientists. The polling of 2009 shows that the population has the highest trust level towards the information of international organizations (53%), and scientists (42%). The respondents showed the lowest trust level towards information provided by the highest state officials — 5%.

The level of perception of the radiation risk is in no way correlated with the behavior of the population residing in the RCT. To generalize the results concerning the study of behavior (lifestyle) the procedure of hierarchical cluster analysis was implemented with the use of the average connection method. The analysis was based on the components of a healthy lifestyle (dietary pattern, work schedule, rest schedule, performance of sanitary and hygienic activities in the households, physical activities), and behavior concerning the performance of the main protective activities due to residence in radioactively polluted territories (control of milk, meat, forest berries, mushrooms, vegetables for radioactive contents, restrictions in consuming game animals, mushrooms, and forest berries).

In the process of experiment, three clusters have been distinguished, which essentially differ from each other according to the peculiarities of behavior (Table 19.5). The 1st and the most extensive is the "situational" cluster — 79.3%. This population stratum has no clearly-defined behavioral strategy, which may vary depending on the changes in the dominating factor of influence. They partially or occasionally keep to the main rules of healthy lifestyle, "in case of necessity", they carry out the protective measures connected with their residence on the RCT in terms of their own level of recognition of the hazards. However the value of health is of high importance for this population category so in case of implementation of consistent educational and informational strategy by the state agencies, their behavioral strategy can be altered for a more responsible one. The 2nd cluster can be called "nihilistic" or "irresponsible". Here person's behavior completely rules out any recommendations concerning health protection and is based on an open confrontation (10.4%). But the nihilistic behavior concerning health protection is not simply based on a principle of confrontation against everything; it is an involuntary choice between the dangerous factors of life: poverty or possible gradual loss of health. This is the very category of people that needs immediate assistance in improving their life quality, and the latter would induce positive changes in their attitude towards health. The 3rd cluster (10.3%) is completely contrasted to the second one. This is the cluster of people "responsible" for their health and with a clearly defined behavior strategy.

Table 19.5. Cluster centers of self-preserving behavior of the population of the radiation contaminated territory and their fill rate

Components	Clusters and meanings of their centers		
	situational	nihilistic	responsible
Behavior concerning health protection	when necessary	when necessary	always; when necessary
Keeping to a dietary pattern	to some extent	do not observe at all	always
Keeping to labor and recreation schedule	to some extent	do not observe at all	always
Performance of sanitary-hygienic activities	always; to some extent	do not observe at all	always
Behavior concerning physical activity	to some extent	do not observe at all	always
Restrictions in consumption of game animals, mushrooms, forest berries	sometimes	sometimes; never	always
Checking of milk, forest berries and mushrooms for radiation before consumption	never; sometimes	never	always; never
Cluster filling rate (in %)	79.3%	10.4%	10.3%

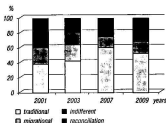


Fig. 19.3. The dynamics of variation in population's plans in connection with residence on radiation contaminated territories (in %)

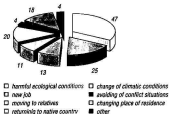


Fig. 19.4. Reasons for migration among the population of the radiation contaminated territories (%)

residing at the CCT and 30% at the RCT counted only on themselves, their activity and ability to adapt. Each third respondent thought the improvements of resolution of general problems were connected with the improvement of the work of the local authorities and with acceleration of economic reforms in the country.

About 21% of population of the 3rd and about 26% of the 4th RCT zones aim at migrating to a different place. The main reasons of this for the population of the RCT remain anxiety about harmful ecological conditions (47%; Fig. 19.4).

Studying the social wellbeing and behavior of the population of the radiation contaminated territories has allowed to find out that the characteristic feature was the presence of depressive realism and decline of the socio-psychological adaptive functions. Research of the psychological state of health using psychodiagnostic testing "General Health Questionnaire — 28" (GHQ) showed that only 24 years after the Chernobyl catastrophe, the general GHQ 28 index, which characterizes the intensity of slight mental disorders and average index of somatization, anxiety, social dysfunction, and depression of the population of the radiation contaminated territories in reality did not differ from the indices of conditionally clean areas (53.0 ± 1.11 and 51.3 ± 0.71) (Fig. 19.5).

Thus the behavior in everyday life, connected with the peculiarities of residence on the RCT is inadequate to the existing health risks. Most of the population is not at all concerned about the protective and prophylaxis measures. The latter is caused by the life aims concerning further residence on the RCT. The strategy of future plans of the RCT population has 4 tendencies:

- traditional for RCT — demanding an increase in compensations from the authorities due to living on a hazardous territory;
- moving to a "clean zone" for permanent residence;
- living the usual way despite the radiation and talks about it;
- living the usual way, observing certain recommendations in connection with residence in this area (Fig. 19.3).

Among the population of all the inhabited localities, which have been compared, the greatest hopes for future were connected with the increase and extension of social privileges and compensations. 45.8% of the population of the 3rd zone of the RCT hope for improvement of ecological situation. Around 40% of the respondents

Among separate groups of the population, the highest GHQ 28 indices, exceeding the average ones, were observed among the divorced (62.25 ± 8.92) and widowers (65.00 ± 5.38) on the RCT. On the CCT, widowers also have a high GHQ 28 index. Among age groups of RCT population the highest index of GHQ 28 was in 40–49 years group (59.00 ± 3.32).

Comparative analysis of structure of GHQ 28 indices according to the somatization, anxiety, and social dysfunction scales for the year 2010 showed that the structure has not changed compared with the years 2003, 2005 [14]: the index of somatization occupies the first place, social dysfunction — the second, anxieties — the third, and depression index — the fourth (Fig 19.6).

In the dynamics according to years, index of physical health of the RCT population according to self-evaluation remains lower than that of the population of the conditionally clean territories (Table 19.6).

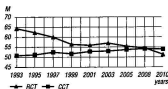


Fig. 19.5. Change of psychological health according to General Health Questionnaire – 28 in dynamics among the population of the radiation contaminated (RCT) and conditionally clean (CCT) territories (Murm)



Fig. 19.6. Average indicators of psychological health state according to GHQ 28 scales among the population of the radiation contaminated (RCT) and conditionally clean (CCT) territories (Murm)

Table 19.6. Indices of health of able-bodied rural population of the radiation contaminated and conditionally clean territories according to self-evaluation in the dynamics according to years

Territories	Indices of health* by years											
	1990	1995	1997	1999	2001	2003	2005	2008	2010	2012	2014	2016
RCT	0.90	0.54	0.43	0.59	0.15	0.21	0.54	0.58	0.62	0.44	0.46	
CCT	0.88	0.82	0.80	0.78	0.78	0.89	0.84	0.78	0.71	0.72	0.56	

*from 0 off 1, where 1 means absolutely healthy; RCT — radiation contaminated territories; CCT — conditionally clean territories

Most of the respondents from the RCT (63%) noted that they have chronic diseases, in particular, 93.1% of women and 52.6% of men. The main diseases were: cardio-vascular (29.9%), bones and joints (24.3%), respiratory organs (24.3%), digestive organs (14.0%), nervous (12.1%) and endocrine (13.1%) systems. 52.1% of the RCT population have chronic diseases, of which 55.6% were women and 45.6% were men. The main diseases also were: cardiovascular (23.5%), digestive organs (18.7%), bones and joints (27.8%), nervous system (11.2%), respiratory organs (17.6%), endocrine system (9.6%).

Thus, the RCT population notes that they have a larger number of chronic diseases in comparison with the population of the conditionally clean territories, especially concerning women. The structure of chronic diseases is similar in both groups under study.

About 76% of the population of the radiation contaminated territories considered their state of health will get worse in future because of the polluted environment. Among the population of the conditionally clean territories, 60% had the same opinion.

Among the reasons, which have a negative influence onto people's health, the respondents defined the following: general ecological situation in the region — 34.8–42.7%; consequences of the Chornobyl catastrophe — 59.8–85.6%; poor quality of work of medical institutions — 29.2–38.9%; unsatisfactory financial status — 29.3–30.5%; poor nutrition and other.

Factorial analysis using the method of main components and factor inversion according to the "varimax" method was performed to find the structural interconnections between the reasons of negative influence onto the RCT population's health. The usage of this method allowed to distinguish three main groups of factors (Table 19.7).

Table 19.7. Factor matrix of reasons of negative influence on radiation contaminated territories population's health

No	Factors	Factor loading		
		I	II	III
1	unstable political situation in the country	0.696	0.197	-0.294
2	residence on the RCT	0.129	0.765	-0.095
3	quality of food products	0.112	0.753	0.398
4	quality of drinking water	0.138	0.807	0.360
5	level of air purity	0.185	0.551	0.582
6	financial status of a family	0.570	0.541	0.016
7	working conditions	0.601	0.318	0.393
8	quality of residential facilities	0.845	0.046	0.164
9	quality of dwelling	0.794	0.122	0.271
10	problems in communication	0.654	0.042	0.407
11	harmful habits	0.467	0.275	0.450
12	heredity	0.265	0.217	0.724
13	personal inaction in preserving one's own health	0.038	0.048	0.795
	General informativeness of the factor	25.45	20.64	19.24
				65.33

The 1st factor — factors of negative influence onto the quality of life (residential facilities, type of dwelling, working conditions, financial status of the family) and also unstable political situation in the country and problems in communication have the main load in the general dispersion of indices. Its general informativeness is 25.45%.

The 2nd factor includes the negative indices of the ecological situation (quality of drinking water, food, level of air purity) and the factor of residence on radiation contaminated territories. This factor unites ecologically dangerous reasons and reflects the opinion of 20.64% of the polled.

The 3rd factor can be named "realistic irresponsibility", where personal inaction in preserving one's own health, heredity and harmful habits has the biggest factor loading. However, this factor is the least informative and reflects the opinion of 19.24% of the polled.

To determine the features of socio-psychological and physical state of health in the main groups of population (those, who remained on radiation contaminated territories and those who have never undergone radioactive influence), the procedure of cluster analysis was used. The analysis was based on the three main components of state of health — physical, mental, and social. Experimentally, it was established that the best quantity of clusters defined is three. According to the characteristics of the polled in clusters defined, the groups of population differ substantially from each other according to the indices of state of health, aims, and values.

Table 19.8 shows the dominant features of the clusters defined. The first cluster is the biggest — 63.6%. It includes people, who consider their state of health as "not quite healthy" (60.3%) and "healthy" (39.7%). According to mental health testing, this cluster mainly includes respondents, who are "almost healthy" (75.0%) and with "prenosological forms" (25.0%). The social wellbeing is characterized as "high" (42.6%), "above average" (22.1%), and "average" (33.8%). Thus it is possible to state that this is a "trouble-free" stratum of the population.

Table 19.8. Cluster centers of physical, mental and social health components of the radiation contaminated territories population

Health components	Clusters and values of their centres		
	1 st	2 nd	3 rd
physical	healthy, not quite healthy	not quite healthy, ill	no quite healthy
mental	almost healthy	prenosological forms	clinically marked symptoms
social	high	average, below average	low, average

According to physical self-evaluation, the second cluster is formed by people who are "not quite healthy" (51.7%) people, and also by a considerable part of the "ill" (41.4%) and "seriously ill" (6.9%). According to the mental features, people with "prenosological forms" (58.6%) and "almost healthy" (41.4%) prevail among those polled. The social component of health is represented with all strata of social wellbeing, but average and below average (86.1%) prevail. Thus, the centers in all the features are moving towards the worse. This cluster was named "relatively unhealthy". It was formed by 27.1% of the polled.

The third cluster, the smallest one (9.3%), is characterized by "ill" people with regards to all components of health. According to physical self-evaluation, these are mostly "not quite healthy" (70.0%) people, but the mental component is represented mostly with people with "clinically manifested symptoms". The level of social wellbeing is characterized as "average" and "low".

Thus, on the RCT, three groups of victims have been defined, for which target assistance methods and rehabilitation means should be adapted: the "trouble-free" — are able to provide themselves and their families with all they need with their own efforts; the "relatively unhealthy" — a share social assistance is needed for their self-sufficiency; the "ill" — are able to survive only through social assistance.

Conclusion

24 years after the catastrophe at the Chornobyl NPP, the level of perception of radiation risk remains high among the RCT population as well as the CCT population. 50–70% of the population of all the inhabited localities consider it possible to lose health due to environment pollution. The evaluations received are largely caused by post-catastrophe events. Deformation of the information flow of the area affected by the Chornobyl catastrophe has changed the classical peculiarities of information perception by the consumer and, as the result, population do not trust the media of the official authorities.

Studying the social component of living has allowed to identify a low level of adequacy in the three spheres of living. According to the factorial analysis, the financial, recreational and cultural, and professional labour spheres carry the main loading in forming the social wellbeing. The general level of social wellbeing of the RCT population is definitely lower in comparison with the CCT population.

Studying the life style of the population on radiation contaminated territories has allowed to find out that its characteristic feature was the presence of depressive realism and reduction of socio-psychological adaptive functions. The latter was triggered by negative feelings caused by failures in everyday life, at work, social despair. Depression acts both as the reason and consequence of the destructive social experience and destructive social behaviour. The latter is manifested in low preventive activity (aimed at preserving one's own life with account of residing on the RCT) in everyday life.

The analysis of the RCT population life style has allowed defining three behavioral types: "situational", "nihilistic", and "responsible". The behavior in everyday life, which is related to peculiarities of living on the RCT, is inadequate to the existing risks for the state of health. Most of the population absolutely neglect protective preventive actions in their life, which is due to their life aim to further live on the RCT.

The reduction of socio-psychological adaptive functions defines the state of health of the affected population. Only after 24 years since the Chornobyl catastrophe, the GHQ 28 general indicator, which demonstrates the depth of slight mental disorders and average indicators of the somatization, anxiety, social dysfunction, and depression scales among the population of the radiation contaminated areas, in reality does not differ much from those of the conditionally clean areas. In the dynamics according to years, the indices of physical health, according to self-evaluation of the RCT population, remained lower in comparison with population of conditionally clean areas. The analysis of the components of the state of health (physical, mental, and social) allowed to define three groups of population: "trouble-free", "relatively healthy", and "ill".

Social protection of victims from the consequences of the Chornobyl catastrophe should be based with account of the differences between the categories of citizens affected by ionizing radiation, and combine the means of social protection with targeted and individual social assistance for every victim. Targeted methods and rehabilitation means should be applied to the defined groups of victims: the "trouble-free" — are able to provide themselves and their families with all they need with their own efforts; the "relatively unhealthy" — a share social assistance is needed for their self-sufficiency; the "ill" — are able to survive only through social assistance.

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CHAPTER 20 RADIATION RISKS PERCEPTION

Since the Chernobyl accident 25 years passed, but the society still is worrying on its consequences. There are many myths and legends about the effects of radiation, supported by rumors and publications in the media. All this increases the individuals' anxiety on his and his family health, that acts as a stress factor, more harmful than a low-dose accidental exposure.

According to the Energy Strategy of Ukraine for 2030 [1] a construction of the nuclear fuel cycle facilities is planned including about 20 reactor units, uranium and zirconium processing facilities, etc.). Construction of the nuclear fuel cycle objects requires the consent of the population according to national and international legislation [2, 3]. The development of nuclear energy in Ukraine may be prevented due to the unwillingness of the population to the construction of new radiation and nuclear facilities. Thus, in Ukraine in 2007, 55.1% of the population were against the building of new nuclear power plants, and only 23.1% of respondents agree on it (in 2005 — respectively 54.9% and 26.8%) [4]. According to studies carried out in 2002–2006 in Russia [5–7], found that from 40% to 69% of the population living in contaminated areas, suffer from fear, intrusive memories, thoughts and experiences in connection 'because of the Chernobyl accident. Thus 51.5–55.6% of apparently healthy people feel fear, anxiety, irritation, and insomnia.

In Russia research on radioanxiety of population living in areas of accidental contamination started about 20 years ago [8, 9]. From 1993 to date from 70% to 90% of the population from contaminated areas indicate that radiation impact is "detrimental" and "very harmful" to their health and the health of their loved ones. High population radioanxiety reduces the quality of life and level of health. To reduce it informational work with the population is conducted and a number of instructive-methodical documents have been developed as well as popular science publications [10, 11].

High levels of radioanxiety impede the development of nuclear energy sector. The population surveys in several countries on the attitude to nuclear energy have shown a significant support for the development of electricity production at nuclear power plants (from 33% in Russia to 80% in the USA) [4].

The objective was to study people's attitude towards various situations of radiation exposure and to determine the basic level of knowledge about radiation risks. To assess factors that influence the perception of radiation risk by population a survey was conducted by questionnaire of secondary school pupils (16–18 years), students and their parents in different regions of Ukraine. The project was initiated by a joint

effort of O.Marzeev Institute of Hygiene and Medical Ecology and the Global Strategic Center for Radiation Health Risk Control of the A-bomb Disease Institute of Nagasaki University.

We have developed a questionnaire consisting of 37 questions and contains a section on the effects of the Chernobyl accident and the safe operation of nuclear power plants, radioactive contamination of territories and food, and the questions on radiation risks. The poll was conducted among young people and their parents in five regions of Ukraine (Zhytomir, Rivne, Kirovograd, Odessa regions and Slavutych city). In each region questionnaires were distributed among 330 respondents, including young people and parents. Parents returned only 861 correctly completed questionnaire (52%), while a vast majority of students (1,536 or 93% of respondents) have answered the questions.

Looking in the near future on the possibility of an accident in Ukraine, similar in scope to Chernobyl, 8.8% of respondents believe that such an accident does not happen, about a quarter — think that this event is unlikely (26.0%) 51.8% — possible and 11.5% — very likely, and for sure to happen in the near future — 1.9% of respondents.

Asked what the consequences of the Chernobyl accident are most relevant for today, the respondents called the most important be a "radioactive soil contamination" (39.4% and 33.6% of parents and of students) and "possible negative effects on health in the future" (34.0% and 31.2% of parents and students, respectively). Each of the variants of "concern for the health of family" and "the existence of Zone of Exclusion" was marked by students with the same frequency — 14.7%. For parents the third biggest was "concern for the health of family" (27.5% of adults). Radioactive contamination of food and water, according to respondents, is losing relevance for today. These factors were noted by 11.1% of all respondents.

70.5% of respondents (69.1% of students and 72.8% of parents) believe that information about the real consequences of the Chernobyl accident is now hidden from the public. However, in Slavutych, where professionals resident in the field of nuclear and radiation safety, the proportion of youth who believe in transparency and impartiality of information about accident rates significantly higher than in other regions (Fig. 20.1).

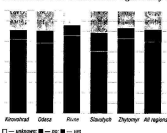


Fig. 20.1. Percentage distribution of high school students' response to the question "Are hidden, in your opinion, the real consequences of the Chernobyl accident from the public?"

The share of respondents in the surveyed regions, who fully approve construction of new nuclear power plants is 7.3%; consider the construction possible 17.3%; consider it possible after public hearings — 24.4%, undesirable — 33.2% and totally unacceptable — 17.8%. The attitude of young people to the construction of new reactors statistically significantly differ according to place of residence (Fig. 20.2).

In questions about the perception of risk, respondents were asked to evaluate each of the 11 risk factors for their own health and fam-

ily health (airplane flights, smoking, AIDS, traffic accidents, accidents on the water, intentional murders, radiation accidents, alcohol intoxications, radon in indoor air, fire, drugs) on a 5-point scale: (1) very safe, (2) slight risk, (3) to some extent dangerous, (4) is quite dangerous and (5) is very dangerous. For each of the factors the response rates were assessed (3), (4) and (5). 79.5% of respondents chose from these options the risks to life due to radiation accidents. Moreover, traffic accidents (76.7%) and alcohol intoxications (65.7%) respondents considered less significant risk factors, although these factors possess leading places in the structure of mortality in Ukraine as a result of external causes.

For high school and university students the subjective risk estimates significantly differ depending on place of residence for smoking,

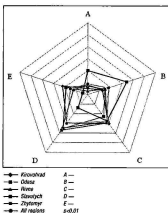


Fig. 20.2. Distribution by region of the youth replies to the question "How do you feel about the prospects of building new nuclear reactors in Ukraine?"

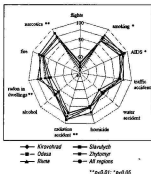


Fig. 20.3. Distribution of responses by region for school and university students who rated these factors as hazardous

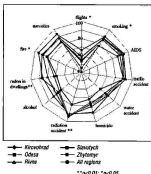


Fig. 20.4. Distribution of parents' responses rating factors as hazardous by regions

AIDS ($p < 0.05$), radiation accidents, radon in indoor air and drugs ($p < 0.01$), while for parents a statistical difference was found for flights, smoking, fires ($p < 0.05$), radiation accidents and radon in indoor air ($p < 0.01$) (Fig. 20.3–20.4).

Summary

1. In the surveyed regions of Ukraine the share of respondents who believe absolutely impermissible construction of new nuclear power plants is 17.8%, undesirable — 33.2%, possible after the public debate say 24.4%, possible — 17.3%, fully approve the construction 7.3% of respondents.

2. 70.5% of respondents (69.1% of students and 72.8% of parents) believe that information about the real consequences of the Chernobyl accident is now hidden from the public.

3. Subjective assessments of the radiation accidents and hazards from radon in indoor air are statistically significantly different for residents of the surveyed regions (both youth and their parents), depending on location.

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CHAPTER 21 MEDICAL CONSEQUENCES OF THE CHORNOBYL ACCIDENT IN EXPOSED IN CHILDHOOD

An unprecedented situation arose, when hundred of thousands of children have been exposed not only to acute radiation, but remained to live under conditions of prolonged exposure to low doses of ionizing radiation and other adverse factors of postaccidental period as a result of the Chornobyl accident [1]. The number of children born from irradiated persons is being increased from year to year.

The researches of a number of authors proved that the worsening health of the exposed pediatric population is one of the most adverse consequences of the Chornobyl accident [2, 3].

Despite the great efforts that were aimed to its preservation and rehabilitation among children who were exposed to radioiodine effect and other negative factors of the Chornobyl accident, the decrease in share of practically healthy person from 27.5% in 1986–1987 to 7.2% in 2005 was observed, as well as the increased number of children with chronic diseases from 8.4% in 1986–1987, to the 77.8% in 2005. The most unfavorable changes occurred in children with thyroid exposure doses over 2.0 Gy.

An integral parameter that reflects all these adverse changes, is an increase in disease incidence and prevalence, as a meter of fact, for all main classes of disease [1].

The causes of an increased somatic pathology remain controversial, and the role of ionizing radiation — the contrary ones. Therefore it is important to establish the characteristic responses of children's body to the effect of radiation and non-radiation factors, accompanying the Chornobyl accident.

Children, irradiated *in utero*, deserve a particular attention. It is known that high radiosensitivity is characteristic for an organism under development and differentiation stages. Children are a critical group of people affected to greatest degree by radiation accidents, especially when the effect of ionizing radiation occurred in the antenatal period of ontogenesis. It was shown that the fetal radiosensitivity was dependent on its age and the period from the ninth to the fortieth day after conception was a critical one [4].

The effect of ionizing radiation on the fetus can occur by the direct influence on its organs and tissues, and by the irradiation disturbances in the mother organism and placenta. Irradiation at preimplantation stage is characterized by a high levels of embryos death *in utero* and absence of radiation injury signs in live-born children. In case of irradiation during organogenesis the antenatal death of embryos is reduced, but a high frequency of stillbirths and congenital defects are noted. Irradiation in the last trimester of pregnancy leads to an increased infant mortality at the first year of life, especially in the first month after birth. In live-borne children, the consequences of intrauterine irradiation can be manifested as the congenital defects, delayed mental and physical development [5], functional disorders of various organs and systems which are found both in the first days after birth and in the subsequent years of life [6, 7, 8].

A low health index is also noted in children born after the accident from irradiated persons. This contingent of children is characterized by a high disease incidence, including every year decrease in the number of practically healthy ones, an increased number of disabled people, and congenital malformations which occupy the first place in the structure of infant mortality [3].

The pathophysiologic mechanisms of all these adverse developments are widely discussed in the literature and there is no unambiguous interpretation. However, some authors suggest a certain role of genetic damage in their occurrence as so-called "small" recessive mutations of polygene viability and DNA disorders of regulatory character in the parents gametes, resulting in birth of "physiologically handicapped children". At the organism level it can be manifested by a shortening of life expectancy, decrease in resistance to adverse environmental conditions, an increased risk of multifactorial diseases and also by the increased risk of spontaneous carcinogenesis as well as under the influence of promoters [9].

The increase of mutation frequency in DNA loci reflecting increased mutation processes in the parent gametes was established by some authors during studies of genetic peculiarities in the Belarusian families living in the contaminated areas [10]. The mutations *de novo* were revealed in the families of Chernobyl clean-up workers who emigrated to Israel. The analysis included a genome fingerprints using PCR and RAPD-PCR of children born before and after the accident in clean-up workers families, and children of the control group. An appearance of new allelic variants was observed among children born in the families of clean-up workers after the accident and that was absent among children born before the accident and in the control group [11].

Within the years elapsed after the Chernobyl accident, children and adolescents, exposed to radiation factors, including thyroid exposure with iodine radioisotopes and other unfavorable factors of the Chernobyl accident, have reached reproductive age and became parents themselves. Every year the number of their descendants is increased. It is logically to assume that the profound changes in health that are found in the individuals irradiated in childhood, can not affect the health of their descendants, as defined in the report of the United Nation Scientific Committee to Assess the Effects of Atomic Radiation (UNSCEAR) for General Assembly (2001), and are one of the most topical problems of radiation medicine.

21.1 EARLY AND LATE CONSEQUENCES IN CHILDREN EVACUATED FROM THE 30-KM ZONE AND RESIDENTS OF RADIATION CONTAMINATED AREAS

For the years elapsed after the Chernobyl accident, we have observed more than 45 thousand children. The thyroid gland dose ranged from 0.1 to 28.53 Sv, the dose of total irradiation — from 10 to 134 mSv. The obtained results allowed to determine the symptom-complexes that are the most common for different terms of post-accidental period, and describe their clinical manifestations [12].

The studies have shown that the most typical reactions of the children body in the acute "iodine" period were as follows: respiratory syndrome (31.1%), hyperplasia of lymphoid tissue (32.2%); peculiar hematologic symptom complex, which was showed by quantitative (34.2%) and qualitative (92.2%) changes in the blood system; disorders of the gastrointestinal tract (9.4%); functional disorders of the cardiovascular system (18.0%); "euthyroid hyperthyroxinemia" without clinical signs of hyperthyroidism.

Children, who had been evacuated from the "alienation area", complained of sensation of irritation in the throat and metallic taste in the mouth (55.7%), tussiculation (31.1%), fatigue (50.1%), headache (39.3%), dizziness (27.8%), sleep disturbance (18.0%), syncope (9.8%), nausea and vomiting (8.0%), defecation disorders (6.9%). Pharynx hyperemia, hyperplasia of rhinopharyngeal lymphoid tissue, a moderate increase in peripheral lymph nodes (22.2%), the liver (9.8%) and the spleen (3.2%) were noted in part of these children. Tachycardia, respiratory arrhythmia, "endless tone" symptom during the measurement of blood pressure were detected in 18.0% of children.

The peripheral blood within one month was characterized by the presence of mild anemia (13.2%), reticulocytosis (7.3%), moderate neutrophilic leukocytosis (14.6%), mild leukopenia (4.4%), thrombocytopenia (13.1%), thrombocytosis (1.5%). A left shift was registered in differential blood count, and a relative lymphocytosis (26.5%), eosinophilia (17.6%), monocytosis (8.8%), an increased number of basophils (7.3%) were revealed in 2–3 months after the accident in 17.6% of children. Enlarged neutrophils with hypersegmented nuclei were found in 92.6% of children among the cellular elements of peripheral blood. 26.5% of neutrophils contained a toxicogenic granulation. Vacuolation of their cytoplasm was observed in 13.2% and of the nucleus — in 7.3% of cases. The cells with pyknotic nucleus, basophilic "fringer" vacuolized cytoplasm predominated over lymphocytes. The solitary binucleated cells were noted. Azurophile inclusions were revealed in the cytoplasm of 17.6% lymphocytes. Anisocytosis of thrombocytes, vacuolization of their hyalomere and granulomere were observed, and the conglomerates were found.

Lymphocyte metabolism was characterized by a sharp activation of oxidation-reduction processes. The levels of the Krebs cycle enzyme and glycolysis, NADPH₂- and NADH₂-dehydrogenases, enzymes of α -glycerophosphate shunt were increased. Lipids and amino acids were more intensively included in the process of energy formation. The activity of inosine-5-monophosphate dehydrogenase (IDH) — a cytochemical marker of nucleic acid synthesis — was increased. Neutrophils in the acute "iodine" period differed by a higher than normal content of peroxidase, lipids, glycogen, alkaline and acid phosphatase. The levels of peroxidase and acid phosphatase were also elevated in monocytes [12].



Fig. 21.1. Erythrocytes from peripheral blood of a child who suffered from ionizing radiation (early phase of the accident). The number of diskocytes is reduced. Erythrocytes with a crest, dome-shaped spherocytes and with pathological form of the surface are observed. Electronogramme. $\times 2300$



Fig. 21.2. Peripheral blood lymphocyte of a child exposed to ionizing radiation (early phase of the accident). Nucleus has an irregular form, euchromatin predominates over heterochromatin. The active nucleolus is placed near the nuclear membrane. Mitochondria are located in the cytoplasm. There are many ribosomes. There are many microvilli on the surface of a number of cells. Electronogramme. $\times 14\,500$

Studies of superficial erythrocyte architectonics by the method of scanning electron microscopy testified that diskocytic number was decreased ($p < 0.001$) during the acute period of iodine (62.5 ± 3.18)% compared with controls (90.86 ± 0.87)%. Content of transient (7.71 ± 0.79)% and prehemolytic (23.82 ± 3.11)% forms was higher than in controls (4.22 ± 0.46) and (4.91 ± 0.53)%, $p < 0.001$, respectively; erythrocytes with degenerative modified (1.36 ± 0.11)% and pathological (4.61 ± 0.69)% structure of the surface have appeared (Fig. 21.1).

The number of lymphocytes with a relatively smooth and villous relief was decreased, but the number of cells with a complex type of surface such as: folds, ruffles, deepening, intermediate configuration of cytomembrane, that could be attributed neither to smooth nor villous forms was increased [13].

An investigation of blood cell ultra-structure showed that erythrocyte content was mostly homogenous; the hemoglobin containing granules and sequesters was sometimes observed. Cells by the type of cupola-shaped curved discs were noted, and echinocytes were more seldom obtained. Erythrocytes with a reduced volume and the separation of cytoplasm areas were revealed. These changes were combined with the presence of loosening or compacting on the surface of areas, erythrocytic sludges were registered. The number of lymphocytes with morphological characteristics of functional activity was increased (Fig. 21.2).

A significant number of them had the nuclei with a complex form by invagination of the nuclear membrane. A formation of nuclear pockets, a nuclear membrane divided into layers and an expansion of

perinuclear space were observed. A significant number of ribosomes, sometimes collected in polysomes were located in the cytoplasm. The number of mitochondria varied from 2 to 18 per a section, making an average of 6.58 ± 0.57 that did not significantly differ from the control indices: 5.00 ± 0.61 ($p > 0.05$). However, mitochondrial structure

was significantly changed. So, mitochondria have an orthodox form ($93.80 \pm 3.01\%$) and a condensed configuration (6.20 ± 0.89) in children of the control group, while a number of the former was decreased up to $83.17 \pm 2.34\%$, $p < 0.05$, and the latter was increased up to $(16.83 \pm 1.39)\%$, $p < 0.05$ in the main group. Giant mitochondria with the light matrix and partial disorganization of the cristae were revealed (Fig. 21.3).

The concentric membranes and osmophilic granules were more often found in the cytoplasm. The reinforced formation of vesicles and clasmatosis were observed.

The number of "active" forms with microfibers and projections on the surface was increased among neutrophils. The high content of granules with mosaic granular component of the cytoplasm was noted. The signs of autophagocytosis with the presence of phagocytic vacuoles in the areas of cytoplasm were sometimes revealed (Fig. 21.4).

A significant number of thrombocytes had condensed matrix and vacuolized hyalomere [13].

The determination of the lymphocyte dry mass during the first month of post-accidental period, found that its magnitude was quite variable with fluctuations from 19 to 110 pg, but the average values (52.14 ± 2.13) pg did not significantly differ from those of the control. At the same time the concentration of solid substances (0.21 ± 0.015) ng/mkm² was significantly lower than in controls (0.22 ± 0.002) ng/mkm², $p < 0.002$, that shows the cell "swelling", an increase of water in its part [13].

In 1987–1990 the presence of various functional disorders of many organs and systems was the most typical for children evacuated from the 30-km zone (group 1) as well as for children who were left to live at the radiation contaminated territories (group 2) [12].

During this period the frequency of complaints to increased fatigue (82.0%), weakness (71.7%), irritability (85.9%), headache (52.0%), dizziness (40.3%), sleep disturbance



Fig. 21.3. Peripheral blood lymphocyte of a child affected by ionizing radiation (early phase of the accident). Fragment of cell. Mitochondria of condensed forms are grouped at one pole of the cell, many ribosomes, centrioles. Electronmicrogramme $\times 14\,500$

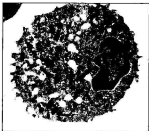


Fig. 21.4. Neutrophil of peripheral blood in a child exposed to ionizing radiation (early phase of the accident). There are granules with different mosaic structure in the cytoplasm. Electronmicrogramme $\times 14\,000$

(29.6%), gastrointestinal discomfort (52.8%), discomfort in the heart (26.4%), pain in muscles and joints (29.6%) was not only diminished, but even increased. Such symptoms as hyperhidrosis, palm and sole acrocyanosis, sweating, mottled skin, thermoregulation disturbances, pulse and arterial pressure labilities, ecuresis, syncope, hyperkinesis and tic were found in 70.3% of children during examination. The convergence insufficiency, asymmetry of facial innervation, disorders of muscle tone, tremor of fingers in a Romberg posture, asymmetry of tendinous reflexes were noted in part of children.

The functional changes from the heart were observed in 40.0% of children. On the ECG sinus tachyarrhythmia was registered in 32.0% of cases, bradyarrhythmia — in 45.0%, moderate and pronounced metabolic disorders in the heart muscle — in 42.4%, and 3.8%, respectively. Data of rheographic studies of regional hemodynamics were characterized by signs of dystonia, the presence of venous stasis, often with blood semisphere asymmetry, a pronounced tendency to increased vascular tone, a decreased pulse rate and a rate of arterial circulation in the extremity vessels. The decrease in the stroke and minute volumes of blood, a significant frequency of hypotonic (22.3%) and dystonic (31.5%) reactions to loading, more intensive and less economic work of ventilation apparatus, a reduction of some effective indices were established in "stable condition" by assessing indices of central hemodynamics and external respiration using dose physical load of the average intensity. The increase of oxygen debt (by 14.8%, $p < 0.05$), total oxygen inquiry to work (by 22.6%, $p < 0.01$), the periods restoring initial indices of pulmonary ventilation (by 18.6%, $p < 0.02$) and oxygen consumption (by 20.1%, $p < 0.01$) after loading were an illustration of that.

Vegetative maintenance of the cardiovascular system activity in children of both major groups differed by the insufficient inclusion of the sympathoadrenal system during physical exercises that can be attributed to the characteristic features of vegetative homeostasis for this period.

An increased frequency of dysadaptation states, that is demonstrated by the results of evaluating stages of adaptation syndrome according to the changes in hemodynamic indices of the coordinate system was established in children of both main groups, comparing to controls. The optimal adaptation developing only in a case of adequate functional reserves of cardiovascular system in children of both main groups was registered two times lesser than in controls (15.4% and 34.8%), $p < 0.02$. The stage of resistance was also determined more rarely (14.6%, 28.4% — in control), $p < 0.01$. By contrast, a significant increase was revealed in the frequency of tension stages (43.8%, 27.6% — in control), $p < 0.01$ and the depletion (26.2%, 9.2% — in control), $p < 0.01$.

A decrease in bronchial permeability mainly at the level of small bronchi, owing to bronchospasm was registered in 53.5% of children (in 18.9% — in control), $p < 0.01$ (Fig. 21.5).

These deviations were combined with changes in some indices of non respiratory lung functions, such as: the reduction of respiratory moisture and aerosol excretions from respiratory organs, that was accompanied by falling concentration of superficial active substances from pulmonary surfactant, by a decrease in phospholipids and total lipids in its content. The intensification of oxidative free radical processes in condensate of expired air (CEA) was revealed, its evidence was the rise of light sum indices and amplitude of instantly flash-initiated chemiluminescence of CEA (Fig. 21.6). The inhi-

bition of antitryptic CEA activity, indicating decrease in the local respiratory protection, was established.

The study of the digestive system showed that the changes in children evacuated from the alienation zone (the 1st group), as well as in the inhabitants of radiation contaminated areas (the 2nd group), had a unidirectional character in the first post-accident years (1987–1990).

Such functional changes as biliary dyskinesia (82.4%), functional disorders of the stomach with disorders of acid production or inadequate response to physiological stimulator (39.6%), reflux esophagitis (20.4%), duodeno-gastral reflux (28.8%), dyspancreatizm with decreased pancreatic exocrine function (59.6%) had the greatest specific weight in the structure of revealed disorders. The chronic pathological processes in the gastrointestinal tract of both children's groups were more rarely diagnosed (16.4%).

Dyspeptic disorders and abdominal pain were observed, in 43.6 and 65.4% cases, respectively, and disorders of asthenic, and vegetative character that were registered in 83.6% children prevailed in the clinical picture.

A morphostructural estimation of the upper gastrointestinal tract mucosa allowed to observe the prevailed catarrhal (superficial) inflammation and hyperplastic character of changes. Subatrophy of gastric mucosa was only found in 10.6%, and duodenum — in 14.0% of cases. The frequency of erosive-ulcerated disorders did not exceed the limits of average population indices (10.8%).

At the same term of post-accidental period, the peculiarity of gastric acid forming uncharacteristic for children age such as the prevalence of hypersthenic response to physiological stimulator, was established.

Quantitative hemogram findings of children, evacuated from the 30-km zone, were approaching to average population ones. Expression of morphofunctional disorders was also decreased. However, they were registered with high frequency in children who remained to live in the zone of obligatory resettlement. Thus, neutrophils with hyper- or hyposegmented nuclei were noted in 78.3% of children, with toxicogenic and immature granulation — in 16.0% and 8.3% respectively, with the vacuoles of the cytoplasm — in 12.0%, and in nucleus — in 4.0%. The cell with pyknotic nuclei, basophilic "fimbria" cytoplasm continued to prevail over the lymphocyte populations. The binuclear elements were sometimes revealed. The level of metabolic processes in lymphocytes was reduced

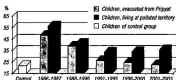


Fig. 21.5. Overall frequency of latent and visible disorders of pulmonary ventilatory function in children of observed groups



Fig. 21.6. Dynamics of light sum indices of initiated chemiluminescence in condensate of expired air in groups of observed children

in comparison with the data of 1986, but was still significantly higher than control parameters. All the cytochemical indices remained elevated in neutrophils [13].

Under evaluating topology of the erythrocyte superficial relief it was established that the number of discoid shapes had further decrease and reached $(48.13 \pm 2.8)\%$, $p < 0.001$ comparing to the data of 1986, while the number of transit $(17.66 \pm 1.24)\%$, $p < 0.01$, and predhemolytic elements $(29.70 \pm 2.17)\%$, $p < 0.001$, was conversely increased. Erythrocytes with pathologic and degenerative changed forms of the surface continued to meet.

The cells with intermediate and complex configurations of the surface relief were more often recorded among lymphocytes than in the control group [13].

Analysis showed that functional disorders were developed against a background of the intensification of free-radical processes, dysproteinemia (36.9%), increased level

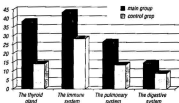


Fig. 21.7. Percentage of children in risk groups for developing pathology of most irradiated organs and systems

of aminotransferase (ALT—12.3%, AST—8.0%), transient disturbances of bilirubinconjugated hepatic function (52.8%), disorders of hepatic (28.3%) and pancreatic echostuctures (19.5%), suppression of T-cell immunity and dysimmunoglobulinemia. The great number of children was determined into risk groups for developing pathology of the thyroid gland, and the immune, the respiratory, the digestive systems (Fig. 21.7).

These risks began realizing since 1989–1990.

For the period of 1991–1995 the transformation of functional disorders into chronic somatic pathology was the most characteristic [1]. There was a significant decrease in a number of healthy children and an increase of the number of children with chronic somatic pathology among evacuated from the 30-km zone as well as children-residents of the contaminated areas in these years (Fig. 21.8).

The increased frequency of ECG disorders (in 68.2% of children in the 1st group, and 63.3% children of the 2nd group) manifesting changes in metabolism and metabolic-recovery processes in the myocardium was established by assessing the cardiovascular system activity.

Data of rheography examination were indicative of existing pronounced change with a high frequency of the regional and central hemodynamics (27.4%), hypokinetic type of organization. The functional reserves of blood circulation caused by decreased blood flow, and also oxygen delivery to tissues with developed hypoxic metabolic disorders were remained decreased [14].

Detected changes were indicative of a decrease in the cardiovascular system reactivity and demonstrate the lack of sympatho-adrenal system inclusion under functional loading due to the depletion of its endogenous reserves. The presence of high frequent dysadaptosis (in 90.7% of children in the 1st group and 83.7% — in the 2nd group) was a reflection of these processes at the organism level. The frequency of latent and visible

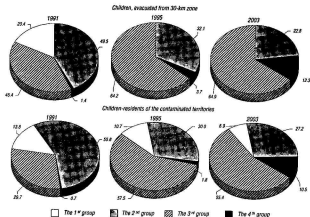


Fig. 21.8. Distribution of children into group of health after accident period

disorders of ventilation pulmonary function, was reduced, but remained higher than in controls, especially in children-residents of the contaminated areas.

As for the digestive system, the frequency of functional disorders was significantly declined in children of both observed groups, but the frequency of chronic pathological processes was sharply increased. Thus, during the examination of children evacuated from the alienation area chronic gastritis was diagnosed in 50.0%, chronic duodenitis — in 40.0%, chronic cholecystocholangitis — in 20.0%.

In children who continued to live in the contaminated areas the prevailed diseases were those of gastroduodenal zone in the structure of revealed pathology: chronic gastritis was diagnosed in 66.8%, chronic duodenitis — in 50.0%, chronic esophagitis — in 18.6% of children. Chronic cholecystocholangitis was detected in 50.6%, chronic pancreatitis — in 3.9% of cases.

Evaluation of structural and functional properties of the hemopoietic elements showed that the cells with hyper- or hyposegmented nuclei (in 30.2% of children of the 1st group and 63.2% of children in the 2nd group) were more seldom revealed among neutrophils. The number of lymphocytes with pyknotic nuclei and hyperbasophilic cytoplasm was decreased. The highest activity of glycolysis enzymes and synthesis of nucleic acids was remained with a tendency to decrease at the level of mitochondrial enzymes.

In 1996–2003 the level of children's health continued to decline. The distribution by groups of health in 2002 was as follows: anyone had the I group of health, the II group was established in 23.4%, the III — in 63.9%, the IV — in 12.7% among the

children evacuated from the 30-km zone; among children-residents of the contaminated areas the I group of health was defined in 6.3%, the II — in 26.1%, the III — in 57.5%, the IV — in 10.1% (Fig. 21.8).

Children who were the residents of the Narodychi district (Zhytomyr region), were divided into 2 subgroups according to dose (600 person in every one), with collective doses in the subgroup 1 — 2.6 man-Sv and subgroup 2 — 9.4 man-Sv. A significantly higher prevalence of respiratory diseases (2.0 times), vegetovascular dysfunctions (1.52 times), dysmetabolic disorders (1.4 times) and hepatic tissue fibrosis (2.3 times), the number of eosinophilias (1.7 times) and lymphocytosis (2.5 times) in the subgroup 2 of children with the collective dose of 9.4 person-Sv [1]. A relationship between the expression of disorder indices in pulmonary ventilation capacity of children and the contamination of soil with ^{137}Cs was established [15]. A negative effect of the Chernobyl accident consequences on the immune system of children-residents in the contaminated areas was observed. The immune imbalance that underlies the formation of many chronic diseases was registered in 56.9% of children in the dose subgroup 1 and 82.5% of the subgroup 2. The more pronounced inhibition of metabolism, phagocytic function, bactericidal activity of neutrophils was revealed in children who belonged to the group with higher collective dose [1].

There was a wide range of fluctuations in the individual indices of humoral immunity in children of the two dose subgroups. However, children with higher collective dose (the dose subgroup 2) had lower levels of classes IgA and IgM immunoglobulins. Under this dose, the activation type of immune disorders was mainly formed in children of the dose subgroup 1 (12.5%); in children of the dose subgroup 2—depression type of immunological disorders (14.06%) (Fig. 21.9).

Taking into account the significant protective role of IgA regarding respiratory mucous membranes, children and adolescents with low level of IgA, were considered at high risk of respiratory diseases with an appearance of pulmonary pathology, which steady increase was observed in the dynamics of post-accidental period. A background for this was the presence of bronchial hyperreactivity, functional disorders of the pulmonary surfactant system, an intensification of free radical processes and reducing factors of local respiratory protection, that are continued to be detected in children-residents of the radioactively contaminated territories by 18 years of age.

Concerning the indices of peripheral blood, an analysis of longitudinal study results of a large cohort of children showed that the levels of hemoglobin, erythrocytes,

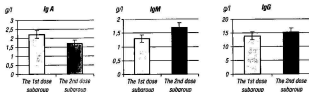


Fig. 21.9. The level of main class of immunoglobulins IgA, IgM, IgG in children of different dose subgroups

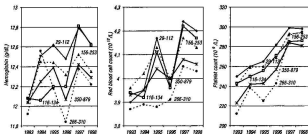


Fig. 21.10. Dynamics of hemoglobin content in erythrocytes and thrombocytes of children who were residents of the territories with different density of contamination

leukocytes and thrombocytes were associated with the ^{137}Cs density in the contaminated areas (Fig. 21.10) [16].

We think that a significant decrease in these indices in 1996 was caused by the withdrawal of measures connected with limited use of local food production.

Cytogenetic studies showed an increased frequency of chromosomal aberrations in lymphocytes of peripheral blood and dependence of cytogenetic effect on radiation dose of the child (Fig. 21.11).

A special attention was focused on the evaluation of the digestive system in the remote space of post-accidental period. It is known that radionuclides, first of all ^{137}Cs , have direct effect on the mucosa of the digestive tract and the parenchymal organs (the liver, the pancreas), their long-term intaking into the organism in combination with deformation of food rations could be one of the leading causes of steady growth in indices of gastrointestinal diseases.

Indeed, a significant increase in the incidence and prevalence of digestive system diseases in children-residents of the contaminated areas and children of the Ukraine population as a whole is observed over the last 15–20 years.

However, basing on the comparative analysis it was shown that children living under conditions of chronic intake of radionuclides into the body through the food chains, had more expressive increase of the incidence and prevalence of digestive diseases. A significant increase of digestive disease indices in children-residents of the radiation contaminated areas was noted at the end of the first year. Beginning from the second five years after the Chernobyl accident,

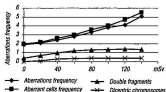


Fig. 21.11. A relationship between at the frequency of chromosomal aberrations in peripheral blood lymphocytes and the total radiation dose.

their level was significantly higher than that of Ukraine pediatric population on the whole, (Fig. 21.12).

Sustainable increase in the incidence and prevalence of digestive diseases in children-residents of the contaminated areas, which rates are still considerably higher than those of the Ukraine in general, indicates the negative effect of chronic intake of radionuclides into the body by food chains.

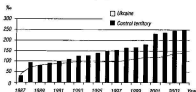


Fig. 21.12. Dynamics of digestive diseases prevalence in children-residents of the contaminated areas and children's population of Ukraine

Dynamic clinical observations showed that pathology of digestive system in 2001–2003, was mostly connected (66.20%) with involving several zones of the digestive tract to pathological process. Chronic gastroduodenitis was a leading form. The hypertrophic pathological changes were prevailed in adolescents evacuated

from the 30-km zone, significant frequency of erosions, hemorrhagic lesions and symptoms of gastro-esophageal reflux were observed too. In adolescents-inhabitants of the contaminated areas, the significant frequency of subatrophic and mixed lesions (22.6% and 26.4%, respectively) was revealed. The hyperplastic (9.3%) and catarrhal changes (8.3%) were less often appeared.

The deterioration of the parameters characterizing structure of the hepatic parenchyma, especially after the abolition of measures limiting use of local food production was observed.

Research of respiratory function did not show significant differences in frequency of bronchial disorders and non respiratory pulmonary functions in children evacuated from the area of "alienation" of the Chernobyl NPP compared with controls in this period. However, children who lived under conditions of chronic intake of radionuclides into the body, had the disorders of ventilatory pulmonary capacity, a decreased pulmonary surfactant production and increased intensity of free radical processes in the lungs that was preserved by 18 years of age [14].

Thus, the results of many year observations suggest that the effect of radiation and non-radiation factors caused the sharp deterioration of health in the children population with a significant reduction of practically healthy persons in the population and increase in the number of children with chronic somatic diseases that are characterized by prolonged, relapsing course with relative resistance to therapy. The most unfavorable changes were seen in children with the irradiated thyroid gland in a dose over 200 cGy. It was established that the most typical were functional disorders of other organs and systems with following transformation into chronic pathological processes in early terms after the accident. This was due to the activation of free-radical processes, an imbalance of pro- and antioxidants in the system, suppression of energy metabolism with a metabolic cofactor deficiency, the presence of autonomic vegetative dysregulation with a prevailed humoral link over that of mediator, disorder of immune homeostasis

and suppression of nonspecific protective factors of the organism, an amplification of mutation process in the somatic cells. Adverse changes in the health were kept in the majority of exposed children throughout all the period of childhood and they enter burdened with multiple chronic diseases into the reproductive age that cannot be safe for the health of their descendants.

(Ye. Stepanova, I. Kolpakov, V. Kondrashova, V. Vdovenko)

21.2 GENETIC AND SOMATIC EFFECTS OF INTRAUTERINE IRRADIATION

In our study the evaluation of prenatal irradiation was carried out in the contingent consisted of 1144 children including three groups: the 1st group — children born from pregnant women at the time of the accident, who were evacuated from Prip'yat; the 2nd group — children born from pregnant women at the time of the accident and left to live in the zones 2 and the 3 of radioactive contamination; the 3rd group — control group — children born in 1986 and lived in the radiation non-contaminated environment

Gestational age that consisted of 0–7 weeks at the time of the accident had 4.3% of the 1st children group and 10.3% — the 2nd group, 8–15 weeks, 9.0% and 29.0%, 16–25 weeks — 41.5% and 25.9% over 25 weeks — 44.3% and 34.8%, respectively (Fig. 21.13).

Dose loading of fetal thyroid in the 1st and the 2nd groups did not significantly differ and ranged from 0.0 to 335.0 cGy. Depending on the average thyroid doses of the fetus were: up to 8 weeks — 0.0; from 8 to 15 weeks — 31.14 cGy, from 16 to 25 weeks — 84.49 cGy, more than 25 weeks — 62.30 cGy. The total equivalent radiation doses of the fetal thymus and the red bone marrow were 10.0–376.0 mSv in 1st group, 4.2–35.9 mSv and 4.7–52.0 mSv, in 2nd group, respectively. In children of 2nd group total equivalent radiation doses of the thymus and the red bone marrow accumulated before 2004 were 14.1–81.7 mSv and 10.5–72.1 mSv, respectively.

The health status of children was evaluated on the basis of complex examination, the health group was established according to these results [17].

The parents' interview with the elucidation of their health status, peculiarities of occupational activity, the presence of bad habits, obstetric and gynecological history, peculiarities of pregnancy and childbirth in the mother, analyses of the medical records of children and their parents were conducted for the detecting antenatal risk factors.

To evaluate the possible mutagenic and teratogenic effects of the environmental factors, including radiation, not only the frequency of congenital defects of development (CDD) and watch phenotypes that can be eliminated in

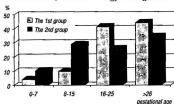


Fig. 21.13. Distribution of children irradiated in utero by gestational age at the time of the Chernobyl accident

the antenatal period were used, but also small abnormalities (SA) — small but persistent disorders of morphogenesis appearing at the early stages of intrauterine development and being not a subject of selection [18].

The protocol designed for accounting SA was included 225 clearly distinguished stigma of dysembryogenesis grouped on the basis of anatomic localization. According to this protocol the phenotypic characteristics of everyone from the 1144 children in the basic and control groups were determined.

Children of basic groups did not differ from the controls by antenatal risk factors (excluding occupational father harmfulness; Table 21.1).

Table 21.1. Frequency of antenatal risk factors in children of basic and control groups (%)

Risk factors	1 st group	2 nd group	3 rd control group
Occupational father hazard	19.2*	3.4	2.8
Bad habits of parents	20.8	21.6	25.1
The presence of a chronic somatic disease:			
– in the father	19.2	17.9	19.4
– in the mother	22.2	19.2	20.5
Late primiparas	12.9	13.4	10.4
Repeated women in child birth older than 35 years	18.5	19.2	20.1
Burden obstetric anamnesis	18.5	26.9	26.4
Complications of pregnancy course	44.9	40.4	41.1
Complications during childbirth	57.4	59.6	61.7
Radiation factor	100.0	100.0	—

*significance of difference with the control group ($p<0.05$)

However, their effect on the fetus in combination with radiation factor has contributed to more frequent formation of morphogenetic variants that were characterized by the presence of multiple small anomalies comparing to the control group. The number of such children in the 1st group as well as in the 2nd group was significantly higher than in the controls (Table 21.2).

Table 21.2. The distribution of children in basic and control groups by the number of small abnormalities (%)

Number of SA	Group I	Group II	Control group
Absent	0.0	4.6*	17.9
Single (1–5)	56.8*	61.8	70.2
Multiple ≥ 6	43.2*	33.6*	11.9

*significance of difference with the control group ($p<0.05$)

A number of SA per child in the 1st group was 5.58 ± 0.26 , in the 2nd group — 5.13 ± 0.32 , in control group — 2.95 ± 0.16 , $p<0.001$.

The most significant differences were observed in the group with locomotor SA, where their average number in children of 1st and 2nd groups two times exceeded the control indices (1.43 ± 0.10 ; 1.22 ± 0.09 ; 0.64 ± 0.06 , $p<0.001$ respectively).

The presence of a direct correlation between the number of SA in child and fetal dose of total radiation ($R=0.61$, $p<0.002$) and reverse ($R=-0.53$, $p<0.003$) — with the age of fetal development at the moment of the effect of radiation factor (Fig. 21.14).

Thus, the largest number of SA was registered in persons exposed to radiation in the terms from 2 to 8 weeks of fetal development ($R=-0.91$, $p<0.0001$).

So, the smaller fetal gestational age was while exposed to radiation factor and the higher dose of this exposure, the greater number of SA was revealed in the child.

A. Sipyagina et al. found a 5,4 times increase of congenital malformations among exposed *in utero* and living in the area of Novozybkivsky district (Bryansk region, Russia) [19].

An increased incidence of congenital malformations observed by Lazyuk G. et al. [20] and stigmata of dysembriogenesis (more than 1.5 times much in comparison with the controls) noted by Lebedev T.V. in the Belarus children after the Chernobyl accident [21].

Taking into account that the phenotype is a genotype function that is realized within the limits of variability of every isolated gene, the growth of mutational loading due to the environmental radioactive contamination is accompanied by the signs of genome instability leading to changes in morphogenetic characteristics that were observed in children irradiated during fetal development.

For analysis of radiation induced chromosomal damages a classical method of continuous (routine) chromosome colouring is widely used [22]. However, this method did not allow to detect all the spectrum of chromosomal damages, therefore the methods of differential G-colouring (G-banding) and *in situ* fluorescent hybridization of metaphase chromosomes with DNA probes (FISH), which have high separate resolution [23] are widely used.

We used a method of the differential G-banding of chromosomes making possible to receive early mitotic chromosomes with a high degree of segmentation (550–1500 in the haploid set) and to determine their structural damages with the identification of break points at the level of segments and subsegments.

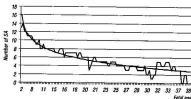


Fig. 21.14. Dependence of small abnormalities number on the fetal age at the time of exposure

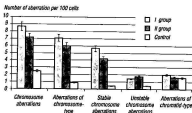


Fig. 21.15. Frequency of chromosomal aberrations in children of basic and control groups

Cytogenetic indices of children in the control group (Fig. 21.15) were in correspondence to the data of modern literature regarding spontaneous levels of chromosomal aberrations in the population and the conclusions of some researchers concerning increase of spontaneous mutagenesis rate for the last 10 years that may be connected with the deterioration of environmental conditions [24].

Over long term after the Chernobyl accident an increase in the frequency of structural chromosomal abnormalities (9.07 ± 1.34 per 100 cells, in the control group — 2.47 ± 0.28 , $p < 0.01$) with a prevalence of stable injuries (5.64 ± 0.94 per 100 cells in control — 0.43 ± 0.19 , $p < 0.05$) and the preferred localization of breaks in the 1, 3, 5, 7, 11, 13, 17 chromosomes was noted in children who were suffered as a result of acute intrauterine irradiation (the 1st group). The most stable-type of aberrations was presented by deletions (4.18 ± 0.71 per 100 cells, in controls — 0.37 ± 0.19 , $p < 0.05$) with a predominance of terminal ones. The translocations and inversions that were registered with approximately equal frequency were observed significantly seldom (mean 0.70 ± 0.16 and 0.75 ± 0.15 per 100 cells, respectively). Unstable chromosomal aberrations (1.43 ± 0.35 per 100 cells, in control — 0.47 ± 0.13 , $p < 0.05$) were presented by acentric fragments (0.66 ± 0.18 per 100 cells), dicentric (0.61 ± 0.15 per 100 cells) and ring chromosomes (0.16 ± 0.05 per 100 cells).

The frequency of chromosomal aberrations in children of the 2nd group was on the average 7.63 ± 2.92 per 100 cells, significantly exceeding the indices of control (2.47 ± 0.28 per 100 cells), $p < 0.05$. There was an increase of stable (4.23 ± 1.06 per 100 cells, in control — 0.43 ± 0.19 , $p < 0.05$) and unstable (1.75 ± 0.25 per 100 cells, in control — 0.47 ± 0.13 , $p < 0.05$) chromosomal aberrations and increased damage of 1, 4, 5, 9, 17, 22 chromosomes. Deletions (3.38 ± 0.87 per 100 cells), mostly terminal, the translocation (0.48 ± 0.14 per 100 cells) and the inversion (0.38 ± 0.06 per 100 cells) were detected among the stable structural reconstruction. Unstable chromosomal aberrations were presented by acentric fragments (1.10 ± 0.19 per 100 cells), dicentrics (0.46 ± 0.09 per 100 cells) and ring chromosomes (0.20 ± 0.08 per 100 cells).

The number of chromatid type aberrations did not differ significantly in children of the 1st (2.00 ± 0.25 per 100 cells) and 2nd group (1.65 ± 0.20 per 100 cells) from control data (1.57 ± 0.28 per 100 cells), $p > 0.05$ (Fig. 21.15).

Chromosome breaks were mainly localized in euchromatin areas where 90% of all the injuries were registered.

The frequency of chromosomal aberrations depended on the dose of red bone marrow irradiation during fetal development. The moderate strength correlation between radiation dose equivalent and the fetal red bone marrow and the number of aberrant cells ($R_0 = 0.51$, $p < 0.02$), chromosome type aberrations ($R_0 = 0.53$, $p < 0.01$), the number of translocations ($R_0 = 0.60$, $p < 0.003$) and deletions ($R_0 = 0.60$, $p < 0.003$) took place in children born to women evacuated from Prip'yat (the 1st group; Fig. 21.16).

The reliable correlations between the total equivalent radiation dose of the red bone marrow accumulated for all the living period at the contaminated territories and the number of aberrant cells ($R_0 = 0.62$, $p < 0.002$), chromosome type aberrations ($R_0 = 0.72$, $p < 0.0003$), stable ($R_0 = 0.64$, $p < 0.003$) and unstable ($R_0 = 0.58$, $p < 0.01$) damage of chromosome apparatus were found in children experienced prolonged exposure to radiation as a factor during fetal development as well as in the subsequent years of life (the 2nd group).

The frequency chroma-tid type aberrations did not depend on loading dose to the red bone marrow.

It is considered that the increased level of chromosomal aberrations in lymphocytes of children living at the contaminated areas can be caused by three mechanisms: a direct hit of radiation quantum/ionizing particle in the nucleus of lymphocyte that circulates in the vascular bed;

the radiation-induced chromosomal instability; intaking into the peripheral blood of a certain part of the aberrant cells derived from irradiated hemopoietic precursors [25].

Mutations in the somatic cells lead to a destabilization of the hereditary structures and formation of genomic instability phenomenon that causes a different disorders of body vital functions, limiting its adaptive capacities, promote the emergence of multi-factorial pathology and increase the risk of carcinogenesis.

One of the most sensitive systems of the body to ionizing radiation is the immune system. According to several studies the changes in functioning different subpopulations of immune cells noted in the mature body under radiation dose 0.01–0.05 Gy and 0.05–0.1 Gy per year is the threshold for the development of non stochastic effects [26, 27].

Taking into account that fetal cells and tissues are characterized by the greatest sensitivity to the radiation effect, the most significant disorders in the development and function of the immune system in postnatal period of ontogenesis in children irradiated *in utero* can be expected.

At present the mechanism inducing congenital disorders of the immune system during fetal development under the influence of adverse factors is proposed. This mechanism is theoretically based on the multidisciplinary comparative analysis of the immune system development in normal embryogenesis and under effect of the harmful factors. In accordance with this, the induced defects of the immune system are realized by embryogenetic homeostasis reactions to change in growth potentialities of cell embryo caused by exposure of harmful agents. According to the proposed theory, these homeostasis reactions receive the paradoxical direction — against the full value development of the immune system, that lead to its defective maturation. The proposed mechanism for the first time explains the widely known phenomenon of “general” vulnerability of the new organism immune system to a significant number of perinatal risk factors [28].

One of such adverse factors is ionizing radiation, that affects all the mechanisms regulating body and causing substantial morphofunctional disorders, especially if its effect is in the critical periods of development.

Increased diversity of proliferative characteristics of embryonic cells is a prerequisite to distort the structure of morphogenic development: the incompatibility of growth

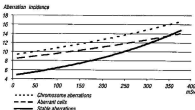


Fig. 21.16. Frequency of chromosomal aberrations depending on the red bone marrow irradiation dose

and formation of various organs and systems in time, including the immune system. Effect of ionizing radiation on the fetus leads to defective maturation, the deterioration of the immune system teaching and the decreased stress stability, that is considered as a system dysontogenetic abnormal immune response [28, 29].

The results of studies showed that an exposure of the central organs of fetal immunogenesis was resulted in postnatal disorders of immune homeostasis. At preschool age the changes in immune status were of an unidirectional nature and were expressed by a moderate suppression of T-cell immunity link, an imbalance of immunoregulatory subpopulations and dysimmunoglobulinemia under acute exposure of central organs of fetal immunogenesis in doses ranging from 10.0 mSv to 376.0 mSv, as well as the prolonged action of ionizing radiation.

An improvement of immunologic test, such as, the use of monoclonal antibodies, that have been using by us since 1994, revealed that not only quantitative changes in the population content of immunocompetent cells were detected, but also the deregulation of immunoregulatory subpopulations with increasing CD4⁺/CD8⁺-ratio mainly by reducing CD8⁺-lymphocyte subsets at primary school age (1994–1995 years) in children of both main groups. There was a clear imbalance of the humoral link, that is characterized by the decreased levels of IgA and IgG, and the increased concentration of IgM.

A functional state of the immune system in children of both main groups was characterized by high variability of the indices, and the amplitude of their fluctuation data significantly exceeded the data of children in control group.

Among variety of immunological abnormalities, we identified three basic types: activated, depressive and undifferentiated types. The first type is characterized by a decrease in CD8⁺, an increased of CD16⁺ and CD22⁺-cells, immunoregulation index, increased levels of IgG and IgM, and it was clinically manifested by allergic syndrome. The second type differed by a decrease in CD3⁺, CD4⁺, CD16⁺, CD22⁺-cell number, the decreased level of IgG and IgM, and had clinical signs of infectious immunodeficiency syndrome. The different deviations of immunological parameters without clearly defined clinical picture were observed in the third type.

At the age of 7 years the frequency of depressive and activated types of immunologic disorders consisted of 26.53% and 23.81% in children of the 1st group and 29.41% and 24.71% — in children of the 2nd group. Their clinical realization in the form of infectious and allergic immune deficiency syndrome was observed in 15.65% and 10.88% children of the 1st group, and in 12.35% and 11.76% children of the 2nd group respectively and was not dependent on the type of immunogenesis organs exposure.

Since 1996, the tendency to improve the immune status was determined in children of the 1st group. In 1999–2004 the majority of immunological parameters and parameters of neutrophil functional activity were approached to the data of children in control group. The frequency of immunological disorders by depressive type decreased up to 17.01%, activation — up to 16.30%, and clinically defined forms of immunological insufficiency in the form of an infectious syndrome was decreased up to 8.16%, allergic — up to 7.48%. However, the functional reserves of neutrophils remained reduced.

The immunological parameters were more slowly normalized in children who lived under adverse environmental conditions, and up to 2004 were received the thymus and the red bone marrow radiation doses 10.5–72.1 mSv and 14.1–81.7 mSv, respectively.

The immunological disorders and clinically defined syndromes of immune deficiency were practically registered with the same frequency. An inhibition of the functional and metabolic neutrophil activity was kept with more pronounced reduction of the functional reserves than in children of the 1st group.

A significant relationship between the health level, the equivalent radiation dose of the thymus, the red bone marrow and the immunological parameters with multiple correlation coefficients 0.74–0.84 was demonstrated. The most pronounced adverse changes in the health status were observed in children exposed at the early stages of fetal development (Fig. 21.17).

An analysis of children's health level in the main and control groups depending on the number of SA showed that practically healthy ones (the I group of health) were as a rule the children of control group without SA (73.1%).

Children with multiple stigmata of dysembryogenesis had the lowest health level. Chronic diseases (the III group of health) were found in 80.0% of children of the 1st group and 59.5% of the 2nd group. Diseases of the gastrointestinal tract, of the respiratory organs, of the cardiovascular system, and the allergic reactions were prevailed in the structure of chronic pathology, that is the greater part of the pathology presented by multifactorial diseases that had irregular heredity, and any mutagenic factor could change the frequency of this pathology manifestation. A moderate correlation between the presence of chronic diseases in children and dose of total radiation ($R=0.45$, $p<0.03$) was established [30].

The thyroid exposure during fetal development significantly affected the health of children. It was shown that chronic somatic pathology more frequently occurred after irradiation of the fetal thyroid in a dose over 0.36 Gy, and in a doses over 1.0 Gy it was registered in nearly all the children (Fig. 21.18) [8].

Irradiation of the fetal thyroid up to 16 week gestation (mean dose 0.31 Gy) formed disharmonious type of physical de-

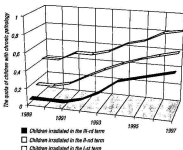


Fig. 21.17. The quota of children with chronic pathology in the dynamics of post-accidental period, depending on gestational age at the time of exposure

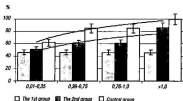


Fig. 21.18. Percentage of children with chronic somatic pathology and the irradiated fetal thyroid at different dose range

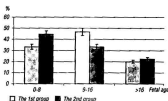


Fig. 21.19. Frequency of disharmonic type of physical development, depending on gestational age at the time of exposure

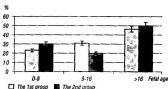


Fig. 21.20. Frequency of pubertal dysplutiarism depending on gestational age at the time of exposure

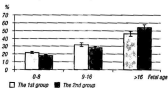


Fig. 21.21. Frequency of increase in thyroid size depending on gestational age at the time of exposure

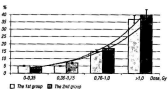


Fig. 21.22. Frequency of disorders in thyroid echostucture in children with its irradiation at different dose range

velopment more frequently than in controls, and after 16 weeks (mean dose 0.85 Gy) the pathological formation of puberty was mainly manifested (Fig. 21.19, 21.20) [8].

An increase of thyroid size was also more often observed in children with intrauterine exposure since 16 weeks of gestation (Fig. 21.21).

An estimation of the thyroid echostucture in children exposed *in utero* showed that the frequency of detecting linear fibrosis elements in all the observation terms exceeded the control data. If the dose of the fetal thyroid exceeded over 0.76 Gy in children, the disorders of its echostucture were significantly more often revealed than in a dose up to 0.36 Gy, as a result of the elements of linear fibrosis (Fig. 21.22).

Thus, the results of our studies indicate that in children exposed to ionizing radiation during intrauterine development, formed the phenomenon of genome instability, that is characterized by morphogenetic variants with multiple small anomalies of development, the increased frequency of chromosomal aberrations in the somatic cells, the decrease in body adaptation possibilities to the action of adverse environmental factors, an increase of the incidence of multifactorial diseases. It has been established that a part of children with chronic diseases was increased more rapidly among children exposed *in utero*. The course of this process was more intense in children exposed in early gestation periods. Proved that The deterioration of health, physical development of phenotypes with multiple minor abnormalities, increasing number of chromosomal aberration

in somatic cells, functional disorder of the immune system and changes in the thyroid echostructure radiation dose are associated with children irradiated during fetal development.

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(Ye. Stepanova, V. Vdovenko, Zh. Misharina)

21.3 PRENATAL IRRADIATION OF THE BRAIN

The developing brain is extremely radiosensitive. Severe mental retardation, Intellectual quotient (IQ) decrease, poor school performance, microcephaly, and seizures have been revealed in Hiroshima and Nagasaki A-bombing survivors exposed to radiation in prenatal period especially at 8–15th and 15–25th gestation weeks. The latest re-appraisal of the dosimetry data showed the 0.06–0.31 Gy dose threshold of a mental retardation after exposure at week 8–15 and 0.28–0.87 Gy at week 16–25 respectively [1]. Despite schizophrenia cases number increased in prenatally exposed to radiation persons who had survived after the Nagasaki A-bombing the issue of an atomic radiation role in this excess remains open [2]. In the meantime schizophrenia risk excess upon prenatal diagnostic X-ray has been found in the Jerusalem cohort [3].

According to the ICRP Publication 84 (2000) 1 Sv prenatal irradiation at a gestation week 8–15 is believed resulting in the IQ decrease for 30 points. Every 100 mSv of prenatal exposure of an embryo or a fetus leads to respective IQ lowering for no more than 3 points. Severe mental retardation excess is estimated as 0.4 per 1 Sv at a gestation week 8–15 and to a less extent at a week 16–25.

Therefore the proven human radioneuroembryological effects are: 1) cognitive deficit i.e. the dose-related intellectual decline down to the severe mental retardation level, 2) microcephaly, 3) seizures. The IQ decrease depends on a cerebrogenesis period when the irradiation occurred. Other potential radioneuroembryological effects (e.g. schizophrenia) require further research.

Prenatal brain damage problem after the Chernobyl catastrophe is utterly debatable. The respective data are summarized in table 21.3. It is clear from the table that cerebral effects had been found almost in all the studies, whereas their radiation origin remains a contradictory issue. At that it must be emphasized that the Japanese and total radiological experience extrapolation to the Chernobyl NPP accident is impossible due to the internal prenatal irradiation by the radioactive iodine in the last case. The UN Chernobyl Forum (2006) experts had recognized the effects on a developing brain among the four priority neuropsychiatric issues of the Chernobyl heritage.

The World Health Organization in 1992–1995 executed the Pilot Project «Brain Damage *in Utero*» within framework of the «International Programme on the Health Effects of the Chernobyl Accident» (IPHECA). Analysis of the data received in Belarus Republic, Russian Federation and Ukraine indicated the following: 1) mild cognitive retardation in prenatally irradiated children exceeds the respective value among the control groups; 2) there was an increasing trend of the emotionally-behavioral disorders incidence in children irradiated *in utero*; 3) prevalence rate of the borderline neuropsychic disorders in parents of the irradiated *in utero* children was higher than in control. However IPHECA was featured by several serious limitations where an absence of individual dosimetry was the leading one. Selection of the exposed and non-exposed study subjects was bases only on the value of soil radioactive contamination intensity at a place of residence irrespective to the individual radiation dose. Therefore an identification of prenatal irradiation role after the Chernobyl catastrophe in the genesis of neuropsychiatric effects in those children remained impossible.

Table 21.3. Summarized principal results of the cerebral effect research in prenatally irradiated survivors of the Chernobyl disaster

Study	Cerebral effect	Radiation effect
International Programme on the Health Effects of the Chernobyl Accident (IPHECA), World Health Organization (WHO), 1992–1995	++	?
Ukraine (Nyagu et al., 1992–2004)	+++	+
Belarus Republic (Igumnov et al., 1994–2010)	++ → ±	—
Russian Federation (Yermolina et al., 1994–1998)	+++	+
USA–Ukraine (Bromet et al., 1998–2010)	±	—
United Nations Scientific Committee on Effects of Atomic Radiation (UNSCEAR), 2000	±	—
French-German Initiative for Chernobyl (FGI), 1998–2004	++	±
Israel (Bar Joseph et al., 2004)	±	—
UN Chernobyl Forum, 2006	+	?
Ukraine (Loganovsky et al., 2005–2011)	++	+
Finland (Huizink et al., 2007, 2008)	++	—
Sweden (Almond et al., 2007)	++	+
Norway (Heliervang et al., 2009–2011)	++	+

A.I. Nyagu and collaborators (1992–2004) had paid high enough attention to the issue of prenatal brain damage as a result of the Chernobyl NPP accident. Their initial reports featured the overestimated incidence of the mental retardation in prenatally irradiated children (up to 77%). It was assumed that thyroid radiation dose *in utero* exceeding 0.3 Gy is a risk factor of neuropsychiatric effects. Further upon the international psychometric methodology application the incidence increase of mild mental retardation, borderline and low IQ, emotional and behavioral disorders was found. Both with that the number decrease of children with high IQ was revealed among the irradiated *in utero*. The radiation-induced dysfunction of pituitary-thyroid system after the 0.3 Gy and more thyroid dose irradiation *in utero* was considered as one among other biological pathways of neuropsychiatric disorders in prenatally exposed children. In conclusion the neuropsychiatric disorders among the prenatally irradiated children were recognized as a consequence of cerebral structure and function alterations resulted from interference of the pre- and postnatal factors against the present radiation effects in a developing brain [4]. The intellectual development disharmony due to the verbal IQ lowering, higher incidence of the electroencephalogram (EEG) low-amplitude and epileptiform patterns with a left-hemisphere lateralization of the dysfunction, EEG delta- and beta-power increase along with alpha- and theta-power decline [5], incidence increase of the paroxysmal and organic mental disorders, somatoform autonomous dysfunction, psychological development abnormalities, both with emotionally-behavioral disorders were revealed in children irradiated in prenatal period after the Chernobyl catastrophe. The prenatal brain damage is considered etiologically heterogeneous [6].

S.A. Igumnov and collaborators (1994–2011) hold their own that sociodemographic and sociocultural factors are the principal sources of mental disorders in prenatally irradiated children. The excessive incidence of the specific speech and emotional disorders

both with lowered general IQ score and increased frequency of a borderline IQ cases among the exposed *in utero* habitants of the Belarus Republic were attributed to the impact of only the social and psychological factors. At that no associations had been found between the prenatal irradiation and decreased IQ as well as with mental health deterioration in children [7]. This stand was entirely supported by the UNSCEAR Report 2000 Annex J experts. They had arrived at a decision that the unfavorable socio-psychological and sociocultural factors had a leading role in the origination of borderline intellectual functioning and emotional disorders among the prenatally irradiated children in Belarus Republic, despite the average IQ score in the most severely irradiated children (thyroid doses *in utero* >1 Gy) was lower vs. all exposed children. The conclusion was reached in the last few years that neurophysiological deviations in prenatally irradiated children in the Belarus Republic had mended and all those persons are not different from the non-exposed control according to EEG data [8].

In the meantime the data were published on intellectual development retardation under the thyroid doses 0.5 Gy and more upon irradiation *in utero* and exposure up to 1.5 years of life [9]. More functional and organic disorders of the central nervous system (CNS), borderline IQ and abnormal EEG explained by the radiation and psychological factors were revealed in children irradiated prenatally especially at gestation weeks 8–15. Higher incidence rate of mental disorders, higher frequency of the borderline intellectual development and mental retardation had been revealed in children irradiated *in utero*. The mentioned phenomena they have associated with just a prenatal exposure [10].

As a result of the prospective American-Ukrainian research and the survey of emigrants to Israel no any signs of a cognitive deficit were found through the applied tests in children exposed to prenatal radiation after the Chernobyl NPP accident [11]. The attention deficit hyperactivity disorder (ADHD) was revealed instead [12]. Noteworthy that evacuated mothers of the prenatally exposed children reported the memory disorders in their children 4 times more often, however no any difference from control was found according to the results of the applied neuropsychological tests and school performance estimates. Alongside with that they have arrived at the conclusion that “Chernobyl anxiety” or mothers’ risk perception was of a decisive role in general and mental health state of the children [13]. It should be emphasized that study authors had no any dosimetry supplementation of the research and have assayed neither verbal intellect nor other functions of the dominant hemisphere (left one in right-handed persons).

The opinion have been offered that the results of “Western” studies indicate to no cerebral disorders in prenatally irradiated persons, whereas “local” research data point out to the cognitive disorder excess in them. Nevertheless the UN Chernobyl Forum experts after all have attributed the effects of a developing brain to the key neuropsychiatric problems of the Chernobyl NPP accident.

In view of the stated topic relevance the thorough study dedicated to the potential cerebral effects of prenatal irradiation was conducted in Ukraine within French-German Initiative for Chernobyl (FGI). The 154 children were examined that were born since April 26, 1986 till February 26, 2007 to mothers evacuated from Pripjat town to Kiev City. The 143 their classmates were involved as a control group members. All pediatric subjects were examined by means of the Wechsler Intelligence Scale for Children (WISC), Achenbach, and Rutter A(2) tests application. The verbal abilities and mental

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United Nations Scientific Committee on Effects of Atomic Radiation (UNSCEAR), 2000	±	—
French-German Initiative for Chernobyl (FGI), 1998–2004	++	±
Israel (Bar Joseph et al., 2004)	±	—
UN Chernobyl Forum, 2006	+	?
Ukraine (Loganovsky et al., 2005–2011)	++	+
Finland (Hutziak et al., 2007, 2008)	++	—
Sweden (Almond et al., 2007)	++	+
Norway (Høivang et al., 2009–2011)	++	+

A.I. Nyagu and collaborators (1992–2004) had paid high enough attention to the issue of prenatal brain damage as a result of the Chernobyl NPP accident. Their initial reports featured the overestimated incidence of the mental retardation in prenatally irradiated children (up to 77%). It was assumed that thyroid radiation dose *in utero* exceeding 0.3 Gy is a risk factor of neuropsychiatric effects. Further upon the international psychometric methodology application the incidence increase of mild mental retardation, borderline and low IQ, emotional and behavioral disorders was found. Both with that the number decrease of children with high IQ was revealed among the irradiated *in utero*. The radiation-induced dysfunction of pituitary-thyroid system after the 0.3 Gy and more thyroid dose irradiation *in utero* was considered as one among other biological pathways of neuropsychiatric disorders in prenatally exposed children. In conclusion the neuropsychiatric disorders among the prenatally irradiated children were recognized as a consequence of cerebral structure and function alterations resulted from interference of the pre- and postnatal factors against the present radiation effects in a developing brain [4]. The intellectual development disharmony due to the verbal IQ lowering, higher incidence of the electroencephalogram (EEG) low-amplitude and epileptiform patterns with a left-hemisphere lateralization of the dysfunction, EEG delta- and beta-power increase along with alpha- and theta-power decline [5], incidence increase of the paroxysmal and organic mental disorders, somatoform autonomous dysfunction, psychological development abnormalities, both with emotionally-behavioral disorders were revealed in children irradiated in prenatal period after the Chernobyl catastrophe. The prenatal brain damage is considered etiologically heterogeneous [6].

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The described above data stand together also with the results of a school performance survey in prenatally exposed persons in Sweden given the radiation contamination levels after the Chernobyl NPP accident. The school performance occurred worse in people that were *in utero* at the time of Chernobyl catastrophe vs. other persons (the most complete database on 562,637 Swedish citizens born in 1983–1988). The poorest educational achievements were in persons exposed at week 9–25 upon conception and born in Swedish regions with most heavy radioactive fallout. Prenatal irradiation therefore alters the cognitive abilities at a level considered before as a safe one [16].

There were statistically lower general and verbal IQ both with more pronounced intellectual disharmony ("asymmetry") due to the verbal IQ decrease in children prenatally exposed after the Chernobyl catastrophe as distinct from children-Kievites (compare group) (see table 21.4). Given the excluded moderate, severe and very severe traditional risk factors it became clear that in prenatally exposed children the named factors are of no effect on a decrease of general and verbal IQ, as well as have no impact on an intellectual disharmony ("asymmetry") due to the verbal IQ decrease [17].

Table 21.4. Intelligence (WISC) of the examined children

Parameter	Prenatally irradiated (n=100)	t	p	Comparison group (n=80)
Complete IQ (M±SD), points	112.2±15.5	-2.7	<0.006	119.1±10.9
Verbal IQ (M±SD), points	105.6±13.6	-4.2	<0.001	115.8±14.3
Non-verbal IQ (M±SD), points	117±18	-0.9	>0.05	119.6±9.4
IQ -asymmetry- (non-verbal IQ — verbal IQ) (M±SD)	11.4±14.2	3.0	<0.003	3.8±14.1

The intelligence of children is a heterogeneous phenomenon defined by the combined effect of mental disorders and nervous system disease if any in a child, economical factors, traditional risk factors, parental educational level (of a father especially), mother's somatic and mental health as well as by prenatal irradiation and stress factors of the Chernobyl catastrophe. The prenatal radiation contribution to the child's intelligence is in average 11% ($p=0.03$). At that the contribution of thyroid radiation dose *in utero* is the highest at the second critical period of cerebrogenesis i.e. week 16–25 at the time of the accident and amounts 20% in the complete IQ ($p=0.04$) and 24% in the verbal one ($p=0.014$).

In a case of the IQ disharmony ("asymmetry") when non-verbal IQ — verbal IQ is >25 points this disharmony goes worth in a statistically reliable dependence on embryo and fetal radiation dose (ICRP-88: (M±SD) 58.6±26 mSv; range: 24.4–116.9 mSv) ($r=0.5$; $p=0.029$; see figure 21.23), as well as on a gestation week at the time of the Chernobyl NPP accident (since week 12: $r=0.6$; $p=0.027$). In prenatally exposed children suffering but more pronounced IQ disharmony (>29 points) the dose-effect relationship becomes substantially stronger. In these cases the IQ disharmony exacerbation is proportional to the embryo and fetal radiation doses and is of a linear correlation pattern (69.5±21.3 mSv; range: 46.6–102.3 mSv; $r=0.97$; $p<0.001$). The power of a relationship between the intelligence disharmony and a gestation week at the time of the Chernobyl NPP accident goes stronger too (since week 14: $r=0.8$; $p=0.035$). The relation of an IQ disharmony in those children on a thyroid radiation dose *in utero* is surveyed too (871.3±330.2 mSv;

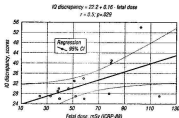


Fig. 21.23. Intelligence disharmony relationship with embryo and fetal radiation doses (ICRP-88) in children prenatally exposed to radiation and suffering an IQ -asymmetry- (non-verbal IQ - verbal IQ) >25 points [17]

range: 421.3–1281.1 mSv $r=0.7$; $p<0.05$) [17].

The intelligence disharmony due to verbal IQ decrease can be considered the radioneuroembryological effect of a prenatal radiation after the nuclear reactor accident. The mentioned effect is surveyed in case of an exposure at week 8 of gestation and later with fetal and thyroid doses *in utero* exceeding 20 mSv and 300 mSv respectively, and in the terms of radiation exposure since week 16 till week 25 with doses >10 mSv and >200 mSv correspondingly.

The EEG theta-range spectral power decrease (especially in the left frontotemporal zone), beta-activity amplification with lateralization to the dominant hemisphere as well as abnormalities of a normal asymmetry of visual evoked potentials and the vertex potential can be considered the neurophysiological markers of a prenatal radiation. The relatively late gestation term (weeks 16–25) is the most critical period for cerebrogenesis under the nuclear reactor accident followed by an intensive environmental release of radioactive iodine compared to the circumstances of a uniform external radiation (weeks 8–15) [17]. The incidence of the organic mental disorders, psychological development abnormalities, paroxysmal states (headache, migraine, and epileptiform syndromes), somatoform autonomous dysfunction, childhood behavioral and emotional disorders was higher in children and adolescents prenatally irradiated after the Chernobyl NPP accident.

The IQ disharmony undergoes an exacerbation along with gestation term that can be explained with the thyroid growth and metabolism intensification. Specific features of radiation exposure after the nuclear reactor accident with an intensive environmental release of radioactive iodine stipulated also the differences in radioneuroembryological risks at cerebrogenesis periods. Weeks 8–15 and 16–25 are the most critical periods of cerebrogenesis that entirely corresponds to radiocerebral effects under purely external exposure as it had been observed after the A-bombings. However after the Chernobyl NPP accident, given the relatively low fetal radiation doses during the most critical period of cerebrogenesis (i.e. gestation weeks 8–15), there were no alterations in neuronal migration. Thyroid size in such a period is small and thyroid metabolism is at a minimal level. It means that the thyroid doses *in utero* and embryo/fetal radiation doses are correspondingly negligible too. At the next critical period of cerebrogenesis (i.e. gestation weeks 16–25) the activation of cellular differentiation and intensification of synaptogenesis occur, cerebral cytoarchitectonics takes shape, the development of main limbic structures both with its pathways is completing as well as cerebral asymmetry and hemisphere dominance appear (ICRP-49). The increase of thyroid mass with thyroid metabolism intensification leads to an *in utero* thyroid radiation dose increase and embryo/fetal dose increase respectively. That is just at the second critical period of cerebro-

genesis (i.e. gestation weeks 16–25) the specific “overlap” of cerebro-genetic criticality occurs as well as the increase of prenatal radiation doses as shown at figure 21.24. Just the abnormalities of neuroembryological events in this cerebrogenetic period can explain the revealed radioneuro-embryological effect of IQ disharmony. They ought to consider that despite the radiosensitivity of a developing brain is lower during further periods of gestation the thyroid and fetal prenatal radiation doses on the contrary increase then resulting in an IQ disharmony also at the later terms of gestation i.e. weeks 26+ [17].

Our initial publications suggested the prevalence of functional disorders of the dominant left hemisphere and especially of its cortico-limbic structures in children irradiated *in utero* after the Chernobyl NPP accident [5]. Later we have concluded that there was an abnormal development of the dominant hemisphere after the prenatal exposure due to the Chernobyl catastrophe. More neuropsychiatric disorders, neurological signs of a left hemisphere involvement, decreased indices of the complete and verbal IQ, the IQ disharmony due to the verbal IQ decrease, disorganized EEG patterns, excess of the EEG delta- and beta-power lateralized to the left fronto-temporal zone along with theta- and alpha-power depression and the interhemispheric inversion of information processing were revealed in the exposed children. Maternal mental health deterioration, stress, prenatal irradiation and routine risk factors are the sources of those effects. The received results should be considered within of a current concern hypothesis on schizophrenia as a cerebral development disorder [17].

Publications in radioneuroembryology field proves the hypothesis of a possible association of prenatal exposure and excessive schizophrenia risk in a further life. Schizophrenia is considered the neurodegenerative disease with possible brain development disorder [18, 19]. Such experimental data have already received the clinical-epidemiological acknowledgement through *inter alia* schizophrenia risk increase after the prenatal diagnostic X-ray in the Jerusalem cohort.

Results of the Norwegian research are especially relevant for an unbiased understanding of the consequences of prenatal brain exposure after the Chernobyl catastrophe especially in the context of schizophrenia risk. The prenatally irradiated adolescents in Norway have the decreased indices of verbal operational memory, verbal learning and memory, information processing speed and executive function vs. non-exposed herd mates. It must be emphasized that study results of the Norwegian authors provide new

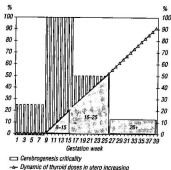


Fig. 21.24. Conventional pattern of relationships between critical stages of cerebrogenesis and dynamic of thyroid doses *in utero* increasing (ICRP-88) in prenatally exposed children as a result of the Chernobyl accident (the square of gray figures is related to brain vulnerability to prenatal exposure by radioactive iodine) [17].

and substantial support to our data on a selective damage to the dominant hemisphere after the prenatal exposure due to the Chernobyl NPP accident [20].

Thus, the radiation damage of human embryo or fetus can therefore be presented with a cognitive deficit and other neuropsychiatric disorders. And these previously unrecognized neuropsychiatric effects can be stipulated by the relatively short-term effect of radioactive fallout levels of which previously had been considered safe. The neuropsychiatric monitoring of people prenatally irradiated or exposed within first years of life after the Chernobyl catastrophe is surely required. These survivors remain under the risk excess of neuropsychiatric disorders including schizophrenia.

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(K. Logunovsky, T. Logunovska, S. Nechayev, O. Napremko, C. Antipchuk, M. Bomko)

21.4 CHILDREN BORN TO RADIATION EXPOSED PARENTS

The consequences of the Chernobyl accident tragically affected the fortunes of millions of people and will affect the health status of subsequent generations.

The statistic data of MPH and the SRU indicate the adverse changes in the health status of children at the age of 0–14, born to radiation exposed individuals (1, 2 and 3 groups of primary registration).

According to these data, there is a significant increase of disease incidence and prevalence in children born to radiation exposed parents (group 4 of primary registration) as well as in child population of the Ukraine at the last 20 years (Fig. 21.25).

However, the increase of these parameters in children of the 4th group of primary registration occurred more rapidly than in the pediatric population of the Ukraine (Table 21.5). The negative tendencies will remain in the near future according to the prognostic rating.

Table 21.5. Growth of diseases prevalence and incidence in affected children and child population of the Ukraine at the postaccidental period

Indices	Average absolute increase		Average growth rate, %	
	Ukraine	affected Children	Ukraine	affected children
Incidence	6.7±10.7	52.3±20.6 *	0.84±1.73	7.03±3.23*
Prevalence of diseases	21.7±20.2	85.8±20.0 *	1.55±1.42	6.30±1.57*

*significant difference between the affected children and children of Ukraine population ($p<0.05$)

A sharp increase of registering certain classes of diseases in affected children regarding child population as a whole in 2007 comparing to 1992 payed attention. For example, endocrine diseases were increased 11.61 times, diseases of the osseomuscular system — 5.34, the digestive system diseases — 5.00, mental and behavioral disorders —

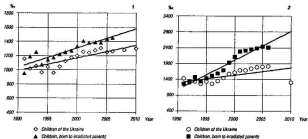


Fig. 21.25. Tendency to disease incidence (1) and prevalence (2) in children born to radiation exposed parents as well as children of the Ukraine

3.83, diseases of circulatory system — 3.75, urogenital system — 3.60 times as much (Table 21.6).

Table 21.6. Indices of disease prevalence by specific classes of diseases in children aged 0–14 years of the 4th group of primary registration (per 1000 persons of corresponding contingent)

Name of disease classes	1992	2007	Increase
Some infectious and parasitic diseases	64.9	69.4	1.07
Neoplasms	2.4	7.3	3.04
Diseases of blood and blood-forming organs	66.1	86.5	1.31
Diseases of the endocrine system	20.4	236.9	11.61
Mental and behavioral disorders	7.8	29.9	3.83
Disorders of the nervous system	79.8	87.0	1.09
Diseases of the circulatory system	15.2	57.0	3.75
Diseases of the respiratory organs	861.6	1070.0	1.24
Diseases of the digestive organs	48.0	240.1	5.00
Diseases of the skin and subcutaneous tissue	70.7	87.6	1.24
Diseases of the osteomuscular system	24.5	130.8	5.34
Diseases of the urogenital system	14.0	50.4	3.60
Congenital defects	16.9	27.6	1.63
All the diseases	1354.0	1879.0	1.39

The results of conducted studies indicate that the processes of adaptation to the environment, were going more intensively in children born to radiation exposed persons beginning from the neonatal period. A numerous group of children who were often ill have been already formed at the first year of life. This group consisted of 49.2–58.7% children at the age under 6–7 years. The immune status of children born to radiation exposed parents was characterized by high frequency of changes in many immunological parameters beyond the physiological variability (75.0–45.7%, in the control 24.2–18.6%), that is the basis for the formation of chronic somatic pathology.

A shortening of specific weight in practically healthy children was shown (from 24.1% in 1992 to 5.8% in 2008). A number of children with chronic diseases was increased (from 21.1% in 1992 to 78.2% in 2008), and the index of pathological lesions consisted of 5.7 in 2008 (Fig. 21.26).

Children, born to parents who took part in the liquidation of the Chernobyl accident consequences in 1986, were required the particular attention among the 4th group of primary registration because they received the highest radiation doses, and also children born to those persons who were exposed to radiation in childhood, as the most significant changes in health status were just recorded in them.

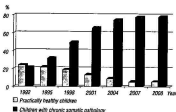


Fig. 21.26. The specific weight of practically healthy children and those with chronic somatic pathology among born to radiation exposed parents in the dynamics of post-accident period

(E. Sirpanova, V. Vdovenko, I. Kolpakov, V. Kondrashova)

21.5 GENETIC EFFECTS OF CHILDREN BORN TO THE CLEAN-UP WORKERS OF CHORNOBYL CATASTROPHE

One of the most complex and controversial problems associated with the action of radiation factor is the evaluation of genetic effects in the first generation of exposed parent descendants. No the conclusive data regarding the increase of congenital defect rate in children born to exposed persons as a result of atomic bombs in Hiroshima and Nagasaki were established according to the results of Japanese authors [1]. The conflicting data were obtained in accordance with the researches of the Chernobyl contingents. The absence of genetic effects in the first generation of exposed parent descendants was indicated in some works [2]. The opposite results were obtained by other authors [3, 4].

There are 13,136 children born to those persons who took part in the liquidation of the Chernobyl accident consequences in 1986, in the SRU, including 1,190 children registered with congenital defects (90.6 per 1000). Their highest frequency was observed in children born at the first post-accident years. Beginning from the time that has been passed after stopping father contact with radiation factor, the number of children with congenital defects was reduced (Fig. 21.27).

The defects of the osteomuscular system took the first place; the second place was occupied by the heart and the vessels diseases; the third — by diseases of the urogenital system; the forth and fifth — by diseases of the digestive tract and neural tube in the structure of the congenital defects [5].

For a more reasonable definition of genetic effects in children whose fathers were participated in the liquidation of the Chernobyl catastrophe, 788 children from 394 families who had a mother, a father and two children, one of which was conceived and

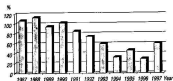


Fig. 21.27. The frequency of congenital defects in children born to the fathers who participated in the liquidation of the Chernobyl accident in 1986.

group consisted of 97 families where parents and children were not exposed to radiation [6].

The established distribution of health groups among siblings of main group was as follows: 13.8% children have the first group of health, 50.8% — the second, and 35.4% — the third, among siblings of the control group these indices were: 15.5%, 57.7% and 26.8%, and among probands — 17.5%, 52.6% and 29.9%. That is, children born before the father's participation in clean-up works, did not differ from children of control group by the level of health.

However, children (probands) who were conceived and born at the post-accidental period in the families of clean-up workers had worse health indices than their older brothers and sisters (siblings), born to pre-accidental period and children of control group. Specific weight of probands with chronic somatic pathology was equal 64.7% in the main group, and a combination of three or more forms of lymphoproliferative pathology (57.3%, in siblings — 23.8%) was more often registered.

The lowest health level was observed in children who were born in 1987–1988 — the number of practically healthy children did not exceed 1.8% and 1.5% among them, and the number of children with chronic somatic pathology consisted of 92.8% and 67.2%, respectively. In contrast, a number of practically healthy children among siblings and probands of control group was not dependent on a year of birth and ranged between 13.6–20.8%.

Table 21.7. Frequency of revealed pathology in siblings and probands who were born to clean-up workers families of the Chernobyl accident consequences

Class by ICD-10	Class name	Siblings	Probands	p
III	Diseases of blood and blood forming organs and some disorders involving the immune mechanism	4.4	13.1	<0.05
IX	Diseases of the circulatory system	4.4	15.5	<0.05
X	Diseases of respiratory organs	34.7	48.8	<0.05
XI	Diseases of the digestive system	23.6	46.1	<0.05
XIII	Diseases of the osteomuscular system and connective tissue	48.5	78.8	<0.05
XIV	Diseases of the urogenital system	10.9	19.9	<0.05

born before the accident, the second — in post-accident period were examined. The main group included 297 families of the Chernobyl accident clean-up workers. The main criterion of family selection for the main group was the fact of ionizing radiation effect on the father in the dose range from 100 to 1000 mSv as a result of his participation in the liquidation of the Chernobyl accident consequences. The control

The diseases of hemopoietic, cardiovascular, respiratory, digestive and osteomuscular systems and diseases of the connective tissue, the urogenital system were more frequently found among the probands of the main group than siblings (Table 21.7).

Thus a father exposure before proband conception increases the risk of development and realization of multifactorial diseases in child. The lowest health indices were noted in children, conceived in the nearest term after the stopping father's contact with the radiation factor.

The clinical and genetic studies have shown that father's exposure contributed to more frequent formation of morphogenetic variations with multiple small anomalies of development in children (Fig. 21.28) [12].

Application of mathematical tools, based on the method of accounting group arguments [7, 8] gave an opportunity to choose the whole complex of classforming features that were characteristic for children born at the post-accidental period in families of the Chernobyl accident clean-up workers, and allow to distinguish them among all the examined patients. With instrumental and software complex for solving numerical assessment of phenotype it was revealed that the morphogenetic variants were characterized by the presence of multiple dysembriogenesis stigmata in majority of siblings, and in 54.2% cases, their number included at least five of SA with the next complex features: an increased extensibility of the skin, teleangiectasias, an increased joint mobility, kyphoscoliotic posture, flat foot, dysplasia of the heart connective tissue structures, abnormalities of the gastrointestinal tract and anomalies in the development of cup-pelvic kidney system.

Among siblings of main group, and siblings and probands of control groups such phenotype was noted no less than in 13.5%, i. e. four times less often than in children born to exposed parents. The probability of correct recognition was 0.8030 [5]. It can be assumed that the so-called adaptophenotype was formed in children born to exposed parents. This term was proposed to identify the real empirically determined repeated discrete complexes of the signs at the different levels of phenotypic polymorphism [9].

As it is known, dysplasia of the connective tissue is the consequence of its disorder in embryonic development and postnatal periods as a result of genetically modified fibrillogenesis in extracellular matrix, leading to homeostasis disorder at the tissue and organism levels in the form of various morphofunctional disorders in the visceral and locomotor organs with progredient course [10]. In our opinion, a higher frequency of "small" connective tissue dysplasia in probands of the main group is a reflection of this process.

Comparison of cytogenetic analysis data showed that the frequency of chromosomal aberrations was higher ($4.15 \pm 0.03\%$) in children born after the Chernobyl accident in families of the Chernobyl accident clean-up workers, than in siblings — ($2.81 \pm 0.04\%$), $p < 0.01$, mainly due to increased damages of the chromosome type — ($3.19 \pm 0.03\%$)

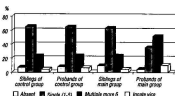


Fig. 21.28. Distribution of children in observed groups by the anomaly presence

and $(1.88 \pm 0.01)\%$, $p < 0.01$, while chromatid aberrations were recorded with the same frequency — $(0.96 \pm 0.13)\%$ and $(0.93 \pm 0.14)\%$, $p > 0.05$. An appearance of the stable chromosomal damage was more often noted in probands than in siblings $(2.83 \pm 0.03)\%$ and $(1.69 \pm 0.04)\%$, $p < 0.05$, respectively. A wide range of different types of cytogenetic abnormalities was observed in children of both groups, but the frequency of proband deletions $(1.88 \pm 0.03)\%$ and translocations $(0.86 \pm 0.03)\%$ was higher than that in siblings — $(1.08 \pm 0.01)\%$ and $(0.59 \pm 0.03)\%$, $p < 0.05$. The positive correlation took place between a number of aberrations detected in the proband and his father ($d = 0.620$) [11].

Molecular and genetic studies using strategy of multilocus DNA-fingerprinting, allowed to reveal the appearance of new bands in probands, that were not observed in either parents, nor siblings born before the father participation in liquidation of the Chernobyl accident consequences [12].

Comparative analysis of individual RAPD-PCR fingerprints and inter-SSR-PCR on DNA matrices in the descendants allowed to reveal an increased (5.6 times) variability of microsatellite associated sequences in the genome of children born at post-accidental period in the families of clean-up workers [12].

Analysis of new band frequency depending on the time that has passed since stopping father's contact with radiation factor before proband conception and father's radiation doses, showed that this dependence was nonlinear and can be described by a model of such form:

$$y = \ln [b_0 \cdot \exp(-\alpha_1 \cdot t) \cdot \exp(-\alpha_2 \cdot D) \cdot \exp(-\alpha_3 \cdot D \cdot t)] \quad (1)$$

where y — the number of new bands; t — time that has passed since the cessation of the father's contact with radiation factor before proband conception (months); D — dose of father's irradiation, mSv; coefficients have the following meanings: $b_0 = 5.11879$, $= 0.0460899$, $= 0.00658849$, $= 0.00001761$.

The coefficient determining R^2 was 0.8435, F-statistics — 19.6574.

Thus, the results of genomic dactyloscopy indicate that father's irradiation in the dose range from 100 mSv to 700 mSv before conception of a child leads to a significant increase in mutation frequency of microsatellite DNA fraction and point to the possibility of transmission into children somatic cells [5].

The most microsatellite locus did not encode proteins, so the majority of mutations did not significantly affect the viability of their carriers. However, it is logical to assume if the number of mutations was increased in one part of the genome as a result of effect, this to some extent can reflect the changes in the whole genetic apparatus.

Radiation disorders induced in the parental germline cells can be detected at different stages of descendant ontogenesis. In postnatal ontogenesis the "small" mutations that are in heterozygous state, cause the destabilization of hereditary structures. Maybe this phenomenon underlies the so-called "physiological inferiority" and reduced viability of exposed parent descendants [13]. The presence of multiple dysmorphias, organ dysplasias, increased frequency of chromosomal aberrations and mutations of microsatellite DNA fraction can be the consequence of genome hereditary instability in the descendants of exposed individuals. All this contributes to the disorder of adaptation processes to living conditions, the increased risk of development and implementation of multifactorial pathology, and reduced health level in children born to exposed parents [3, 14].

(Ye. Stepanova, O. Skvarsha)

21.6 HEALTH STATUS IN FIRST-GENERATION DESCENDANTS OF PERSONS, EXPOSED IN CHILDHOOD AS A RESULT OF THE CHORNOBYL ACCIDENT

For the years that were passed since the Chornobyl catastrophe, children and adolescents exposed to radiation factor, including exposure of the thyroid gland with iodine radioisotopes, and other unfavorable factors of the Chornobyl catastrophe, have reached the reproductive age and became parents themselves. The results of many researchers suggest the profound changes in the health status of this victim contingent, that can be affected on the health of their newborn children.

The examination of children born to the parents evacuated from Prypiat city and 30-km zone of Chornobyl (the 1st group) in childhood; the children-inhabitants of the 2nd and the 3rd zones of radioactive contamination whose parents were children at the time of the accident, were lived and are still living in these populated areas (the 2nd group). The thyroid doses of parents in the 1st group of children were ranged from 0.40 to 2.70 Gy, total irradiation doses — from 8.4 to 271.3 mSv; and from 0.11 to 7.66 Gy, from 4.29 to 44.70 mSv, respectively in the 2nd group. The control group consisted of children born to the individuals of similar age, that were not related to the affected contingents.

Children of both main groups were characterized by the low levels of health, a number of healthy children at school age among them did not exceed 10.0% in accordance with a complex examination and the index of pathological lesions reached 5.39.

Physical development, which is one of the main criteria characterizing health status was disharmoniously different in 62.40–62.58% of children. The frequency of harmonious disorders in physical development was increased owing to children with the body mass deficit relative to the growth in the 1st group, and additionally due to the increased number of children with growth deficiency in the 2nd group.

Nearly a quarter (24.6%) of the children-residents in radiation contaminated areas had disharmony of the physical development connected with a backlog of biological age from passport (Fig. 21.29) [15].

Some features of the phenotype in the main groups of children were revealed by clinical and genetic studies. An increase in a number of small anomalies on average per one child in the main groups compared with controls (in the 1st group — 9.74 ± 0.28 ; in the 2nd group — 9.81 ± 0.19 ; in control — 4.23 ± 0.10) attracted the attention. An increased frequency of morphogenetic variants with multiple dysmorphias among children of main groups, including SA of the musculoskeletal system, the connective tissue and organ dysplasias was shown by the structural analysis of revealed small anomalies [16].

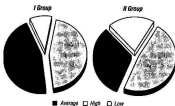


Fig. 21.29. Distribution of children in the main groups by indices of growth

The significant differences of the functional state in the respiratory system were found only in children-residents of the contaminated areas comparing to control. The increased frequency of deviations from the normal indices of bronchial patency, caused by bronchial hyperreactivity was noted. A frequency of bronchial hyperreactivity (total frequency of visible and latent bronchospasm) exceeded their control indices 2.2 times (40.0% and 18.3%, respectively), $p < 0.05$.

The research of non respiratory pulmonary function shown that a decrease in specific pulmonary moisture (SPM) an increase of the superficial intention of a drop hanging (Sldh) in the condensate of expired air (CEA), a decreased content of total lipids (TL) in CEA, the tendency to the phospholipid (PL) decrease, were evidence of the superficial-active substance content in CEA pulmonary surfactant. The index of nonspecific respiratory protection — CEA antitryptic activity (ATA), had the tendency to declining relative index of the control group in the children of the 2nd group and was equal to (4.66 ± 0.69) IU/ml and (5.39 ± 0.78) IU/ml. $p > 0.05$, respectively (Table 21.8) [17].

Table 21.8. Indices of the functional state of respiratory system in children born to persons irradiated in childhood

Indices	the 1 st group	the 2 nd group	control group
Frequency of deviations in pulmonary ventilation capacity	17.5	21.4*	9.1
Frequency of bronchial hyperreactivity, %	27.5	40.0*	21.2
SPM, ml/l	29.7 ± 3.5	$27.3 \pm 2.6^*$	34.6 ± 2.4
CEA Sldh, ml/m	69.02 ± 0.55	$70.16 \pm 0.48^*$	68.54 ± 0.61
TL, mg/ml	3.08 ± 0.61	$2.79 \pm 0.44^*$	4.17 ± 0.53
PL, mg/ml	0.356 ± 0.077	0.303 ± 0.052	0.434 ± 0.056
ATA, IU/ml	5.22 ± 0.71	4.66 ± 0.69	5.39 ± 0.78
S of CEA, imp.	50011 ± 9144	$52964 \pm 7012^*$	30576 ± 5223

*significant differences from controls, $p < 0.05$

Some pronounced changes in CEA initiated chemiluminescence that were indices of the intensification of pulmonary free radical processes had been revealed in the children-residents of the radioactive contaminated territories. Thus, the light sum index of CEA initiated chemiluminescence (S) significantly (1.73 times) exceeded the index of control group, $p < 0.05$. An activation of body free radical processes was also established according to light sum of initiated chemiluminescence of blood serum — $(126,535 \pm 16,018)$ imp. in controls — $(79,322 \pm 10,145)$ imp., $p < 0.05$.

A decrease in the adaptive-compensatory mechanisms was obtained in children of younger and middle school ages of both main groups. An adaptation of the cardiovascular system to environmental conditions was satisfactory in the elder school age. However, it was ensured with the inadequate processes of vegetative regulation of the heart rate, mainly due to humoral channel of regulation under insignificant participation of the parasympathetic vegetative nervous system (active mechanisms of self-regulation). This was confirmed by results of modelling prolonged functional loading, that indicate the dominance of vegetative variant for ensuring activity with the lack of sympathoadrenal system connection, namely, asympathokinetic in the majority of children [18].

The changes in the hepatobiliary system were characterized by high frequency of dystonic and dyskinetic echosymptoms in the biliary tract, the presence of the gall bladder deformities in children of both main groups. A prevalence of pancreatic reactive disorders and parenchymal dysmetabolic processes was observed. Endoscopic semiotics of gastroduodenal damages in the children-inhabitants of the contaminated areas was characterized by more extensive spectrum of mucosal changes owing to increased frequency of disorders of atrophic, erosive, ulcerative and hemorrhagic nature in comparison with children born to persons evacuated in childhood from Pripjat and the 30-km Chernobyl zone.

The unidirectional changes in the immune status were registered in children of two main groups: a decrease in the relative number of $CD3^+56^+$ — lymphocytes, an imbalance of immunoregulatory subpopulations, the decreased level of IgA, a decrease in the number of phagocytes and more higher frequency of deviations of individual parameters beyond the limits of physiological variations (Fig. 21.30). These changes were more pronounced in children of the 2nd group [19].

To test the hypothesis of the probable connection between the presence of child's respiratory pathology of immunodeficiency states and the factors of endogenous and exogenous origin, data of family history and familial social status, negative factors affecting the parents before child birth, including the dose of total irradiation and thyroid dose loading, the correlation analysis using correlation coefficients of Spirmen and Pearson was performed, and also the coefficient of relative risk was calculated.

It was established that the formation of multifactorial pathology in children born to irradiated parents was under the influence of complicated negative factors, the leading of which are burden heredity, unfavorable micro-social environment, numerous medical and biological risk factors of mother and father, some pathological conditions of the child in the infant term, certain features of early child age. A significant correlation between the thyroid dose of mother, a dose of total irradiation of mother and/or father and the development of immunodeficiency states in their children was established. The content of ^{137}Cs in the child body has negative influence on the functional status of broncho-pulmonary system, that caused the increased risk of chronic nonspecific pulmonary diseases [19].

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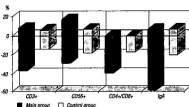


Fig. 21.30. Frequency of deviations of some immunological parameters beyond the physiological fluctuations

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CHAPTER 22 THYROID AND REPRODUCTIVE SYSTEM IN CHILDREN EXPOSED TO IONIZING RADIATION AFTER THE CHORNOBYL NPP ACCIDENT AND IN OFFSPRING OF EXPOSED PARENTS

The highest doses of radiation after thyroid exposure to radioactive iodine in 1986 occurred in pediatric population of several regions of Ukraine including in particular the Prypiat and Chornobyl cities and some other settlements of the 30-km zone and surrounding territory. Radioactive iodine within an entire spectrum of its isotopes with various half-time periods was a principal dose source there. Dose range was rather wide in survivors; there were no dose enough for thyroid destruction [1]. Any radiation doses however are recognized as hazardous from sad mankind evidence in this field [2].

Nowadays hundred thousand of children reside under low radiation dose impact upon the episode of exposure from radioactive iodine after the Chornobyl NPP accident. All that makes an unfavorable impact on the regulation of maturation processes in a child and on the onset and course of some diseases consequently.

High-risk groups of radiation effects from radioiodine exposure to thyroid and other endocrine glands are therefore persons who had received high thyroid radiation doses in childhood or prenatally, as the named life periods are critical for the function establishing of hypothalamic-pituitary-thyroid regulatory system.

Children been exposed to the short-living isotopes of radioactive iodine within first round-the-clock after the accident are the supreme-risk group for thyroid disease.

Endocrine disorders possess an important place within growing morbidity of the Chornobyl NPP accident survivors. Those disorders are represented by thyroid and other endocrine organ radiation effects, both with abnormalities that define, follow or compensate the appeared functional and structural disorders of several organs and systems.

From the evidence of studies of health consequences of past massive population exposure from radioactive iodine in particular the thyroid cancer, nodular goiter and hypothyroidism were an expected thyroid disease [3].

22.1 IMMEDIATE EFFECTS OF THE CHORNOBYL NPP ACCIDENT

Pathways of thyroid disease in children survived from the impact of radioactive iodine after the Chernobyl catastrophe are to some extent represented by an abnormal regulation from hypothalamic-pituitary complex. Thyroid here is significantly dependent on radiosensitivity of the specific circuits of hormonal regulation within loop hypothalamus-hypophysis-thyroid [4]. Ionizing radiation can impact on both direct and reversed links in this system i.e. on pathways tropic hormone — target gland, peripheral gland hormone — respective central endocrine body regulating peripheral function [5]. Function of this complex is critical for the adequate response of organism on all its level of integration and extent of adaptation-protective reactions. That's why it is logical to suppose the hypothalamus-pituitary involvement in thyroid disease onset in remote terms after the radiation exposure.

F prenatal period and early postnatal one are recognized as critical frames of ontogenesis for the radiation damage to thyroid. Duration of postnatal time interval depends on species-specific peculiarities of a final establishing of hypothalamic-pituitary-thyroid system function in systemic regulation [6]. Thyroid exposure in an early postnatal period is followed by a transient hyperfunction that eventually can transform to the hypofunction [4].

Hypothyroidism as one among other effects of pediatric exposure after the Chernobyl NPP accident can also be the result of thyroid autoimmunity [7].

Thyroid parenchyma is slowly proliferating and radiation effects therefore can occur a long time later upon the irradiation.

Benign and malignant neoplasm can be expected as the effects of 0.1–15 Gy radiation dose range exposure. Higher doses can lead to structural abnormalities of thyroid resulting in chronic autoimmune disease and hypothyroidism [8]. Hyperthyroxinemia with no clinical presentation accompanied by a "stress-type" hyperthyrotropinemia followed by restoration of normal TSH-thyroxin interdependencies was the early radiation response to radiation within first year in children survives after the accident. In one year of year and a half the serum thyroxin content had become normal and remained so further [8].

Abnormal serum TSH concentration not compliant with the feedback circuit dependencies indicates to other besides thyroid pathways of accident factors impact on thyroid system. Keeping in mind the study results for some other endocrine pathways in survivors the elevated TSH level in pediatric evacuees from the 30-km zone 7–8 months upon evacuation were interpreted as an abnormal regulation within hypothalamic-pituitary-thyroid system. Later on the average TSH values were not different from normal values against remaining hyperthyroxinemia [8].

Hyperthyroxinemia was typical mainly for the persons having the thyroid radiation doses 2 Gy and more. Average values of thyroxinemia then became normal too. So there was a thyroid system hyperfunction within first years upon the accident that can be regarded on the one hand as a feature of general stress reaction to the accident and on the other as an effect of incorporated radiiodine leading to phase deviations in thyroid function. It means stimulation followed by a functional depression. That is why

thyroid hyperactivation within first year upon the accident can be regarded as at least partly stipulated by radiation.

Severity of hyperthyroxinemia in children within 6 months after the Chernobyl NPP accident was dependent on age i.e. the average serum thyroxin concentration in kids up to 3 years exceeded the normal threshold in 2.8 times, in 4–6 years was 2.7-fold higher whereas in 7–10 years and 11–15 years being in 2.3 and in 2.1 times higher respectively. It is the pattern of age-related thyroid morphological and functional peculiarities (table 22.1).

Table 22.1. Total thyroxine content (nmol/L) in serum of children and adolescents within first year after the ChNPP accident

Age		Time since the ChNPP accident			
		6 months	8 months	10 months	12 months
0–3 years	n	38	49	38	42
	M±m	251.9±11.8	151.2±10.5	109.5±4.0	143.0±12.8
	p	<0.001	<0.01	<0.01	<0.01
4–6 years	n	64	70	83	34
	M±m	245.1±8.5	141.4±8.9	106.3±3.5	137.9±14.2
	p	<0.001	<0.01	<0.05	<0.01
7–10 years	n	124	220	162	253
	M±m	210.2±6.1	131.0±4.0	107.1±2.8	128.6±8.1
	p	<0.001	<0.01	<0.02	<0.01
11–15 years	n	147	214	253	158
	M±m	188.4±5.5	113.2±3.9	95.4±2.0	108.9±4.5
	p	<0.001	<0.01	>0.1	<0.02
Control	M±m	91.3±5.1			

Control is the non-exposed population; p — significance of difference vs. control

Incidence of hyperthyroxinemia in children evacuated from the Prypiat city had decreased within 2–3 years after the accident along with simultaneous normalization of average values. At that thyroxin content was a bit higher in the groups of young and intermediate age children, and hyperthyroxinemia incidence was exceeding one of hypothyroxinemia. Hyperthyroxinemia and hypothyroxinemia incidence was just equal in older children. However the review of individual cases brings to the fact of a remaining hyperthyroxinemia up to 1988 in 18% of youngest children, 23.8% of intermediate age ones and in 6.5% of adolescents. Hypothyroxinemia to that date was found only in 4.5% of youngest children having thyroid radiation doses over 2 Gy, and in 8.6% it was found in adolescents. In parallel the hyperthyroxinemia incidence in kids having thyroid radiation doses up to 0.3 Gy was not different from control (non-exposed persons) with however a significant incidence increase in case of dose over 0.3 Gy (0.3–6 Gy range). Anyway no clear dose dependence at that was found.

Time variations of TSH serum content in pediatric evacuees from the Prypiat city was undulating with its average values being within normal range. Hyperthyroxinemia was only a bit more frequent at the end of second year of survey.

The observed hormonal disorders were not at that time presented as symptoms as no any change of thyroid disease incidence was found in survivors even having the long-term hyperthyroxinemia after more than 0.3 Gy thyroid radiation dose.

In a whole the some kind of "phase-type" pattern of thyroid dysfunction surveyed in 1986–1987 was the reflection of an acute primary response to radiation followed by a pendulous course of pathophysiological processes. The detailed description of the pathways here has remained unstudied. There was an assumption of the partial thyrocyte lysis under radiation otherwise as a result of autoimmune process.

In thyroid dose over 2 Gy the average thyroxin concentration was higher along with dose value reaching maximum in doses exceeding 15 Gy. Again no clinical presentation was found there i.e. no change in thyroid disease incidence occurred [8]. Moreover that excessive thyroxin secretion within a couple of years upon the accident among children and adolescents evacuated from the 30-km zone of Chernobyl NPP or residing in contaminated territories can be regarded as a prolonged autonomous thyrocyte reaction to radiolodine impact not followed by significant disorders of hypothalamic-pituitary activity.

Further multiannual study of thyroid status in pediatric survivors of the Chernobyl NPP accident revealed no significant disorders. Thyroxin and TSH concentrations were within normal range except decreases free thyroxin in 0.8% and elevated TSH in 0.2% again with no any clinical presentation. There were some cases of chronic thyroiditis and hypothyroidism however with no significant increase of their incidence being explained by the effective recovery/restoration processes of growing organism.

Because of no significant manifestations of hypothyroidism in survivors and necessity however to assay the state of hypothalamic-pituitary-thyroid system the thyroliberine challenge test was applied [9]. Medication "Relefact TRH 200" the synthetic thyrotropin-releasing-hormone (TRH) was applied. Single dose was 1 µg per 1 kilogram of body mass. Children having a diffuse non-toxic goiter, chronic thyroiditis or those from risk group of thyroid disease but having no signs of thyroid dysfunction were involved. All children have been evacuated from the 30-km exclusion zone.

Diffuse goiter was featured with thyroid volume elevation with no structural abnormalities in ultrasound scan. Thyroid disease risk was assayed in case of thyroid structure abnormalities regarded as a reactive response i.e. inhomogeneous decrease of tissue echogenicity, hydrophilic inclusions, and few linear or single small echo-positive inclusions. Such abnormalities were peculiar to the children evacuated from Prypiat city.

Thyroliberine challenge test in diffuse non-toxic goiter patients been evacuated from the Prypiat city had revealed the 3–5-fold increase of serum TSH concentration vs. basal values 30 minutes after infusion, 5–6-fold increase in the risk group children and 7–8-fold elevation in chronic thyroiditis (Fig. 22.1).

Excessive increase of TSH concentration is considered the latent hypothyroidism marker. There was a hypersecretory reaction only in 15% of the diffuse goiter patients, whereas in 30% and in 50% it occurred in thyroid disease risk group and chronic thyroiditis patients. Incidence of hypersecretory reaction is to the full extent is dependent on the severity of thyroid injury. Tissue abnormalities are most pronounced in chronic thyroiditis. No significant difference of the TRH-challenge test results was found between the diffuse non-toxic goiter patients and thyroid disease risk group members. Difference was significant between the chronic thyroiditis cases and two other study groups.

Thyroid susceptibility to radioiodine impact is much exaggerated under environmental/alimentary deficiency of stable iodine. Thus similar to the described above challenge test was applied to the children born in 1984–1986 and been residing in radionuclide-contaminated locations of the Rivne region of Ukraine. Soil and natural water there are impoverished for iodine.

TRH-challenge test had resulted in a 5–6-fold increase of TSH content in diffuse goiter and thyroid disease risk group children, and in 9–10-times elevation of TSH in chronic thyroiditis group (Fig. 22.2).

Hypersecretory TSH reaction was found neither in diffuse goiter cases nor in a thyroid disease risk group. In chronic thyroiditis it had occurred in 40% of cases.

Dramatic increase of serum TSH content in chronic thyroiditis group was due to irreversible abnormalities of thyroid parenchyma.

To prove the fact that environmental iodine deficiency can potentiate a hazardous impact of the Chernobyl catastrophe factors the same challenge test of the hypothalamic-pituitary-thyroid functional capacity was applied in children born in 1987–1991 and been resident in Lviv region under an environmental iodine deficiency.

TSH elevation had reached 5–6 times in diffuse non-toxic goiter, 6–7 times in thyroid disease risk group and 8–10 times in chronic thyroiditis cases vs. basal level (Fig. 22.3). Hypersecretory response indicative for a subclinical hypothyroidism was found in 20% of the diffuse non-toxic goiter cases and thyroid disease risk group, whereas in chronic thyroiditis it was found in about 40% of children. Again, the excessive TSH response to TRH administration was the sign of a latent thyroid dysfunction.

In absolute values the TSH response over 9–21 mU/L was typical for thyroid dysfunction in any group of children i.e. diffuse non-toxic goiter, thyroid disease risk group or chronic thyroiditis. In some cases of a chronic thyroiditis the magnitude of TSH elevation was 22.4 mU/L in 30-km zone evacuees, 26.8 mU/L — in an iodine-deficient radionuclide-contaminated territory population and even 34.7 mU/L in purely

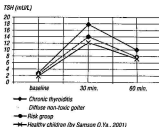


Fig. 22.1 Serum TSH concentration in Chernobyl NPP 30-km zone evacuees after thyrotropin-releasing-hormone administration 1 µg per 1 kilogram of body mass.

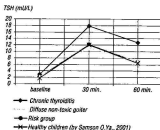


Fig. 22.2 Serum TSH concentration in pediatric population of environmentally iodine-deficient radionuclide-contaminated territories after thyrotropin-releasing-hormone administration 1 µg per 1 kilogram of body mass.

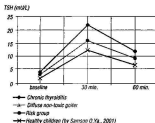


Fig. 22.3. Serum TSH concentration in pediatric population of environmentally iodine-deficient territories after thyrotropin-releasing-hormone administration 1 μ g per 1 kilogram of body mass.

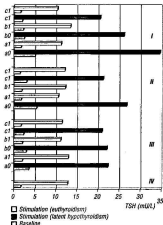


Fig. 22.4. Serum TSH concentration after thyrotropin-releasing-hormone administration 1 μ g per 1 kilogram of body mass in children having thyroid disease. I — environmentally iodine-deficient zone residents (Lviv region), II — environmentally iodine-deficient and ^{137}Cs radioisotope-contaminated zone residents (Rivne region), III — evacuees from the Chernobyl NPP 30-km zone, IV — healthy children; I — euthyroidism; II — latent hypothyroidism; a — chronic thyroiditis; b — diffuse non-toxic goiter, c — thyroid disease risk group

severe environmental iodine deficiency (Fig. 22.4).

Hypersecretory reaction despite the study group was found in subjects from each of them i.e. in 31.7% of the Chernobyl NPP 30-km zone evacuees, in 26.7% of the Rivne region residents (environmental iodine deficiency and soil radioactive contamination) and in 26.7% of subjects in the compare group (Lviv region population, environmental iodine deficiency). Pronounced hypersecretory reaction in latter was probably stipulated by the ethnic features. Children born and living on the radionuclide-contaminated territory are the thyroid disease risk-group members because, among other issues, of latent thyroid deficiency. Long-term and even life-long iodine deficiency leads ultimately do decreased production of thyroid hormones resulting in pituitary hyperproduction of TSH. Thyroid function undergoes adjusting in its turn under TSH impact towards hyperactivation of iodine uptake by thyrocytes and modification of intrathyroid metabolism. Proliferation of thyroid parenchyma leads to increase of thyroid mass and volume with thyrocyte amount elevation and appearing of a non-toxic endemic goiter.

According to [10] the iodine deficiency leads to thyroid depot depletion being a background of thyroid disease. The TRH-challenge test results in children exposed to radiiodine, in those living in ^{137}Cs -contaminated zone under environmental iodine deficiency, and in the inhabitants of purely iodine-deficient territory prove the assumption.

Cases of chronic thyroiditis where a hypersecretory response was found actually do suffer the subclinical hypothyroidism not yet clinically evident because of high compensatory capaci-

ties of pediatric thyroid. However the latent thyroid dysfunction can potentiate the radiation carcinogenesis of thyroid. Just combination of radiation injury and hormonal abnormalities are responsible of thyroid cancer onset [11].

Thyroid parenchyma has a high plasticity providing a pronounced regeneration ability. The formation of new follicles occurs during the entire life a becoming bit slower with age. In case of a partial radiation damage to thyroid tissue the extent of reparation and compensation depends on the size and function of remaining tissue. Dead cells are replaced with a connective tissue and the remaining ones response with hypertrophy and cell division to pituitary TSH, excessively produced under thyroid hormone deficiency. Compensatory hypertrophy at the background of cellular mitotic apparatus injury can also lead to malignant transformation [12].

Hence, the impact of radioactive iodine on thyroid after the Chernobyl NPP accident accompanied by some hazardous amplification factors i.e. environmental iodine deficiency and internal radiation from incorporated cesium isotopes results in:

- decreased synthesis and secretion of thyroid hormones through multiple pathways including the intrathyroid autocrine regulation, that ultimately results in pituitary TSH hyperproduction;
- decreased thyroid depot of a stable iodine;
- strained function of hypothalamic-pituitary-thyroid system (latent thyroid dysfunction);
- amplified thyroid tissue proliferation.

22.2 THYROID IN CHILDREN EVACUATED AND LIVING IN CONTAMINATED TERRITORIES

There were 6,000 children 12–17 years old checked-up for a few years. Among them 1,470 were evacuees from the Chernobyl NPP 30-km zone, 1,332 reside in Chernihiv region, 1,220 lived in Kyiv region, and 1200 — in Rivne one (see above). Original PC software have been applied for the registration of clinically evident cases of thyroid disease and respective risk-group compilation aiming the long-term survey [13]. Native pediatric population of Kyiv city were the compare group ($n=778$).

Thyroid disease i.e. cancer, chronic thyroiditis, hypothyroidism, and nodular goiter were diagnosed in 1.9–7.4% of cases. Thyroid cancer was found in 0.4–0.8% of study subjects, chronic thyroiditis — in 0.6–4.8%, nodular goiter — in 0.6–1.21%, hypothyroidism — in 0.3–0.6%.

Noteworthy the highest incidence of chronic thyroiditis (4.8%) was in Rokytna district of Rivne region. A bit rarer the disease was found in Kyiv region residents. No significant difference at that was found vs. other study groups (Table 22.2) except Kyiv city residents ($p<0.05$).

Revealed discrepancies in a chronic thyroiditis incidence among children living on contaminated territory (Kyiv, Chernihiv, and Rivne regions) and in Kyiv city are almost apparently related to the complex impact of radiation and iodine deficit being most pronounced in Rivne region.

Incidence of a nodular goiter in all the study groups including the control one was not significantly different.

Table 22.2. Incidence (%) of thyroid disease in children evacuated from the ChNPP 30-kilometer zone, residing on variably contaminated territories, and in a compare group (Kyiv city residents)

Disease	Exclusion zone evacuees (n=1470)	Population			
		of Rivne province (n=1200)	of Chernihiv province (n=1332)	of Kiev province (n=1224)	of Kiev city (n=773)
Diffuse non-toxic goiter grade 1a-1b	51.8	65.7	50.2	59.7	48.8
Chronic thyroiditis	3.8	4.8	3.1	4.2	0.6
Nodular goiter	1.0	1.2	0.6	0.8	1.0
Hypothyroidism	0.4	0.6	0.4	0.3	0.3
Cancer	0.4	0.8	0.4	—	—

Thyroid cancer incidence in the Chernobyl NPP 30-km zone evacuees was 4:1000, in Rokytne district residents — 8:1000. No thyroid cancer cases were found in children from Kyiv city and Kyiv region.

Diffuse non-toxic goiter in kids exposed to incorporated radiiodine was diagnosed in 50.2–65.7% of cases. In Kyiv city population (compare group) the thyroid enlargement according to ultrasound scan data was found in 48.8% of cases that can be stipulated by the impact of a range of unfavorable environmental factors.

No significant increase of hypothyroidism incidence in pediatric survivors of the Chernobyl catastrophe accident can be due to the not enough period since the accident. Thyroid hypofunction after radiation impact can appear much later than cancerogenesis [5, 14].

Comparative analysis of study results taking into account the hospital morbidity indices revealed no significant difference in thyroid disease incidence.

Application of the elaborated PC software provided the risk groups compilation aiming the long-term survey. The most plural risk group appeared among the exclusion zone evacuees i.e. 16.8% and 37.5% in years 1995 and 2000 survey respectively. In Chernihiv, Kyiv, and Rivne regions the corresponding values were 10.4% & 14.8%, 10.6% & 18.4%, and 12.4% & 24.5% (Fig. 22.5).

Thyroid ultrasound screening in 1040 children from various population groups,

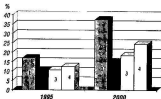


Fig. 22.5 Incidence of thyroid enlargement according to ultrasound scan data in children evacuated from the exclusion zone (1), residing in Chernihiv (2), Rivne (3) regions, and Kyiv city (4) in 1995 and 2000 study periods

namely 30-km zone evacuees (n=290), residents of Chernihiv (n=236) and Rivne (n=250) regions endemic with goiter, and native Kyiv residents (n=264), had shown the increased incidence of thyroid enlargement in all study groups vs. Kyiv city population however with no statistical difference.

Thyroid echostructure abnormalities were seen in 43.3–56% of children who had survived after the Chernobyl NPP accident. In the native Kyiv residents the irregular echostructure were visualized in 15.5% ($p<0.05$).

Age analysis of data received had revealed the most pronounced structural abnormalities in children that were up to 3 years of age at the time of accident and in those exposed to ionizing radiation in prenatal period.

Thyroid echostructure after the exposure to incorporated radioactive iodine isotopes was peculiar with small (3-4 mm) echo-positive and echo-negative inclusions both with fibrotic elements [13].

There was a difference in the incidence of thyroid nodules and cysts between the evacuees, Chernihiv, and Rivne region residents (1.5-2.5-6% respectively) and 0.7% in Kyiv population (table 22.3).

Table 22.3. Incidence of thyroid ultrastructure in children evacuated from the ChNPP exclusion zone, residents of Rivne and Chernihiv provinces and Kiev city.

Indices	30-kilometer zone #EVACUEES	Population		
		Rivne region	Chernihiv region	Kyiv city
Volume – normal	7.2	13.3	16.9	34.0
Volume – increased	92.8	87.7	83.1	66.0
Echogenicity – normal	84.4	68.0	67.3	75.6
– decreased	5.5	8.0	15.2	18.0
– increased	10.1	24.0	17.5	6.4
Echostructure – homogenous	58.7	44.0	49.1	84.5
– inhomogenous	43.3	56.0	50.9	15.5
Nodules	1.5	6.0	2.5	0.7

Ultrasound pattern typical for the chronic thyroiditis i.e. linear echogenous structures was revealed in 12.1% of the survived children having no clinical presentation or hormonal abnormalities, whereas no such abnormalities were found in Kyiv city residents. Appearing of linear echogenous structures are considered the sign of a connective tissue proliferation in thyroid parenchyma, probably as a consequence of radiation and non-radiation factor impact with environmental iodine deficiency [15]. The revealed thyroid echostructure abnormalities can be considered the risk factors of thyroid disease.

Age-related analysis had revealed the more pronounced susceptibility of children the second period of puberty when the thyroid disease actually becomes evident. At that the high enough percent of thyroid disease revealed in children of all the study groups can be due to a complex impact of a range of the unfavorable environmental factors including ones stipulated by the Chernobyl catastrophe and iodine deficiency.

Studies were conducted among pediatric population of the Ukrainian Polissia region (woodlands) [16]. In particular the Rokytne and Dubrovitzia districts of Rivne region are peculiar with a severe non-homogeneity of radiation environment, deficiency of iodine and some other macro- and microelements, both with demographic features i.e. a lot of large families and numerous pediatric population in general. At that the Rokytne district is different with a significantly higher soil contamination with ^{137}Cs .

The proportion of thyroid disease and thyroid disease risk factors is increasing along with age in Rokytne district inhabitants. Thyroid disease was found in 3.4% of children 4-7 years old with a risk group being of 12.6%. In 8-10 years the children suffered thyroid disease in 5.3% and risk group was 22.9%. Incidence in 11-15 years

old ones was 9.5% and risk group had been 19.8%. Thyroid disease incidence among the entire studied population was 7.4% (including 7 cases of thyroid cancer upon the surgery), namely of diffuse goiter — 0.4%, of nodular goiter — 0.2%, of hypothyroidism — 0.4%, of chronic thyroiditis — 4.4%.

Thyroid volume excess over the age threshold in 4–7 years old children was found in 41.8%, in 8–10 years old ones — in 58.5%, in 11–15 years old kids — in 76.2% respectively. Echostructure abnormalities i.e. 3–4 mm long echonegative inclusions and large clusters of hydrophilic ones were most frequent in 8–10 years old children. Thyroid enlargement accompanied by the mentioned above structural abnormalities was peculiar for 11–15 years old group of subjects. Thyroid nodules were found in 1% of cases. Ultrasound pattern of thyroiditis i.e. tissue fibrosis, numerous hydrophilic clusters was found in 22.4% of children. The diagnosis of chronic thyroiditis itself was established in 4.4% of cases. Fibrous elements in thyroid parenchyma specifically the amount of small echopositive inclusions was the highest in children 5–10 years old who were 0–5 years at the time of accident. Elevated serum TSH content was found in 4.5% of cases with no any clinical presentation of hypothyroidism.

The number and proportion of nodular goiter cases among the Rokytne district kids had increased in 1998 up to 2.4% vs. 0.2% in 1996. The risk group had increased by 1.5 times since 1996 and reached 32.2% in 1998.

Further survey had revealed the correlation ($r=0.36$) of thyroid disease incidence and soil contamination density by radionuclides. The specific role of ^{137}Cs as a hazardous environmental pollutant is potentiated by the significantly higher doses of an acute radiation exposure from radioactive iodine in 1986.

Survey of children 4–15 years old from the Dybrovitsa district (is different from Rokytne district with dramatically lower soil ^{137}Cs contamination) had revealed the similar to mentioned above increase of thyroid disease incidence with age. In children 4–6 years old the thyroid disease was found in 7.9% and risk group numbered 15.8%, in 7–10 years old the respective values were 1.2% and 13.9%, in 11–14 years old — 3.7% and 16.3%, and in 15 years — 4.2% and 13.5%. Thyroid disease among all the surveyed children of Dybrovitsa district was found in 2.5% (including a one case of thyroid cancer after surgery). Namely there were 0.9% of a diffuse goiter patients, 0.7% of a nodular goiter ones, 0.5% suffering hypothyroidism, and 1.2% having chronic thyroiditis. Cases of a diffuse non-toxic goiter Grade Ia-Ib with no abnormal tissue structure (55%) were considered as almost healthy subjects. Risk group numbered at that 14.9%.

According to thyroid ultrasound data the volume increase was found in all children aged 4–6 years, in 86.7% of 7–8 years old, and in 87.5%–81.4%–87.1–97% of 9–10, 11–12, 13–14, and 15 years old respectively. In a whole the increased thyroid volume was found in 88.5% of studied persons, thyroid tissue non-homogeneity — in 24%. Tissue echogenicity increase was not found, whereas a decrease was identified in 25.5%. The most pronounced abnormalities of tissue echogenicity were revealed in 6–10 years old children. Small (3–4 mm) echonegative and echopositive inclusions were present most often i.e. in 35.5% and 48.5%. Fibrous elements (linear fibrotic fold) were revealed in 7%, nodules — in 3%. Ultrasound pattern of thyroiditis (thyroid tissue fibrosis with plenty hydrophilic clusters) was found in 34.5% of children. Chronic thyroiditis was diagnosed at that in 3.5%.

Significant difference was found in thyroid disease indices between Rokytne and Dubrovitsa regions of the Rivne region pediatric population groups (table 22.4).

Table 22.4. Incidence of thyroid disease in per cent among children from Rivne region of Ukraine, year 1998 (14)

Diagnosis	Region	
	Rokytne n=751	Dubrovitsa n=363
Goiter Ia-Ib with no structure abnormalities	41.9	54.8
Goiter Ia-Ib with structure abnormalities	35.2	27.9
Chronic thyroiditis	2.1	2.2
Nodular goiter	2.5	1.3

Higher incidence of a goiter with abnormal thyroid structure and of a nodular goiter was found in children living in settlements of territories with highest ^{137}Cs -contamination intensity and highest levels of incorporation ($p<0.05$).

Check-up of the children from Kyiv and Slavutych cities was held in 1998–2003. High proportion of the abnormal thyroid structure and chronic thyroiditis cases (fibrosis, non-homogeneously decreased echogenicity patterns were assayed as signs of chronic inflammation been therefore included here) is noteworthy. Summarized data are shown in table 22.5.

Table 22.5. Thyroid in studied population depending on demography

Population subgroups	1	2	3	4	5	6
n	4421	2804	257	722	245	393
No disease	88.4%	88.3%	79.0%	88.09%	93.1%	92.88%
Simple diffuse goiter	1.72%	2.1%	2.2%	0.69%	0.8%	1.53%
Diffuse goiter with abnormal tissue structure and chronic thyroiditis	9.07%	9.6%	15.8%	19.28%	5.31%*	3.56%*
Hypothyroidism	1.7%	2.0%	3.5%	10.55%	0.8%	1.02%
Nodular goiter	2.65%	2.0%	2.3%	2.91%	0	1.78%

1 — all surveyed children; 2 — native Kiev residents; 3 — Prypiat city evacuees living upon that in Kyiv; 4 — Slavutych city population evacuated elsewhere from contaminated territories; 5 — control group of Kyiv population (migrants elsewhere from the former Soviet Union); 6 — similar control group of the Slavutych city residents;

* significant difference ($p<0.05$) vs. summarized values and indices of the native Kyiv pediatric population.

Less pronounced prevalence of immune-inflammatory disease in the control groups is quite natural because of no impact of radioactive iodine in spring-summer period of 1986. In its turn the high values in Prypiat city evacuees are stipulated by the exposure to the short half-life iodine isotopes resulted in a high dose-rate irradiation. At that the supreme proportion of a chronic thyroiditis cases among all children born after the Chernobyl NPP accident appeared peculiar for the Slavutych population migrated there from contaminated territories. The long-term impact of radiation had led to onset of immune system disorders against the background of primary reaction of thyroid parenchyma on radiation. High proportion of a chronic immune-inflammatory disease is peculiar to the evacuees from Prypiat city exposed to radiation in prenatal period. Significantly lower respective values are in control and among the former inhabitants of contaminated territories being explained by a principal difference in radiation dose-rates.

Thyroid disease risk groups are to be structured according to the radiation/demographic history of a child (dwelling place before and after the accident), state of thyroid (goiter Ia-Ib with structural abnormalities), serum content of a free thyroxine less than 8 nmol/L and TSH content over 3 mU/L both with identified circulating antithyroid antibodies. The latter were assayed in the exclusion zone evacuees (table 22.6).

Table 22.6. TSH, FT₄, and thyroglobulin antibodies in the Chernobyl exclusion zone evacuees 10-15 years after the accident

Parameters	Age groups (years)		
	10-11	12-14	15-17
TSH (mU/L)			
N	57	78	87
Mx±m	2.05±0.18	1.84±0.17	1.88±0.08
Xmax	3.0	3.8	4.8
Xmin	1.0	0.6	0.2
FT₄ (nmol/L)			
N	57	78	87
Mx±m	15.0±1.01	13.6±1.12	12.6±0.84
Xmax	21.0	31.5	31.9
Xmin	8.9	8.3	6.0
Antithyroglobulin antibodies (mg/L)			
n	42	42	59
Mx±m	53.16±19.70	53.22±16.06	71.83±30.85
Xmax	206.3	206.3	1575.0
Xmin	0.0	0.0	0.0

Antithyroglobulin antibodies in a vast majority of children was normal except elevation in 0.5% of cases.

Elevation of TSH serum content was found in 3.6% of children from Rokytné district 15 years after the accident (table 22.7). Serum FT₄ was within a normal range. However under no signs of thyroid dysfunction a general trend to decrease of a FT₄ level was surveyed towards lower threshold limit.

Table 22.7. Thyroid function indices in 14-17 years old adolescents living in Rhine region and having the thyroid disease of thyroid disease risk factors

Study group	n	TSH (mU/L)	FT ₄ (nmol/L)
Chronic thyroiditis	31	2.47±0.24	11.48±0.75
Chronic thyroiditis risk group	57	3.24±0.22	12.02±0.43
Diffuse goiter Grade Ia-Ib	50	1.82±0.19	14.47±0.51

No significant dependence of thyroid hormone content on thyroid disease, simple diffuse goiter Grade Ia-Ib or risk factors presence was found. There was a trend of serum FT₄ decrease along with TSH elevation in 38.7% of chronic thyroiditis cases and in 14% of subjects having thyroid disease risk.

Summarizing the received data one can assume that low incidence of thyroid dysfunction (0.5-0.8%) in children having chronic thyroiditis or the respective risk factors in 1998-2003 period is the reflection of powerful compensation capacities in a child.

So, no significant increase of thyroid dysfunction or any evident thyroid disease except thyroid cancer incidence 15 years upon the Chernobyl NPP accident was found in survived children i.e. evacuees from the exclusion zone or dwellers of contaminated territories been 0-3 years old at the time of accident. It is most probably stipulated by the age peculiarities of children i.e. despite radioactive iodine induce thyroid structure abnormalities the age-related tissue proliferation provides normal gland function. The structural state and function of thyroid and thyrocytes as elementary units was uneven resulting in different radiation doses and polymorphous pattern of radiation injury. Thus the remote effects of radiation related to thyroid dysfunction and hypothyroidism in particular are expected to onset along with growth and maturation of a child or adolescent.

22.3 SOMATOSEXUAL DEVELOPMENT IN PEDIATRIC SURVIVORS OF THE CHORNOBYL NPP ACCIDENT

Exposure to radioactive iodine in a child can lead not only to thyroid disease but also to abnormal rates of physical and sexual development. Fetal growth in prenatal period is genetically determined. After birth and up to a pubertal period the human growth is under control of thyroid hormones, growth hormone and insulin. After puberty onset the role of mentioned hormones decreases whereas body growth appears under the increasing control of sex hormones that accelerate bone differentiation much more than linear growth.

Disorders of hypothalamic-pituitary-gonadal system can lead to pronounced abnormalities of reproductive system. Ionizing radiation effects the cerebral structures including hypothalamus responsible for sexual differentiation of a fetal brain. Clinical presentation of an abnormal sexual development occurs as a rule in puberty.

Testicular damage by ionizing radiation can substantially depend on age and period of puberty. Radiation resistance increases along with cellular differentiation. Therefore reproductive function in males upon exposure to medium radiation doses is not decreasing immediately as spermatozooids remain comparatively intact. Radiation dose of 0.1 Gy can lead to decrease of spermatozoid quantity within 1 year, after what their recovery occurs. Spermatogonia are most radiosensitive. If all A-type spermatogonia are injured the complete sterility occurs soon [11]. Interstitial (Leydig) cells producing androgens are resistant to radiation and remain morphologically unchanged even under exposure to radiation with cumulative doses of 50 Gy [17]. Experimental studies have shown however slowly renovating tissues (gonadal ones in particular) are still susceptible to radiation impact but manifestation of respective abnormalities appears delayed in time due to a specific biological issues [18].

Puberty in girls to the rather extent is defined by ovarian function providing feminine phenotype and establishing of reproductive function. Abnormalities of somato-sexual development can be linked rather than gonadal injury to dysfunction of central regulation of reproductive system, particularly of hypothalamic-pituitary regulation [19].

It is clinically evident that slow physical development in adolescents closely correlates with pubertal retardation.

Environmental deterioration can lead to endocrine disorders including the hypothalamic-pituitary-gonadal system that is capable to make an impact on both physical and sexual development of a child and his health in a whole [20].

Therefore an abnormal thyroid function induced by radiation can lead to disorders of somato-sexual development.

Study of somato-sexual development was held 15 years after the Chernobyl NPP accident in 4500 children 14–16 years old exposed to incorporated radioactive iodine, namely 1400 evacuees from the 30-km exclusion zone, 940 dwellers of Kyiv region, 1200 residents of Rivne region, and 960 native residents of Kyiv city.

Normal physical development in pediatric survivors of the Chernobyl accident depending on a study subgroup was within range 63.1–85% of subjects, short stature was found in 2.5–6.8%, subnanism — in 0.5–1.2%, nanism — in 0.2–1.2%, high stature — in 4.9–19.5%, subgigantism — in 2.8–12.2%, and gigantism — in 0.4–1.54% of cases (Table 22.8).

Table 22.8. Proportion of physical development (%) in children evacuated from the exclusion zone, residents of Rivno, Chernihiv, Kiev provinces and Kiev city as a compare group

Physical development categories	Evacuees from the 30-kilometer zone	Residents			
		Rivno province	Chernihiv province	Kiev province	Kiev city
n	1470	1200	1332	1220	778
Gigantism (>3 σ)	1.5	0.4	—	—	1.54
Subgigantism (>2 σ)	8.1	2.8	6.7	5.2	12.2
High stature (>1 σ)	19.4	6.4	7.6	4.9	19.5
Normal range ($\pm 1 \sigma$)	68.0	82.2	79.8	85.0	3.1
Short stature (<1 σ)	2.5	6.8	5.9	4.9	3.6
Subnanism (<2 σ)	0.5	1.2	—	—	—
Nanism (<3 σ)	—	0.2	1.2	—	—

Increased incidence of a short stature was found in children residing on the contaminated territories vs. evacuees that was stipulated by a detrimental impact of iodine deficiency which consequences are most evident in childhood because of a dietary peculiarities, both with some other unfavorable environmental factors. Increased incidence of a high stature in the 30-kilometer zone evacuees and Kyiv city population was surveyed in 1990th and in 2000–2003. Incidence of growth retardation remained at that stable. Acceleration phenomenon among pediatric population evacuated from the 30-km exclusion zone and among Kyiv city residents can be explained, besides radiation, by urbanization, chemical exposure etc.

Survey of a sexual development in evacuees from the exclusion zone having a range of thyroid disease i.e. chronic thyroiditis, diffuse toxic and non-toxic goiter, nodular goiter had revealed puberty deviations in 44% of them. Namely ovarian-menstrual cycle abnormalities were diagnosed in 12% of girls, pubertal gynecomastia in its turn was found in 14.1% of boys.

Testicular volume decrease was found at a testicular ultrasound examination in about one-third of boys having thyroid disease and pubertal gynecomastia, abnormal testicular location i.e. stable or unstable inguinal retention was revealed in about 4% of them. Abnormal testicular echostructure through vascular dilation, wall thickening and small cysts of epididymidis were found in 17.4% of boys having evident thyroid disease.

Analysis of puberty rate indices had shown a normal index of pubertal maturation in 93% of girls evacuated from the 30-km zone, retardation was found in 6.3%, acceleration — in 0.7% of cases. Normal index was calculated in 96% of boys, retardation — in 4% and no acceleration was found.

In Kyiv region residents the pubertal maturation index was normal in 99.4% of girls with retardation found in 0.6% of cases. Normal index was calculated in 97.3% and retardation — in 2.7% of boys.

Normal index of pubertal maturation was estimated in 97.7% along with retardation or acceleration in 2.3% of Kyiv city dwellers.

Pelvic ultrasound in girls 15–17 years old evacuated from the 30-km zone indicated a slight decrease of uterine and ovarian dimensions vs. normal values. Irregular ovarian echostructure was found at that, namely the “microfollicular” ovary in 18.4% and dense capsule — in 9.4% of girls. Such abnormalities were typical in high-stature girls suffering pubertal-juvenile dyspituitarism (Table 22.9).

Table 22.9. Dimensions in mm of internal genitalia of girls exposed to ionizing radiation prenatally and in the age up to 3 years old

Study group	Uterus			Right ovary		Left ovary	
	Length	Width	Height	Length	Width	Length	Width
Evacuees from 30-km zone	64.60±5.42	41.67±3.07	32.43±3.67	31.52±3.92	17.82±2.78	32.52±2.86	18.47±3.42
Kiev province residents	50.83±5.79	41.46±6.49	29.38±6.22	32.17±5.14	15.83±3.13	31.71±8.09	17.00±5.06
Kiev city population	60.60±5.67	39.40±4.10	34.70±3.72	32.90±3.31	18.16±2.17	34.36±3.46	17.72±2.06

Similar research of internal genitalia in Kyiv city and Kyiv region female children also showed a slight decrease of uterine and ovarian size vs. normal values. Irregular echostructure was found in 12.4% and 15.2%, dense capsule — in 6.4% and 8.1% respectively.

Testicular ultrasonography in boys having thyroid disease had shown the abnormal structure and small size in 9.7% and 12.2% respectively.

No any significant difference was found between the 30-km zone evacuees, Kyiv region and Kyiv city residents.

Peculiarities of a later pubertal phase have been identified in children having thyroid disease. Namely there is an increase of pubertal diencephalic syndrome incidence represented by obesity grade I–II, multiple stretch marks (*striae cutis distensae*), ovarian-menstrual cycle abnormalities in girls, and pubertal gynecomastia in boys.

Study results coincide with data from scientific publications on the point that hypothalamic-pituitary-gonadal dysfunction through gonad size decrease, increased incidence of hypoechotic structures and “microfollicular” ovary is peculiar for children evacuated from the Chernobyl NPP exclusion zone.

Biological age is to the most extent correlating with maturation parameters of a child/adolescent (body length, growth rate, sexual development). Identification of a biological (otherwise “bone”) age by means of hand X-ray is the most recognized

approach in clinical pediatrics. Value of approach is doubtless as it precisely reflects physical state and personal body type features.

Calculation of the growth age index (GAI) indicated the harmonious (concordant) development of children both evacuated from the 30-km zone and native Kyiv residents (Table 22.10).

Table 22.10. Indices of biological age in adolescents exposed and non-exposed to ionizing radiation in prenatal period and age up to 3 years

Indices	Evacuees from the 30-kilometer zone n=51	Kyiv city residents n=50
Growth age index (GAI)	1.01±0.02*	0.97±0.015
Ossification index (OI)	0.96±0.01*	0.82±0.017
Growth ossification index (GOI)	0.98±0.02	0.92±0.01

*significant intergroup difference ($p<0.05$)

Calculation of the ossification index (OI) in children having thyroid exposed and non-exposed to ionizing radiation i.e. evacuees from the 30-km zone and Kyiv city residents indicates to no any abnormalities of skeletal growth and development. Significant intergroup difference of OI however indicates to more grade of skeletal maturation in persons having exposed thyroid vs. those not exposed to ionizing radiation.

22.4 THE THYROID AND THE SEXUAL MATURATION IN CHILDREN BORN FROM PERSONS BEING CHILDREN THEMSELVES AT THE TIME OF CHORNOBYL NPP ACCIDENT

Survey of children born to persons being children themselves at the time of Chernobyl NPP accident had revealed the pubertal diencephalic syndrome in every third child that is stipulated by neuroendocrine dysfunction at the hypothalamic-pituitary-thyroid and hypothalamic-pituitary-gonadal levels. All that results in a prolonged unorthymical stimulation of adenohypophysis and amplified secretion of tropic pituitary hormones. Therefore target organ stimulation occurs resulting in dysregulatory abnormalities of pubertal period and possibly in thyroid disease and disorders of somatic-sexual development.

Thyroid disease possess a key place amongst the radiation-induced health disorders in Chernobyl accident survivors and their children in whom incidence of those diseases numbers 45.3% vs. 28.8% in children of non-exposed parents. Therefore the descendants of parents exposed to radioactive iodine and other radioactive sources especially in a childhood are the risk group of both thyroid disease and disorders of somato-sexual development.

Incidence of body mass excess and obesity in such children is 24%. Disorders of carbohydrate metabolism i.e. glucose intolerance and type 2 diabetes mellitus are pathogenetically stipulated just by insulinresistance being the factor of numerous systemic metabolic disorders as a some kind of "vicious circle" fundamental for metabolic syndrome X.

Pubertal diencephalic syndrome was found in 79.6% of cases, glucose intolerance — in 61.1%, obesity grade I — in 28.7%, grade II — in 45.4%, grade III — in 22.2%, grade IV — in 3.7% of children respectively. Thyroid disease namely diffuse non-toxic

goiter grade Ia was diagnosed in 44.4%, grade Ib — in 25%, chronic thyroiditis — in 11.1% of them. Risk group of chronic thyroiditis numbered 20.4% of children. Abnormal ovarian-menstrual cycle was diagnosed in 16.6% of girls, pubertal gynecomastia — in 32.4% of boys respectively.

Dysfunction of hypothalamic-pituitary-gonadal system was found as the obesity grade I-IV, multiple stretch marks (*striae cutis distensae*) on chest, shoulder, abdomen and hip skin, asymmetry of the arterial blood pressure up to 15 mm Hg. There were complaints on increased appetite (about 50% of cases), fatigue (42%), memory deterioration (22.5%), headache (44.4%), hairloss (11.2%).

Thyroid function tests indicated the abnormal (increased) TSH levels in 17.5% of cases numbering in average 5.12 ± 1.6 mU/L, free thyroxin concentration was at that normal i.e. 12.6 ± 4.2 nmol/L in average.

Biological age acceleration was identified in 18.4% that can be linked to amplified growth hormone secretion, hyperandrogenia and some indirect signs of adaptation/compensatory pathways in a pubertal period.

Deviation of the sexual development rates is a reflection of abnormal hypothalamic regulation of pituitary gonadotropic function, particularly of changed normal ratio between luteinizing and follicle stimulating hormones specially in children and adolescents suffering the pubertal diencephalic syndrome. Such hormonal abnormalities lead to ovarian sclerocystosis with consecutive reproductive disorders.

The held studies in children having disorders of carbohydrate and lipid metabolism diagnosed through the assay of C-peptide, immune-reactive insulin, and leptin had shown the insulinresistance and leptinresistance in 38% of them. Glucose intolerance is pathogenetically stipulated by insulinresistance being a causative factor of a range of systemic metabolic disorders. In obesity it is followed by the leptinresistance. Moreover, obesity in children in 62% of cases is accompanied by the leptinemia at the background of hyperleptinemia (Table 22.11).

Table 22.11. Serum content of immune-reactive insulin, C-peptide, leptin and glucose depending on obesity grade in children born from persons exposed to ionizing radiation in a childhood after the ChNPP accident (M±m)

Parameters	Obesity		
	Grade I (n=55)	Grade II (n=27)	Grade III (n=25)
Immune-reactive insulin, pmol/L	14.8 ± 4.6	12.3 ± 5.8	19.7 ± 6.1
C-peptide, µg/L	1008.6 ± 397.2	1218.4 ± 345.8	$1854.1 \pm 378.0^*$
Leptin, µg/L	14.2 ± 4.6	$19.6 \pm 8.8^*$	$24.2 \pm 9.4^*$

*significance intergroup I & 2, I & 3 difference ($p < 0.05$)

Compare of the immune-reactive insulin and C-peptide indices had led to conclusion that there is a discrepancy in 18.2% of obesity grade I cases, in 15.5% of grade II, and in 20.3% of grade III. In our opinion the matter is that rates of insulin and C-peptide degradation upon split-up of proinsulin molecule are to a much extent variable under the impact of ionizing radiation or after that.

Sexual maturation index calculated according to Tanner's scale. In 88.4% of girls it was within normal range, 7% of them suffered a pubertal retardation, 4.6% were peculiar with acceleration.

Abnormal hypothalamic regulation of pituitary gonadotropic function, namely changes in a normal ratio of luteinizing and follicle stimulating hormones can be considered the main cause of deviation of sexual maturation rates. Menstrual cycle disorders including anovulatory cycles were most frequently found in girls. Possibly it can be explained by a relative hyperestrogenemia due to an amplified conversion of androgens to estrogens in adipocytes, serum progesterone decrease and abnormal secretion of luteinizing hormone. Such events lead to ovarian sclerocystosis followed by reproductive disorders later (Table 22.12).

Table 22.12. Indices of sexual development in children born from parents exposed to ionizing radiation in a childhood after the ChNPP accident (Mism)

Study group	Gender	LH (mIU/mL)	FSH (mIU/mL)	Estradiol (pg/mL)	Testosterone (ng/mL)
Children having obesity with insulinresistance and leptinre- sistance	M	0.62±0.14	1.14±0.21	18.5±1.4	0.43±0.16
	F	1.92±0.12	1.37±0.15		
Children having obesity with neither insulinresistance nor leptinresistance	M	1.43±0.11	1.74±0.14	54.6±6.2*	4.72±0.19*
	F	3.82±0.18*	4.96±0.21*		

*significant intergroup difference of LH ($P<0.05$) in girls; significant intergroup difference of FSH ($P<0.05$) in girls; significant intergroup difference of testosterone ($P<0.05$) in boys.

Uterine and ovarian size decrease was found by means of ultrasound of internal genitalia in respectively 25.9% and 16.6% of girls 12–14 years old been born from persons exposed to ionizing radiation in a childhood after the Chernobyl NPP accident. Irregular ovarian echostructure was noticeable as a "microfollicular" ovary in 14.8% of cases and dense capsule in 9.3% of girls. Extent of revealed structural ovarian abnormalities is not enough to conclude of disease onset, but it is indicative for considering such cases as a risk group of polycystic ovary syndrome. Irregular echostructure through the "microfollicular" ovary pattern was more often in the high-stature girls suffering pubertal diencephalic syndrome.

Conclusion

No significant data on thyroid dysfunction and increased incidence of thyroid disease except thyroid cancer were evident 15 years after the Chernobyl NPP accident for children who were 0–3 years old at that time. Obviously it is related to age peculiarities of the children. Exposure to radioactive iodine certainly leads to structural thyroid abnormalities, but the age proliferation of the organ tissue provides normal function within a rather long time. Taking into account the mankind experience of the past radiation accidents and events the hypothyroidism can be expected in young pediatric survivors within dozens of years upon.

Minimal latent period of thyroid cancer onset is about 5 years, whereas thyroid cancer risk may persist for up to 50 years.

The revealed discrepancies in a chronic thyroiditis incidence in children evacuated from the exclusion zone, living in Rivne, Chernihiv, Kyiv regions and Kyiv city

are almost probably related to an impact of radiation on the background of iodine deficiency.

Radiation after the Chernobyl NPP accident induces the dyshormonal pituitary-thyroid disorders namely the latent thyroid dysfunction. Through the feedback pathway such a dysfunction leads to TSH hyperproduction inducing a thyroid tissue hyperplasia that can ultimately result in a focal tissue growth.

Physical development of children after the thyroid exposure to radioactive iodine is not significantly different from normal range indices.

Formation of a peak bone mass is accelerated in children exposed to high thyroid radiation doses. Concept of a radiation impact on maturation processes is worth an attention as the premature aging can be the consequence here.

Results of ultrasound examination of gonads indicates to the trend of decreased ovarian size and increased incidence of microfollicular ovarian structure in evacuated girls. There is also some decrease of testicular volume and abnormal testicular structure (vascular dilation, wall thickening, small epididymal cysts), both with an abnormal location i.e. stable or unstable inguinal retention in 4% of evacuated boys having an evident thyroid disease. The latter is indicative for an uncoordinated function of the hypothalamic-pituitary-gonadal system.

Survey of the children born from parents exposed to ionizing radiation in a childhood after the Chernobyl NPP accident had revealed some disorders of the central and peripheral pathways of hormonal regulation being clinically evident as obesity, insulinresistance, leptinresistance, abnormal thyroid metabolism, disorders of a menstrual cycle establishing at a background of some decrease of ovarian size and their irregular echostructure all resulting in the dysfunctional disorders being risk factors of female and male infertility.

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CHAPTER 23 GENETIC MONITORING OF CONGENITAL MALFORMATIONS AND THE INHERITED PATHOLOGY

After Chernobyl NPP accident some authors reported on the increased number of cases of birth defects in regions of Ukraine with radiationnuclide contamination. At the same time, some scientists are inclined to refute this fact and prove that the increase of birth defects frequency and inherited pathology takes place both on radiationnuclide contaminated and uncontaminated territories of Ukraine [1]. It is known that alterations in the genetic health of population are conditioned by both direct radiation influence and complex of factors of chemical, biological and social nature. Sometimes the non-radiation factors give maximal negative input in forming of birth defects.

Annually up to 13 thousand children are born with innate and inherited pathology in Ukraine. Its frequency is about 30 per 1000 born alive, the risk of its origin increased 1.3 times for the last decades. Genetic diseases stably occupy the second place in the structure of infant morbidity according to the data of Ministry of Public Health of Ukraine. The genetic monitoring, which is determined as a system of the multicomponent dynamic supervision of frequency and prevalence of innate and inherited diseases, control of factors, which can present a threat for the gene pool of population with simultaneous realization of measures, aimed to diminish genetic load for next generations takes an important place in researches in Ukraine.

In an ideal situation the genetic monitoring of birth defects (BD) must have the detailed and high-quality information about BD, which occurred not only in live-born but also in stillborn, as well as information, about the induced abortions, conducted after the revealing of development defect in foetus. The account of all cases can considerably change the indices of birth defects [2]. In addition, the genetic monitoring includes research of intercommunication of environment contamination and frequency of BD.

Such monitoring projects were conducted in Ukraine, Russia, Belarus and in some regions of the CIS. Different methods for the estimation of frequency of this pathology such as statistical data of health protection agencies, current registration with diagnos-

tics of "model forms", retrospective methods with the analysis of documentation of maternity hospitals, etc. were used by researchers. The range of BD frequency in these investigations was wide enough and varied from 4.2 to 55 per 1000 new-born (1000 n/b) [2–4].

This index oscillates in wide limits: from 15:1000 n/b to above 50:1000. Probably, not only geography of the investigated regions, state of ecology, demographic processes, but also level of social environment, development of society, system of health protection and, in particular, medical-genetic help to the population are important. The frequency of BD goes down in new-born in Japan in spite of permanent urbanization, senescence of population and decline of birth-rate. It is explained largely by development and realization of the programs for prevention and treatment of birth anomalies. The frequency of BD for new-born children as the average presents as 16–20 per 1000 n/b according to the data of the European researchers from France, Spain, Hungary, and Poland, although its expected frequency would be considerably higher — 60:1000 [5–7].

General prevalence of BD in new-born demonstrates the regional differences in Ukraine: 1.9:1000 — in Ternopil, 12–19:1000 — in Lviv, 7.7:1000 — in Simferopol, 11.5:1000 — in Zaporizhzhya, 20.2:1000 — in Mariupol. These differences are explained by the different level of environment contamination in the cities like Zaporizhzhya and Mariupol, where ecological situation approaches to catastrophic [8, 9].

Since 2002, a joint Ukrainian-American research program "Prevention of birth defects" started in the regions of Ukraine, which suffered from the action of factors of the Chernobyl catastrophe. Extraordinarily high frequency of birth defects was shown convincingly for children, whose mothers lived on territory with chronic influence of low doses of radiation. Large-scale researches of preventive action of folic acid were also conducted in relation to the neural tube defects in new-borns.

Materials and methods

The reports of regional centres of medical statistics and medical-genetic centres (form No 49-healthy, approved by the order of the Ministry of Public Health of Ukraine from 16.06.1993, No 141) were analyzed. Annual statistical collections of Department of statistics of Ukraine were used for determining the amount of new-born. The registers, created according to international standards in district medical-genetic consultations served as basic materials.

Frequencies of birth defects [10] were estimated in Kyiv, Zhytomyr, Chernihiv, Volhynia and Rivne regions. In addition, the comparing analysis of this indices on every district of the investigated region were conducted. The environmental analysis in regions was conducted by using the data from the RCRM, State Committee for Statistics of Ukraine, Governmental portal and data of literature [11–17].

In accordance with recommendations of the International monitoring systems for BD, all defects including Down syndrome, anencephaly, spina bifida, encephalocele, hydrocranium, microtia, transposition of large vessels, hypoplasia of the left heart, palate cleavage, total lip cleavage, imperforation of gullet, imperforation of anus, kidneys' underdevelopment or failure development, hypospadias, epispadia, extrophy of urinary bladder, limb reduction defects, diaphragmatocele, omphalocele, gastroschisis were registered.

Environmental influence was estimated by the dose for settlements calculated in dosimetric passportization of Ukraine [12] and analysis of chemical contamination.

23.1 THE BIRTH DEFECTS FREQUENCY ANALYSIS IN REGIONS OF UKRAINE

Volhynia region. Frequency of birth defects was analysed for the period from 1997 to 2006 using statistical materials of regional medical-genetic consultation (Fig. 23.1). The oscillation of mean indices of BD frequency in region within indicated period from 33.13 to 46.03 per 1000 new-born (n/b) is presented on the histogram. There was a gradual increase of frequency of these pathology in region within 1997–2006 with peaks of increases in 1999 and in 2004 (45.50 and 46.03 cases per 1000 n/b respectively). In 2005, a tendency of diminishing of BD frequency among new-born (33.13 per 1000 n/b) was registered. In 2006 frequency of pathology grew to 37.32 per 1000 n/b again.

Within the investigated period, the BD frequency in region considerably (1.52 times) exceeded the midfrequency of BD in Ukraine every year. In peaks 1999 and 2004, BD exceeded the mean indices of Ukraine 1.75 and 1.77 times.

Dynamic of BD frequency by administrative districts of region from 2004 to 2006 revealed a wide differences (Fig. 23.2) with the lowest indices in Turijsky district (20.09 per 1000 n/b) and with the maximal level in Kovel'sky district (56.61 per 1000 n/b).

Two from three radiation contaminated districts, Lubeshivsky, Kamin-Kashyrsky, Manevyrsky are situated in north-eastern part of region, where the ecological situation is unfavorable as a result of chemical contamination. Frequency of BD in these districts did not differ from frequency of BD in other territories of region (Table 23.1).

It should be noted that the indices of these pathologies in the radiologically controlled districts considerably exceed the midfrequency of BD on Ukraine. In addition, megascopic frequency of BD in other districts of region can be predefined by the high level of chemical contamination. Therefore, frequencies of BD in districts with the high level of chemical contamination, and also in ones, where the combined influence of factors of radiation and chemical nature takes place, were analyzed. The results of analysis are presented in the Table 23.2.

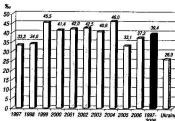


Fig. 23.1. Birth defects frequency in the Volhynia region

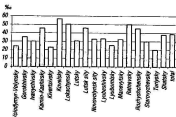


Fig. 23.2. Frequency of birth defects in administrative districts of the Volhynia region.

Table 23.1. The comparative analysis of birth defects frequency in the districts of the Volhynia region

Territories	2004		2005		2006		2004–2006	
	new-born	birth defects absolute per 1000	new-born	birth defects absolute per 1000	new-born	birth defects absolute per 1000	new-born	birth defects absolute per 1000
Radiation contaminated districts, total	2,232	43.01	2,347	45	2,431	106	7,010	267
Lubashivsky	562	38.8	548	16	607	19	1,657	55
Kamini-Kashynsky	954	55.55	954	32	1,008	45	2,916	133
Manevyivsky	776	29.60	845	17	816	35	2,437	79
Other districts, total	9,651	48.73	10,121	348	10,325	370	30,087	1,169
Region, total	11,883	46.03	12,468	413	12,756	476	37,107	1,436

The data hereinabove explained that midfrequency of BD in Volhynia region was very high and considerably exceeded average indices in Ukraine (38.7 vs 26.0 per 1000 n/b respectively) for period of 1997–2006. The analysis of frequency of BD at the districts showed, that this index was higher in the radiologically controlled districts in comparison with relatively ecologically clean districts of region. The greatest level of frequency of BD in Volhynia appeared in chemically contaminated districts of region (46.09 and 30.42 per 1000 n/b respectively). Frequency of BD in districts, where the combined influence of radiation and chemical factors took place, exceeded such observed on radiation contaminated territory without chemical contamination. The high level of BD frequency in Ratnivsky district (50.16 per 1000 n/b), can be conditioned by the unknown ecological factors influence.

Chernihiv region. Frequency of birth defects in Chernihiv region was analysed for period from 1997 to 2006 using statistical materials of regional medical-genetic consultation. The data presented in Table 23.3 and on Fig. 23.3 demonstrate the increase of BD frequency among new-born in the Chernihiv region for period 1997–2001. In 2001, 41.3 cases of birth of children with birth defects per 1000 new-born took place, 1.66 more than in 1997. A gradual decline of frequency of these pathologies to 21.9 cases per 1000 new-born was registered from 2002 through 2006. The obtained data were based on the average indices of values of the investigated pathologies for the whole region.

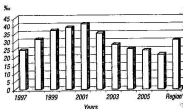


Fig. 23.3. Frequency of birth defects in the Chernihiv region.

Table 23.2. Frequency of birth defects in the districts of Volhynia region with different ecological state

Territories		Number of		Birth defects frequency per 1000 n/b
		new-born	birth defects	
District of radiological control	Manevytsky	2,437	79	32.42
Districts with the high level of chemical contamination	total	16,187	746	46.09
	Kovelsky	3,886	220	56.61
	Lutsky	6,806	312	45.84
	Novorolynsk city	1,681	55	32.72
	Rozhyschensky	1,393	63	45.23
	Gorochivsky	1,706	60	35.17
	Lokachynsky	715	36	50.35
Districts with the high level of radiation and chemical contamination	total	4,573	188	41.11
	Kamin-Kashyrsky	2,916	133	45.61
	Lubashivsky	1,657	55	33.19
Other districts	total	12,917	393	30.42
	Volodymyr-Volynsky	2,091	51	24.39
	Kivertivsky	2,390	55	23.01
	Lutsky	2,189	67	30.61
	Lubomytsky	1,540	39	25.32
	Staromyzhivsky	1,033	31	30.01
	Turijsky	896	18	20.09
	Shatsky	605	23	38.02
	Ratnivsky	2,173	109	50.16
Total for Volhynia region		37,107	1,436	38.70

Table 23.3. Frequency of birth defects in the Chernihiv region

Parameter	Years									
	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
New-born	11,035	10,442	9,582	8,957	8,818	8,451	8,684	8,853	8,922	8,826
Live-born	10,646	10,369	9,522	8,908	8,567	8,415	8,449	8,813	8,646	8,791
Still-born	66	73	60	48	44	36	34	40	32	35
Died to 7th day	51	43	40	32	34	25	18	12	33	—
Have BD*	275	329	354	290	313	300	246	226	219	193
Frequency of BD among new-born	1:40	1:32	1:27	1:31	1:28	1:28	1:35	1:39	1:40	1:45
Amount of BD per 1000 births	24.80	31.60	37.26	39.00	41.30	35.50	28.30	25.50	24.50	21.90

*BD birth defects

Frequencies of BD from 2003 to 2006 in every district of region were analyzed to find possible regional differences in their distribution and relation to ecology. Data by districts are presented in the Table 23.5. Generalized data of BD frequency are shown in the Table 23.6 and Fig. 23.4.

Table 23.4. Frequency of birth defects in the districts of the Chernobyl region

Territories	2003			2004			2005			2006		
	new-born	birth defects		new-born	birth defects		new-born	birth defects		new-born	birth defects	
		absolute	per 1000		absolute	per 1000		absolute	per 1000		absolute	per 1000
Chernobylsky	3,171	108	34.06	3,552	87	27.31	3,614	100	27.67	3,696	85	22.99
Bachmachsky	255	9	35.29	254	4	15.75	234	2	8.54	244	1	4.09
Borovytsky	217	8	36.86	270	10	37.03	231	6	25.97	245	6	24.49
Borzhansky	243	10	41.15	210	8	38.09	209	10	47.84	194	4	20.61
Vanytsky	111	6	54.05	119	2	16.81	86	4	46.51	105	1	9.52
Carodniansky	178	5	28.09	156	5	32.05	189	2	10.58	155	0	0
Irzhynsky	217	1	4.61	183	0	0	204	7	34.31	151	2	13.25
Kozelsky	337	3	8.90	336	1	2.98	306	5	16.34	333	7	21.02
Koropsky	180	6	33.33	173	7	40.46	144	3	20.83	93	1	10.75
Korlukovsky	280	11	42.31	251	8	31.87	234	4	17.09	267	7	33.82
Kulykovsky	133	2	15.04	84	6	71.43	89	3	33.71	60	3	50.00
Mansky	246	9	36.59	238	7	29.41	198	3	15.15	221	5	22.62
N. Shvetsky	235	5	21.28	231	6	25.97	284	4	15.15	278	4	14.49
Nizhynsky	902	21	23.28	995	32	32.16	946	31	32.77	941	28	29.76
Nosivsky	197	5	25.38	232	6	25.86	280	15	57.69	229	9	39.30
Pryluksky	878	17	19.36	830	22	26.51	846	30	35.46	808	22	27.23
Ripkynsky	121	7	57.85	106	4	37.73	108	7	64.81	72	4	55.56
Semenivsky	155	0	0	142	3	21.26	154	4	25.97	124	0	0
Talativsky	118	3	25.42	105	1	9.52	84	0	0	73	0	0
Schorsky	136	7	51.47	165	4	24.24	104	5	48.54	154	4	25.97
Sosnytsky	139	1	7.19	136	7	51.47	111	1	9.01	91	0	0
Shchutinsky	78	1	12.82	64	0	0	88	1	11.36	82	1	12.19

Table 23.5. Frequency of BD in the districts of the Chernihiv region on average for 2003–2006.

No	Districts	New-born total	BD	Frequency of BD per 1000 n/b
1	Chernihivsky	14,033	390	27.82
2	Bachmachsky	987	16	16.21
3	Borovytsky	979	30	30.64
4	Borzniansky	856	22	25.70
5	Varvynsky	421	13	30.88
6	Gorodniansky	658	12	18.24
7	Itchniansky	585	10	17.09
8	Kozeletsky	1,311	16	12.20
9	Koropsky	590	17	28.81
10	Koriukovsky	932	30	31.51
11	Kulykovsky	366	14	38.25
12	Mensky	903	24	26.58
13	N. Shvetsky	1,006	19	18.89
14	Nizhynsky	3,765	112	29.75
15	Nosivsky	918	35	38.13
16	Prylutsky	3,362	91	27.10
17	Ripkynsky	407	22	54.05
18	Semenivsky	575	7	12.17
19	Talalajivsky	380	4	10.53
20	Schorshy	589	20	35.78
21	Sosnytsky	477	9	18.87
22	Sribniansky	213	3	14.08

Data presented show a wide difference on frequency of BD in every separate district for investigated period.

The least frequency of BD for this period was registered in Talalajevsky district (10.53 per 1000 n/b), and the highest one was in Ripkynsky district (54.05 per 1000 n/b). Such level was not the consequence of some sharp increase in one of the investigated years, it stably remained high enough during 2003–2006 and almost two times exceeded the average level for region. In 2005, this index increased to 64.8 per 1000 n/b, that was also the highest index among all districts for investigated period.

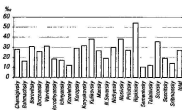


Fig. 23.4. Frequency of birth defects in the districts of the Chernihiv region on average for 2003–2006.

The frequency of BD was compared among the urban population having usually higher indices [19], and other population of district in the Chernihivsky and Prylutsky districts (Table 23.6), as the Chernihiv and Pryluky are industrial foci bringing the most for regions' atmospheric air contamination (31.2% and 10.3 % of all extrass in atmospheric air respectively).

Table 23.6. Frequency of birth defects in the urban and other settlements of Chernihivsky and Prylutsky districts

Years	Indices	Chernihivsky district			Prylutsky district		
		Chernihiv city	rural settlements	total	Pryluky city	rural settlements	total
2003	new-born	259	580	3171	514	364	878
	absolute	101	7	108	11	6	17
	per 1000	38.98	12.08	34.06	21.40	16.48	19.36
2004	new-born	2,481	671	3,552	500	330	830
	absolute	90	7	97	16	6	22
	per 1000	31.24	10.43	27.31	32.00	18.18	26.51
2005	new-born	2,958	656	3,614	510	336	846
	absolute	92	8	100	13	17	30
	per 1000	31.10	12.20	27.67	25.49	50.59	35.48
2006	new-born	3,347	349	3,696	466	342	808
	absolute	82	3	85	16	8	22
	per 1000	24.45	8.60	22.90	34.33	17.54	27.23
Total 2003–2006	new-born	11,777	2,256	14,033	1,990	1,372	3,362
	absolute	365	25	390	56	35	91
	per 1000	30.99	11.08	27.82	28.14	25.51	27.10

Higher frequencies of BD both in Chernihiv and in Pryluky than for other population in the districts are shown in the Table 23.6. In Chernihiv city it exceeded 2.8 time the level of other population of district and 1.7 times the mean value for region.

In Pryluky on the average for period of 2003–2006 the frequency of BD also was more than for other population of district, though not so large, as in the Chernihiv.

At the same time, the dynamics of frequency of BD among new-born in Pryluky and other settlements of district for this period showed that such situation is conditioned by the sharp increase of BD frequency in the Prylutsky district in 2005 year, that was equal to 50.59 per 1000 n/b. In other years, the frequency of BD among new-born in Pryluky approximately 2 times exceeded this index among new-born in the district. Although it was shown on the example of the Chernihivsky and Prylutsky district that frequency of BD among new-born in cities which were district foci was high than among other population of these districts, the high level of BD frequency in the Prylutsky district (except for Pryluky) in 2005 year and extraordinarily high level of the pathology in Ripkynsky district specify that for this there were other etiologic reasons than the difference of industrial contamination between city and rural locality.

The contamination by radiation as one of possible factors of BD frequency increase was not the same in different districts of Chernihiv region. The districts which have

radiation contaminant as a result of Chernobyl NPP accident are situated in western and north-eastern parts of region, and have different radioecological state. Similar situation is about chemical contamination. To find the possible influence of these factors on frequency of birth anomalies, the analysis of frequency of BD was conducted in the radioecologically controlled districts in comparison with other districts.

There are seven radioecologically controlled districts in the Chernihiv region. Passported districts in western part of region include Ripkynsky, Chernihivsky, Kozeletsky, and in north-eastern part include Novgorod-Siversky, Semenivsky, Koriukivsky, Sosnytsky districts.

Even somehow lower frequency of BD among new-born on the average on all radiologically controlled territory (26.28 patients per 1000 n/b) in comparison with other districts of region (26.97 patients per 1000 n/b) is shown in the Table 23.7.

Table 23.7. Frequency of birth defects in the radiation contaminated and uncontaminated districts of Chernihiv region

Territories	New-born number	Birth defects	
		absolute	per 1000 n/b
Radiation contaminated districts, including:	18,761	493	26.28
– western districts	15,751	428	27.17
– north-eastern districts	3,010	65	21.60
Uncontaminated districts	16,019	432	26.97
Region in total	34,780	925	26.61

Although in the passported districts in western part of the region, which are situated closer to the epicentre of Chernobyl NNP, frequency of BD (27.17 per 1000 n/b) was higher than in uncontaminated districts (26.97 per 1000 n/b) and for the region as a whole (26.6 per 1000 n/b). Exactly in this part is situated Ripkynsky district which have the highest frequency of BD for region. Frequency of BD in the Chernihivsky district also exceeds average indices for region. The lower than average for region index of frequency BD took place only in Kozeletsky district.

From 120 settlements of Ripkynsky district 71 were with known level of radiation contamination. The soil contents of ^{137}Cs were from 185 kBq/m² to 370 kBq/m² for period of 2003–2006 only in the district center Ripky, levels from 37 kBq/m² to 185 kBq/m² were found in 16 settlements, at levels below 37 kBq/m² — in all other passported inhabited localities.

In the Chernihivsky district there were 128 settlements, of which 44 were with known level of radiation contamination. The soil contents of ^{137}Cs were above 370 kBq/m² for period 2003–2006 was in only one village Malijky, levels from 37 kBq/m² to 185 kBq/m² were characteristic to 19 settlements and levels below 37 kBq/m² were found in all other passported settlements.

Direct dependence between the level of ^{137}Cs soil contamination and frequency of BD in these districts was absent. At the same time these indices were higher in districts with higher BD frequency (Chernihivsky and Ripkynsky) than in Kozeletsky district, where BD frequency was less than average for the region.

There are technical unsatisfactory depositories with radiation contaminated wastes in Ripkynsky district which follows the special attention.

Although during 2001–2006, a general tendency to radiation contamination diminishing was observed, but exactly Ripkynsky and Chernihivsky districts were most radiation contaminated according to data of the dosimetric passportization system in 1992 and 1996. Thus, it is possible to assume, that influence of radiogenic factor on frequency of BD carries was not linear, but had complicated character and prolonged influence on frequency of BD remote consequences.

Direct dependence on the certain type of contamination was not observed for BD. However, in the district of Bachmach, where a critical situation was with contamination of aquatic resources, frequency of BD was below than average for regions. Only in those districts, where considerable multivariable chemical contamination of atmospheric air, soils, water were, the increased frequency of BD was marked. Especially it touched districts, where combination of chemical and radiation contamination was present.

The data hereinabove explained a tendency to the increase of frequency of birth defects among new-born in region from 24.9 to 41.3 case per 1000 n/b for period from 1997 to 2001, and from 2002 where was the gradual diminishing of frequency of BD among new-born to 21.9 cases per 1000. There was a permanent increase of infant mortality rate as a result of birth defects (included abortions by medical reasons) from 1997 to 2006 in region. The higher frequency of BD among new-born in cities which are district foci (and also industrial foci of region) in comparison with new-born in rural settlements of district was shown districts of the Chernihivsky and Prylutsky districts.

Considerable districts' differences in distribution of BD frequency (from 10.53 to 54.05 cases per 1000 n/b) within period from 2003 to 2006 was found in the Chernihiv region. It was shown that frequency of BD in the radiologically controlled districts of the Chernihiv region (taking into account midfrequency for period from 2003 to 2006) did not differ from frequency of these pathologies in the districts of region, which were not subjects of radiological control. Frequency of BD exceeded the mean values of these pathologies for region in districts with increased level of chemical contamination (in Schorsky this index is equal 35.78, in Ripkynsky — 54.0, in Koriukivsky — 31.5, in Chernihiv city — 31.0, in Varvynsky — 30.9), but direct dependence between the certain type of contamination and frequency of BD was not found. At the same time, the high frequencies of BD in districts with combination of high levels of chemical and radiation contamination (54.0 for Ripkynsky and 31.5 for Koriukivsky) were shown.

Rivne region. Frequency of birth defects in Rivne region was analysed for period from 1997 to 2006 using statistical materials of regional medical-genetic consultation. The average indices of BD frequency oscillated from 31.7 to 50.2 per 1000 n/b. For period from 1997 to 2001 the frequency of these pathologies in region was approximately the same (oscillation within the limits of 28.4–33.8 per 1000 n/b), however with exceeding of midfrequency of BD in Ukraine (26.0). From 2002 to 2004 there was a considerable increase of BD frequency in region, the increase peak took place on 2004 (50.2 patients per 1000 n/b). In next years there was a tendency to diminishing of frequency of birth defects among new-born (37.7; 35.0 and 36.7 per 1000 n/b), but it remained considerably higher in comparison with the whole Ukraine and with region for period from 1997 to 2001 [20] (Fig. 23.5).

It is important to notice that every year frequency of BD in the Rivne region considerably exceeded the midfrequency of BD in Ukraine at the investigated period. So, midfrequency of BD for region 1.41 times exceeded the mean value of this index in Ukraine (36.7 and 26.0 per 1000 n/b respectively) for period 1997–2006. But in 2004 year it exceeded the mean value of frequency of BD in Ukraine 1.93 times.

Frequencies of BD in every district of region separately were analyzed to find out possible regional differences (Table 23.8).

Table 23.8. Indices of birth defects frequency in the administrative districts of the Rivne region

District	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
Bereznivsky	54.7	63.3	48.7	41.3	40.2	38.4	57.8	77.6	67.0	51.9
Volodymyrsky	11.9	10.0	15.9	18.6	16.3	16.6	17.4	18.0	33.4	46.7
Goschansky	20.0	13.4	15.4	52.5	50.3	54.3	41.8	56.5	44.3	67.9
Dubensky	34.4	23.3	25.6	51.8	40.9	43.0	30.0	34.0	29.8	23.9
Dubrovysky	72.8	88.9	77.4	40.5	54.7	59.8	68.4	86.3	65.0	63.7
Domydivsky	14.8	14.3	5.7	31.9	51.4	47.1	75.8	107.0	24.7	40.9
Zaritchnensky	66.3	19.3	22.2	11.2	6.8	6.8	17.1	35.6	18.3	21.2
Zdolbunivsky	17.4	28.8	5.4	19.8	18.1	17.3	37.1	32.5	45.6	13.7
Koretsky	21.6	31.5	14.3	4.9	27.5	30.4	34.8	43.0	18.4	17.6
Kostopilsky	28.2	11.7	20.8	11.4	17.5	17.4	19.6	25.7	13.5	13.0
Mlynivsky	17.9	18.5	20.4	33.8	14.3	15.4	51.5	45.6	44.3	23.9
Ostrozky	17.4	20.9	37.0	42.3	75.4	74.0	74.9	63.7	35.0	31.3
Radyvylivsky	74.6	91.2	73.3	60.2	30.4	101.0	126.5	138.4	92.1	71.4
Rivnensky	6.7	5.2	8.1	19.9	26.7	43.3	27.5	10.7	23.3	8.7
Rokytivsky	6.8	7.8	7.9	16.5	6.7	24.6	17.7	13.3	16.2	17.6
Samivsky	65.5	55.3	40.3	38.6	48.1	38.0	35.7	37.9	43.3	31.1
Rivne city	58.3	67.7	45.1	44.0	29.3	35.8	22.1	27.2	27.0	50.5

A wide difference of BD frequency in every separate district for investigated period was shown. The least frequency of BD took place in Rokytivsky district, most frequency of BD was in Dubrovysky district. Frequency of BD was less than middle one for region in Volodymyrsky, Koretsky, Kostopilsky districts. The higher than middle for region levels of BD frequency were observed in Goschansky, Bereznivsky, Samivsky districts and in Rivne city. The indices of BD frequency in these districts exceeded a middle for region level 1.7–1.82 times. It comes into the special notice that even in districts, where BD frequency was less than middle index for region, however, remained at higher level compared to the Ukraine as a whole [6, 18].

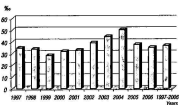


Fig. 23.5. Frequency of birth defects in the Rivne region

Kyiv region. Differences of BD frequency in the Kyiv region were analysed for 10 years (from 1995 till 2004). The highest frequency of BD was registered in 1995 and was equal to 38.5 per 1000 n/b, the lowest one was registered in 2004 and was equal to 27.8 per 1000 n/b. The fact that the BD frequency declined at the end of 2004 can indicate the improvement of diagnostics of birth defects (include prenatal) and also improvement of radioecological situation with decreasing of passport doses.

Table 23.9. Indices of birth of children with BD in districts and cities of the Kyiv region

№	Districts & cities	Children		Indices (per 1000) in	
		total	with birth defects	1995-2004	2004
1.	Baryshivsky	2,496	57	22.8	16
2.	Bilotserkivsky	4,378	67	15.3	13
3.	Boguslavsky	3,236	52	16.1	38
4.	Boryspilsky	6,620	140	21.1	25
5.	Borodiansky	4,086	70	17.1	5
6.	Brovarsky	12,652	667	52.7	28
7.	Vasylkivsky	6,637	278	41.9	15
8.	Vyshgorodsky	3,981	285	71.6	140
9.	Volodarsky	2,277	70	30.7	17
10.	Zguriivsky	1,437	25	17.4	19
11.	Ivankivsky	2,379	73	30.7	16
12.	Kagarlytsky	2,942	40	13.6	20
13.	K-Sviatoshyivsky	8,350	445	53.3	49
14.	Makarivsky	3,115	112	36.0	41
15.	Myronivsky	3,536	86	24.3	16
16.	Obukhivsky	5,125	136	26.5	8
17.	Perjaslav-Khmelnytsky	5,242	194	37.0	23
18.	Polisky	795	43	54.0	18
19.	Rokytniansky	3,403	58	17.0	16
20.	Skvyrsky	3,268	53	16.2	21
21.	Stavyschensky	2,460	34	13.8	10
22.	Taraschansky	2,903	121	41.6	17
23.	Tetlivsky	4,160	105	25.2	28
24.	Fastivsky	6,588	254	38.6	14
25.	Yahotynsky	4,064	147	36.2	18
26.	Bilotserkivsky	17,804	512	28.8	3
27.	c.Irpin	5,310	56	10.5	14
28.	c.Berezan	1,748	44	25.2	11
29.	c.Rzhyschiv	495	13	26.3	0
30.	c.Slavutych	1,466	57	38.9	33

The results of analysis of BD frequency in the Kyiv region on districts separately, presented in the Table 23.9, indicate that these indices oscillate greatly and depend on a concrete district. The highest frequency of BD was observed in Vyshgorodsky district

and was equal 71.8 per 1000 n/b. At the same time frequency of BD in Irpin city was almost 7 times less and was equal to 10.5 per 1000 n/b. This index did not exceeded 20 cases per 1000 n/b in Bilotserkivsky, Boguslavsky, Borodiansky, Kagarlytsky, Rokytynsky, Skvytsky and Stavyschensky districts. Most prevalence of birth anomalies was observed in Brovsky, Vasytkivsky, Kyievo-Sviatoshytsky and Polisky districts.

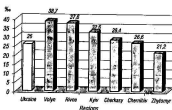


Fig. 23.6 Indices of frequency of birth defects in radiation contaminated regions for ten years

Such range of divergences can be explained by efficiency of prenatal diagnostics of birth defects and in time induced abortions in the cases of BD presence in foetus, and by different genetic education degree of neonatologists and obstetricians-gynaecologists, qualification of geneticists, or unfavorable ecological state of environment, by the unsatisfactory life conditions, and also quantitative difference in the population contingent, which overcame by a medical-genetic help. In addition, not more than 50% of children with BD were revealed according to the documents of maternity hospitals. Other part in polyclinics, child's hospitals, pathologic-anatomic departments [3].

In the Kyiv region the midfrequency of BD was equal to 30 cases per 1000 n/b and was comparable to the level in Ukraine, and also in European populations [21].

The districts of Kyiv region were grouped (Table 23.10) according to dose intervals against the background of dosimetric passportization [12].

Table 23.10. Frequency of birth defects in the Kyiv region (1:1000)

Districts with intervals of passport doses, mSv per year							
>5		1-5		0.5-1		<0.5	
name	birth defects*	name	birth defects	name	birth defects	name	birth defects
Polisky	54.0	Bilotserkivsky	15.3	Boguslavsky	16.1	Baryshivsky	22.8
		Vyshgorodsky	71.6	Borodiansky	17.1	Boryspilsky	21.1
		Ivankivsky	30.7	Brovsky	52.7	Volodarsky	30.7
		Makarivsky	36.0	Vasytkivsky	41.9	Zgurevsky	17.4
		Taraschansky	41.6	Kagarlytsky	13.6	Pereslavl-Khmelnytsky	37.0
				K.-Sviatoshytsky	53.3	Tetijivsky	25.2
				Myronivsky	24.3	Yahotynsky	36.2
				Obuchivsky	26.5		
				Rokytynsky	17.0		
				Skvytsky	16.2		
				Stavyschensky	13.8		
				Fastivsky	38.6		

*per 1000 new-born

A tendency to increased BD frequency in more intensively contaminated districts was revealed.

Zhytomyr region. Data resulted in analysing the indices of BD frequency in the Zhytomyr region (Table 23.11) indicate mean frequency of BD in region which was equal to 21.5 case per 1000 n/b within 10 years from 1995 to 2004. The highest frequency of BD was registered in 1995 and was equal to 25.9, the lowest one was equal to 17.7 in 2004.

Table 23.11. Frequency of birth defects and in the Zhytomyr region.

Year	Born alive (abs)	Born alive with birth defects (abs)	Frequency of birth defects (per 1000)
1996	16,170	407	25.1
1997	15,071	323	21.4
1998	14,172	304	21.4
1999	11,774	262	22.2
2000	11,815	222	18.8
2001	11,010	229	20.8
2002	11,282	221	19.6
2003	11,849	217	18.6
2004	12,072	214	17.7

The received data of BD frequency and inherited pathology in the Zhytomyr region confirm once again the absence of influence of radiation contamination of the territory to this index. It is known that the Zhytomyr region considerably exceeds Kyiv region on the amount of settlements which were subjects of the dosimetric passport system. For example in the Zhytomyr region there were 60 settlements, which corresponded the dose interval 1–5 mSv per year, but in the Kyiv region there were only 3 such settlements in 2004 year.

An analysis of the Zhytomyr region districts separately was not conducted.

Discussion

In each of four investigated regions districts were divided as: much chemically contaminated, much radiation contaminated, conditionally clean and much chemically and radiation contaminated to analyse the BD frequencies and to compare these indices with the ecological state (Table 23.12.).

It was found, that high BD frequencies as 45.2–56.6 per 1000 n/b in the Volhynia region (at a midfrequency 38.7), 23.6–85.9 per 1000 n/b in Rivne (at a midfrequency 36.7), 26.5–52.7 per 1000 n/b in Kyiv (at a midfrequency 3.0 per 1000 n/b), 30.9–35.8 per 1000 n/b in Chernihiv (at a midfrequency 26.6 per 1000 n/b) were observed mainly in much chemically contaminated districts for all investigated regions.

The least frequencies of BD were registered mainly in conditionally clean districts. In most cases, frequency of BD does not exceed middle indices for regions in the radiologically controlled districts. However, frequency of BD was high and exceeds mean values for regions in districts with the combined influence.

But some features of the noted tendencies should be noted. For example, in some conditionally clean districts such as Ratnivsky (Volhynia region), Ostrozky (Rivne region), Nosivsky (Chernihivsky region) frequencies of BD exceeded middle ones for regions (50.2; 47.2; 38.1 respectively). Although on average for all conditionally

clean districts frequency of BD was low (in other districts it oscillated from 10.5 till 25.7 per 1000 n/b, that was less than for all Ukraine). It can indicate difference from the investigated etiologic factors, which influence on BD level in these districts. It is not eliminated, that such situation also can be conditioned by the insufficient public authorized institution's territory contamination monitoring level and insufficient official and scientific sources' information.

As for Kyiv region, the highest BD frequency was observed in a much chemically and radiation contaminated Vyshgorodsky district and was equal 71.6 per 1000 n/b for investigation period. In the Chernihiv region (in accordance to the general tendencies of BD frequency distribution in differently contaminated districts) the highest BD frequency was observed in radiation contaminated Ripkynsky district and was equal 54.0 per 1000 n/b. Although in radiation contaminated Semenivsky district frequency of BD was less than middle for region and was equal 12.2 per 1000 n/b. Here it should be noted that in spite of the similar level of radiation contamination in these districts (according to the passport doses) there were settlements with the level of soil contamination by ^{137}Cs from 185 kBq/m² till 370 kBq/m² in Ripkynsky, unlike Semenivsky district for investigated period.

Generalized results of analysis

Summarizing the results of analysis of the investigated districts' ecological state it should be noted that regions take place as follows: Kyiv > Zytomyr > Rivne > Chernihiv > Volhynia in accordance to the size of territory, which is the subject of radiological control. However distribution of passported settlements by dose intervals in these regions is irregular. Therefore regions ranged by another character: Rivne > Volhynia > Kyiv > Chernihiv in accordance to the level

Table 23.12. Comparison of BD frequency with the ecological state of regions

The region and its administrative districts	Level of contamination	BD
UKRAINE		26.0
Volhynia		38.7
Kovel'sky	Ch	56.6
Lokachyn'sky	Ch	50.4
Luts'k city	Ch	45.8
Rozhyshchyn'sky	Ch	45.2
Kamin-Kashir'sky	R+Ch	45.6
Lubyshev'sky	R	33.3
Turly'sky	CC	20.1
Kivertsi'sky	CC	23.0
Lubomyl'sky	CC	25.3
Ratniv'sky	CC	50.2
Rivne		36.7
Rivne city	Ch	40.7
Zdolbuniv'sky	Ch	23.6
Goschansky	Ch	41.6
Radyvyliv'sky	Ch	85.9
Dubrovyl'sky	R+Ch	67.5
Berezni'sky	R+Ch	54.5
Volodymyrets'ky	R+Ch	20.5
Sarnansky	R+Ch	43.4
Rikytniv'sky	R	13.4
Zarichniansky	R	22.5
Korosty'sky	CC	24.4
Ostrozh'sky	CC	47.2
Kyiv		30.0
Brovary'sky	Ch	52.7
K.-Sviatoshyn'sky	Ch	53.3
Obuchiv'sky	Ch	26.5
Fasliv'sky	Ch	38.6
Vyshgorod'sky	R+Ch	71.6
Ivankiv'sky	R	30.7
Taraschansky	R	41.6
Zgurliv'sky	CC	17.4
Tetliv'sky	CC	25.0
Volodarsky	CC	30.7
Chernihiv		26.6
Varvyn'sky	Ch	30.9
Schorsky	Ch	35.8
Chernihiv'sky	R+Ch	27.8
Korukiv'sky	R+Ch	31.5
Ripkyn'sky	R	54.0
Semeniv'sky	R	12.2
Tatohiv'sky	CC	10.5
Sribniansky	CC	14.1
Bachmat'sky	CC	16.2
Borzniansky	CC	25.7

Ch — means chemical contamination, R — means radio contamination, CC — means conditionally clean districts.

of radiation contamination, and Kyiv > Volhynia > Rivne > Chernihiv in accordance to the level of chemical contamination. The data hereinabove explained the higher level of radiation contamination in the Rivne and Volhynia regions, which resulted to the birth defects of children exposed to its action in the prenatal period. The highest frequencies of BD in the Volhynia and Rivne regions are shown in Fig. 23.6.

Conclusions

1. Frequency of birth defects and the inherited pathology in the regions of Ukraine, which suffered from the influence of the Chernobyl NNP accident factors, exceeds the average indices in Europe (26.6–38.7 and 23.6–32.3 respectively per 1000 n/b).

2. The analysis of BD frequencies in the investigated regions indicates the considerable differences of these indices and gives possibility to range regions on a chart: Volhynia > Rivne > Kyiv > Chernihiv > Zhytomyr.

3. The lowest level of frequency of BD, which is observed in the Zhytomyr region indicates not their better ecological state, but the imperfect search of innate pathology in obstetric and medical-genetic centres.

4. The level of oscillation of BD frequency and inherited pathology for 10 years in different regions does not allow to estimate the increase of this index due to the mutagenic or teratogenic action of ionizing radiation or chemicals. Such differences can be conditioned by different exactness of registration.

5. Frequency of BD in the Volhynia region exceeds a mean value on Ukraine (38.7 per 1000 n/b and 26.0 per 1000 n/b respectively) and has divergences on districts; the greatest level of this index was appeared in chemically-contaminated districts and arrived at 56.6 per 1000 n/b; in districts with the combined influence of radiation and chemical contaminations it arrived at 45.6 per 1000 n/b.

6. Considerable differences of frequency of BD depending on the ecological state of separate district of region were exposed: so in the Chernihiv region with the high level of chemical and radiation contamination there is the greatest frequency of innate pathology — 54.0 against 29.7 for region; in Volhynia it was 56.6 in a district with chemical contamination, 45.8 in Lutsk city and 50.2 in a conditionally clean district against 38.7 for region; in Rivne it was 85.9 in a chemically contaminated district, 67.5 in a district with the combined action of radiation and chemical contamination against 36.7 per 1000 n/b for region.

7. Under the increased level of innate and inherited pathology in Ukraine introduction of effective preventive measures include the protection of population from the action of additional anthropogenic factors, vitaminization of population, timely medical-genetic advision and prenatal diagnostics with the modern methods usage is necessary.

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CHAPTER 24 PECULIARITIES OF THE INFLUENCE OF HIGH AND LOW DOSES OF IONIZING RADIATION ON TOOTH HARD TISSUES

Ionizing radiation was remaining the most significant environmental factors, causing severe impacts on human health on the vast territory of Ukraine for 25 years.

One of the most important is the problem of "low doses" (LD) of ionizing radiation (IR), mechanisms of their effects on human body and the way to find effective methods for correction of radiation exposure negative effects.

Low doses of ionizing radiation act as an alarm signal [1]. Medical and biological consequences of their impact will be significantly different from high doses effects [2, 3]. Radiation impact of bone system, including its mineral component, and especially its structural and metabolic disorders over time has not lost their relevance. Throughout the human life resorption and remodeling processes at human bone system take place. Also radiation stress which causes premature aging is one of the effects of ionizing radiation on human. These processes lead to disruption of compensatory ability of bone tissue and, consequently, reduce its functionality and its ability to restore [4].

After the catastrophe of the nuclear plant in Chornobyl it was observed, that persons who took part in the clean-up works in Chornobyl zone were suffering from atypical stomatological problems — pathological dental abrasion, wedge-shaped defects, enamel erosion and enamel scissuras. The main pathology was additionally worsening while carious changes occurred, although they were not directly related to irradiation.

Monitoring of dental health of more than 1500 dwellers of radiation contaminated areas showed significant deterioration of the periodontal tissue and skeletal system in this contingent compared with control. Medical examination of people from contaminated territories demonstrated high level of hyperesthesia of hard dental tissues (around 63% of all patients), increase of enamel abrasion (the first and the second degrees) for 36% of examined patients, non-carious dental teeth (wedge-shaped defects, pitting) for 47% of examined patients [5]. Study of mechanisms of periodontal diseases (non-carious diseases and parodontal changes in dental tissues) provoked by ionizing radiation) is an actual problem for last years.

The task of this investigation was to compare the intensity of osteoporosis process in solid teeth tissues of persons exposed to low and high doses of ionizing radiation during the clean-up works in the Chernobyl zone.

The objects of study were clean-up workers of the Chernobyl NPP accident, exposed to external radiation doses in the range of 0.5–1.0 cGy — 26 persons of low dose group (LDG) and 18 people, exposed to doses in the range of 6–22 cGy (high dose group, HDG). All of them were under medical examination of RCRM with assessment of teeth index, depth of dental tissues damage and the correlation between caries and non-caries lesions during medical observation. Teeth were extracted according to medical recommendations from patients of HDG (15) and LDG (8).

The potentials of infrared spectroscopy were used as a fingerprinting tool in disease diagnosis.

Samples and experimental technique.

Infrared (IR) absorption spectroscopy has been applied to investigate tooth tissues (enamel, cement, dentin, alveolar). It must be emphasized that only patients without the bone marrow affection were investigated. For comparison the same tooth tissues of people who had not been exposed to radiation (control group, CG)) were investigated too.

To prepare samples for infrared spectroscopy analysis it was necessary to dry them, because molecule of water had broad absorption spectrum in the investigated spectral region. Teeth were washed by distilled water and dried in drying chamber at 60° C during 2 hours. After that enamel, dentin, cement and alveolar were sawn down by diamond dental drill.

Then 0.2 g of tooth tissue powder was mixed with 50 μ l of vaseline oil and put in cuvette of KBr. All spectra were obtained by one-beam infrared spectrometer IKS-31 with accumulation (10 accumulations have been applied) of spectra to increase ratio signal to noise.

Spectral measurement has been fulfilled at low vacuum camera (10^{-1} Pa) to minimize spectral components due to atmospheric CO₂ and H₂O in ambient air. An air exhaust pump has been used. The infrared intensity transmitted by the cuvette containing the sample (I_s) and that transmitted by empty cuvette (I_0) were measured in the 500–3000 cm⁻¹ spectral range with resolution of 2–4 cm⁻¹. Spectral resolution depended on diffraction grating (number of grating groove per mm) for each spectral interval. Absorption intensity $I_A(\nu)$ which is approximately proportional to the sample absorption was obtained as

$$I_A = (1 - I_s/I_0) \quad (1)$$

where ν is wavenumber in cm⁻¹, of investigated samples.

For a quantitative analysis, the $I_A(\nu)$ spectra were fitted using a standard procedure which describes the $I_A(\nu)$ spectrum as a sum of Lorentzian components [6].

Diagnosis of structural changes of hard tissue of teeth using infrared spectroscopy

The organic matrix of dental tissues are formed by collagen which is controlling the calcification of bone and crystallization of apatite in bones [7]. Collagen is rich of glycine, proline, and hydroxyproline [8]. Collagen is presented by amide groups of pro-

teins — (CONH_2) . The amide absorption bands are well evident at 1660 cm^{-1} (Amide I — C=O stretching vibration), $1540\text{--}1550\text{ cm}^{-1}$ (Amide II — C-H deformation), and weak band can be detected at 1240 cm^{-1} (Amide III — C-N , C-N-H stretching vibration) [6].

Mineral component of bone and dental tissues causes the presence in the IR spectra bands assigned to carbonates (CO_3^{2-}) 880 , 1430 , 1460 cm^{-1} and orthophosphates (PO_4^{3-}) 610 , 1030 , 1050 , 1080 cm^{-1} [7]. PO_4^{3-} tetrahedral ion has four fundamental vibrations: symmetric degenerate, asymmetric valency three degenerate and degenerate deformations thrice. Typical bands for orthophosphate anions are (cm^{-1}):

H_2PO_4^- — $300\text{--}570$; $700\text{--}900$; $840\text{--}910$; $915\text{--}970$; $1010\text{--}1080$; $1130\text{--}1190$; $1220\text{--}1370$; $1580\text{--}1700$; $2350\text{--}2400$; $2750\text{--}3250$.

HPO_4^{2-} — $350\text{--}580$; $700\text{--}900$; $860\text{--}913$; $940\text{--}1010$; $1040\text{--}1170$; $1210\text{--}1400$; $1400\text{--}1750$; $2300\text{--}2450$; $2600\text{--}3250$;

PO_4^{3-} — $410\text{--}480$; $510\text{--}670$; $930\text{--}990$; $975\text{--}1140$.

The simplest condensed phosphate ion is the ion-diphosphate (pyrophosphate-ion) formed by two tetrahedral PO_4^{3-} ions to form $\text{P}_2\text{O}_7^{2-}$ ion. Absorption bands typical for the bond P-O-P appear at $700\text{--}740$, $920\text{--}940$ and $960\text{--}980\text{ cm}^{-1}$.

Discussion

During medical examination of people of HDG and LDG specific pathological dental abrasion, wedge-shaped defects, enamel scissuras and enamel erosion were detected. Dystrophic (non-carious) changes of teeth were typical for patients of HDG. Enamel and cement were easily split and fragile.

The most common pathologies for patients of HDG were pathological tooth wear and severity-III (around 78%), wedge-shaped defects constituted around 71% of enamel erosion (around 39%) and cracks (around 60%), see Fig. 24.1. Carious processes were secondary in nature and mostly souring course of non-carious lesions of teeth (Fig. 24.2).

The most common pathologies for patients of LDG, 63% of examined patients, were non-carious diseases. Dental hyperesthesia and inflammations were characteristic features of dental problems for patients exposed to low doses irradiation. Enamel attrition of varying degrees was observed in 32%, wedge-shaped defects — in 26% and erosion — in 15% of examined people. Increasing of caries pathology and aftereffects of caries



Fig. 24.1. Pathological abrasion of dental enamel



Fig. 24.2. Irradiated enamel syndrome. Non-carious damage of hard dental tissues

were observed in these patients as well (Fig. 24.3). Caries, tooth filling, removal (CFR) index showed the following values: C — 2.08 ± 0.22 , F — 8.79 ± 0.54 , R — 3.26 ± 0.36 , with the sum amount of 14.14 ± 0.67 ($p < 0.05$).

Probably influence of radiation on teeth has different mechanism on dental tissues changes in case of high and low doses.

Enamel, dentin and cementum are complex bioinorganic materials which form the hard component of teeth. Tooth enamel (Fig. 24.4) is the hardest and the most crystalline tissue of teeth. It contains about of 95–97% of hydroxyapatite. Teeth enamel directly contacts with the oral liquid.



Fig. 24.3. Teeth belonging to people exposed in low doses of radiation. Odontogenic pathology



85% — mineral matrix
 75.4% — $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$
 12.06% — $\text{Ca}_{10}(\text{PO}_4)_6\text{CO}_3$
 4.4% — $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$
 12% — organic matrix
 (38% protein — Ca-protein — 3D structure which links with hydroxyapatite crystals (Petrovich D.O., 1983); 42% — lipids with low amount of lactate, saccharide and citrate ions)
 3.8% — water

Fig. 24.4. Chemical composition of enamel

IR spectra of dental enamel taken from examined people consist of strong bands which belong to phosphate group and very weak bands in Amide region (Fig. 24.5). Absorption bands typical of $\text{Ca}_3(\text{PO}_4)_2$ have been observed at 950–970 and 1030–1080 cm^{-1} in the IR spectra of enamel belonging to people of HDG (Fig. 24.5). These spectra showed increasing of the area of the band typical of anion CO_3^{2-} (at 880 cm^{-1}) up to 9.7 ± 0.4 (a.u.), in comparison with the area value of 8.4 ± 0.4 (a.u.) for patients of CG. The changes in the phosphate-carbonate ratio lead to the enamel strength decreasing.

A comparison of enamel infrared absorption spectra typical of LDG and CG showed only some changes in organic spectral region (Fig. 24.6 and Fig. 24.7).

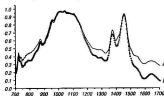


Fig. 24.5. Infrared spectra of the dental enamel taken from the persons of CG (curve a) and of HDG (curve b). Abscissa axis corresponds to wave numbers (cm^{-1}), the axis of ordinates — absorption in absolute units (a.u.).

The absorption bands at 750 and 710 cm^{-1} arise from P-O-P bond, the bands at 880 and 1430 cm^{-1} are typical of CO_3^{2-} ion, and the band at 540–570 cm^{-1} belongs to Ca-O bond.

For the patients of LDG the ratio between mineral components of dental enamel was presented like 140 a.u. for phosphate and 8.95 a.u. for carbonate compounds, that was around 89.40 wt.% and 5.60 wt.%, respectively. Organic component of tooth enamel belonging to people of LDG

corresponded to 8.8 a.u. (5.0 wt.% of total enamel's content).

Qualitative changes in enamel taken from exposed patients demonstrated appearance of the band at 1238 cm^{-1} , typical of Amide III (C-N/H-N bond). Area of the Amide III band was around of 2.7 a.u. (23% of organic matrix). Area of the Amide II bands at 1539 and 1547 cm^{-1} was around 5.3 a.u. (46% of organic matrix).

The wide band of carbonyl bond C=O has been detected at $1640\text{--}1670$ and belonged to Amide I. Area of this band was around 3.3 a.u., which corresponded to 29% of organic matrix content. High doses of radiation also provoked changes in the Amide I and Amide II ratio caused by C-O bond tears. Amount of Amide I and Amide II were the main characteristics of organic matrix therefore their ratio was important characteristics of dental tissue. The ratio of the area of Amide groups

$$S_{\text{Amide I}}/S_{\text{Amide II}}=3.3\text{ a.u.}/5.3\text{ a.u.}=0.62\text{ [9]}.$$

This result demonstrates that N-N bonds were typical of enamel of HDG.

Dentin and cementum differ from enamel and they look like coarse-fibered bone. Diversities of enamel, dentin and cementum are provided by nature of the tissues' embryogenesis.



70% — mineral matrix
 $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, CaCO_3 ,
 MgCO_3 , MgSO_4 ,
 $\text{Ca}_{10}(\text{PO}_4)_6\text{CO}_3$, $\text{Fe}_2(\text{PO}_4)_3$,
 $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$, Mg_3CO_3 ,
 ZnCO_3 , *et al.*

20% — organic matrix
80% — collagen (I type)
18% — noncollagenous compounds)

10% — water

Fig. 24.8. Chemical composition of dentin

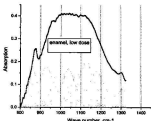


Fig. 24.6. Infrared spectrum of the dental enamel typical for the persons of LDG. Abscissa axis corresponds to wave numbers (cm^{-1}), the axis of ordinates — absorption in absolute units (a.u.).

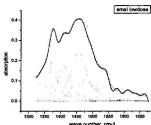


Fig. 24.7. Infrared spectrum of the dental enamel typical for the persons of LDG, spectral region of organic matrix ($1300\text{--}1650\text{ cm}^{-1}$). Abscissa axis corresponds to wave numbers (cm^{-1}), the axis of ordinates — absorption in absolute units (a.u.).



62–65% — mineral matrix
 $\text{Ca}_{10}(\text{PO}_4)_6\text{OH}_2$, CaHPO_4 ,
 $\text{Ca}_{10}(\text{PO}_4)_6\text{CO}_3$, $\text{Ca}_2(\text{H}_2\text{PO}_4)_2$,
 $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$, CaSO_4 ,
 $\text{Ca}_2(\text{PO}_4)_2$, MgCO_3 ,
 FeCO_3

20% — organic matrix (collagen of the I type)

12% — water

Fig. 24.9. Chemical composition of cementum

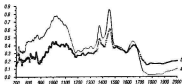


Fig. 24.10. Infrared spectrum of dentin taken from people of control group (curve a) and from patients exposed to external radiation (curve b). Abscissa axis — wave numbers in cm^{-1} , ordinate axis — absorption in absolute units.

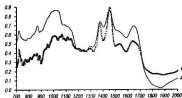


Fig. 24.11. Infrared spectrum of cement taken from persons of control group (curve a) and from patients exposed to external radiation (curve b). Abscissa axis — wave numbers in cm^{-1} , ordinate axis — absorption in absolute units.

$\text{H}_2\text{PO}_4^-/\text{PO}_4^{3-}$ increased and was of 0.06 ± 0.01 a.u. (for CG the ratio was of 0.03 ± 0.01 a.u.), $p < 0.05$. Intense absorption bands typical of the soluble phosphates (like pyrophosphates) appeared in the spectral range $1070\text{--}1150\text{ cm}^{-1}$.

Area of absorption band at $800\text{--}900\text{ cm}^{-1}$, typical of anion CO_3^{2-} increased to 10.5 ± 0.4 a.u. for dentin taken from exposed patients in comparison with area of that band of 9.7 ± 0.4 a.u. for control group, $p < 0.05$. Significant increasing ($p < 0.005$) of organic component content of cementum and dentin taken from people of HDG has been registered. Increasing of Amide I band area to 7.5 ± 0.3 a.u. (in comparison with Amide I band area of 8.6 ± 0.2 a.u. for CG) and Amide II band area to 9.4 ± 0.2 a.u. (for control group the area was 8.6 ± 0.2 a.u.) have been shown. Changes in the ratio between protein components have been observed:

$S_{\text{Amide II}}/S_{\text{Amide I}}$ was equal to 0.81 ± 0.05 for exposed patients and 0.61 ± 0.04 for people from control group [9].

Infrared spectra of dentin belonging to people of LDG consisted of bands typical for phosphate ion vibrations: totally symmetrical valence vibrations at 970 cm^{-1} , asymmetrical valence vibrations at 1040 cm^{-1} (Fig. 24.12) and deformation vibrations at 540 and 615 cm^{-1} . Besides these, the absorption bands typical of P-O-P bond appeared at 770 and 880 cm^{-1} for dentin belonging to exposed patients. Intensities of P-O-P bond

The tooth dentin and cementum are grown up from mesenchyme while the tooth enamel is grown up from ectoderm, so the enamel almost does not contain organic matrix.

Analysis of infrared spectra typical of dentin (Fig. 24.10) and cementum (Fig. 24.11) taken from people exposed to high doses of ionizing radiation showed three bands of vibrations typical of ion PO_4^{3-} : symmetrical valence vibration at 970 cm^{-1} , asymmetrical valence one at $1035\text{--}1080\text{ cm}^{-1}$ and asymmetric deformation vibration at $605\text{--}610\text{ cm}^{-1}$. Changes of infrared absorption bands provoked by external radiation have been found out for dentin and cementum. Area of the absorption bands typical of insoluble phosphates decreased to 9.1 ± 0.4 a.u. in comparison with the area of those bands of 11.0 ± 0.5 a.u. for control group ($p < 0.05$). The ratio between soluble and insoluble phosphates

bands in dentin spectra of HDG were higher than those in enamel of HDG. It demonstrated high amount of pyrophosphate ($\text{H}_2\text{P}_2\text{O}_7$)²⁻ in irradiated dentin. The appearance of the more soluble phosphate forms (with solubility of 1.8 mol/L) leads to elimination of these soluble inorganic components from the dental tissues by intercellular liquids and saliva. Low intensities of the absorption bands at 880 cm^{-1} and 1430 cm^{-1} showed lower content of carbonate ions, CO_3^{2-} , in dentin in comparison with enamel for all groups.

Calculation of the bands' area values showed the following results: the area of phosphate ions band was around of 229 a.u. (71% of inorganic matrix), and the area of carbonate ions bands was around 13.35 a.u. (4% of inorganic matrix).

Organic matrix of dentin was represented by three bands typical for amides (Fig. 24.13). Total area of these bands was around of 79 a.u. (25% of dentin). Weak band of Amide III at 1238 cm^{-1} arised from N-H deformation vibration and had area of 3.4 a.u. (4.3% of organic matrix of dentin). Infrared spectrum of dentin also consisted of characteristic band of Amide II (C = N- bond), which was split into two bands at 1539 and 1547 cm^{-1} with the areas of 22.65 a.u. and of 6.65 a.u. respectively, the total area of Amide II bands was of 29.3 a.u. (37% of dentin organic matrix). The band at 1680 cm^{-1} belonging to Amide I (C = O connection) was the strongest amide band and has area of 46.6 a.u. (59% of the total content of dentin organic matrix). Besides the mentioned amide bands another band at 1300 cm^{-1} was detected, corresponding to CH_2 bond. The area of this band was around of 1.5 a.u. (2% of dentin organic matrix). Ratio between different amides showed the following results:

$$S_{\text{Amide III}} / S_{\text{Amide I}} = 3.41/46.61 = 0.07;$$

$$S_{\text{Amide III}} / S_{\text{Amide II}} = 3.41/29.31 = 0.12;$$

$$S_{\text{Amide I}} / S_{\text{Amide II}} = 46.61/29.31 = 1.59.$$

Obtained results demonstrated dominance of C = O bond which is typical of collagen coll. Contribution of C = N-C = N / N-N — bond to organic matrix was much lower then contribution of C = O bond.

Infrared spectrum of cementum taken from people of LDG is presented in Fig. 24.14. Mineral matrix of cementum was characterized by three types of orthophos-

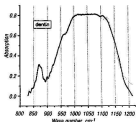


Fig. 24.12. Infrared spectrum of tooth dentin belonging to patients of LDG in spectral range 850–1200 cm^{-1} .

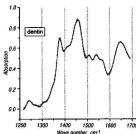


Fig. 24.13. Infrared spectrum of human tooth dentin taken from patients of LDG in the range 1200–1650 cm^{-1} .

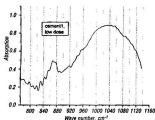


Fig. 24.14. Infrared spectrum of cement belonging to people of LDG in the range 850–1120 cm^{-1} .

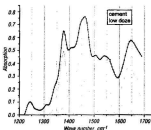


Fig. 24.15. Infrared spectrum of cement teeth belonging to people of GDO in the range 1200–1650 cm^{-1} .

phate's vibrations: totally symmetrical valence vibrations at 970 cm^{-1} and asymmetrical valence vibrations at 1040 cm^{-1} (Fig. 24.14). Deformation of phosphate ion vibrations appear at 540–580 and 605–615 cm^{-1} .

Area of absorption band at 820–850 cm^{-1} is lower than area of the same band of dentin. This fact indicates a difference in the structure of the phosphate crystal caused by external radiation. Carbonate-ion band at 880 cm^{-1} was stronger than the in dentin. For patients exposed to low doses of external radiation area of phosphate and carbonate bands was of 208 a.u. (64% of inorganic matrix of cementum) and 14.8 a.u. (4.6% of inorganic matrix of cementum), respectively.

Area of bands typical for organic matrix of cementum was equal to 99.64 a.u. (31% of the total cementum tissue). Area of the absorption band at 1241 cm^{-1} arose from Amide III (C = N / N–N bond) was around 6.98 a.u. (7% of organic matrix). Areas of the absorption bands at 1537 and 1562 cm^{-1} belonged to Amide II (C = N bond) were around 14.08 and 11.48 a.u., respectively. The total sum of these areas was 25.56 a.u. (25.6% of the organic matrix of cementum). Area of the absorption band at 1641 cm^{-1} arose from

Amide I (C = O bond) was around 51.7 a.u. (51.9% of organic matrix). This value of the band area was the same as for dentin and did not depend on absorbed radiation doses. Besides the mentioned amides' bands weak bands typical for CH_2 bond at 1303–1306 cm^{-1} , asymmetric valence vibrations of C–O–C bond at 1200–1275 cm^{-1} , pendular oscillations of $-\text{CH}_3$ bond at 1250 cm^{-1} , and symmetric valence vibrations of $-\text{NO}_2$ bond were observed in infrared spectra of cementum taken from patients of LDG.

These links were usually structural elements of protein, in this case probably paracollagen proteins and glycosaminoglycans. The total area of these bands was 6.5 a.u. (6.58% of organic matrix of cementum).

The ratio between Amide bands areas indicated protein components increase:

$$S_{\text{Amide III}} / S_{\text{Amide II}} = 6.98 / 25.56 = 0.27,$$

$$S_{\text{Amide III}} / S_{\text{Amide I}} = 6.98 / 51.70 = 0.14,$$

$$S_{\text{Amide I}} / S_{\text{Amide II}} = 51.70 / 25.56 = 2.02.$$

The obtained results showed that late effect of ionizing radiation on dental tissues depended on exposed dose. For patients from HDG non-carious dental diseases dominated. For patients from LDG carious disease and its complications were more typical instead of non-carious diseases. Different radiation doses impact on dental tissues could be explained by changes in hydroxyapatite crystal caused by phosphate-carbonate bonds rebuilding.

The results of spectral analysis of teeth taken from people of HDG showed decrease of hydroxyapatite crystal perfection. Pathological changes in phosphates were caused by transformation of basic phosphates into more soluble acid phosphates, such as pyrophosphates. Soluble phosphates elimination from dental tissues led to resorption within the dental inorganic matrix.

Low doses of external radiation provoked insignificant decreasing of hydroxyapatite bands' intensities ($p > 0.5$) and therefore elimination of Ca^{2+} (as for carious process) took place [12]. High doses of external radiation activated in cementum pathological processes linked with changes in protein conformation, protein molecules' shattering and the relative increasing of organic matrix content in the tissue. Additional absorption band belonging to soluble pyrophosphates P-O-P bond and C-O bond appears at $1170\text{--}1306\text{ cm}^{-1}$ in infrared spectra of cementum taken from patients of LDG.

At the same time changes in intensity of Amide I and Amide II, split of Amide II band were results of some bonds' breakage and new bonds' formation in collagen coil. These processes led to changes in organic matrix architectonics. In spite of the mentioned changes caused by low doses irradiation the ratio between the protein components of enamel, dentin and cementum, and the ratio between the organic and inorganic constituents of dental hard tissue were relatively similar to those for the control group. Infrared spectra presented in Fig.16 demonstrated different structural changes of cementum provoked by high and low doses of external radiation.

Thus, the results of clinical and spectral studies have shown different influence of high and low doses of external radiation on dental tissues. While high doses provoked and stimulated launch of dystrophic (non-carious) processes at dental tissues, low doses of external radiation impacted mouth cavity and activate odontogenic oral pathology.

The present results demonstrated that the pathological effects of radiation touched upon all dental tissue and obviously all bone system of irradiated people. We detected an increase of the CO_3^{2-} content (imbalance between phosphate-carbonate phase levels) in enamel, dentin, cement and alveolar for persons exposed to high doses of radiation. This disorder had an effect on the dental matrix strength. High doses of radiation also lead to mineral composition changes: more soluble phosphate forms (pyrophosphate) were accumulated in the mineral part of the

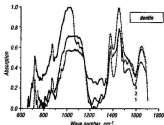


Fig. 24.16. Infrared spectra of cementum taken from persons of HDG, LDG and CG.

teeth, and afterwards they were washed out by intracellular liquids. As a result of this process, the content of mineral phase decreased and hard dental tissues were easily split and fragile. On the other hand, low doses of radiation did not induce noticeable changes in the mineral part of all tooth tissues.

Changes of the organic composition of dentin, cement and alveolar were noted for both high and low radiation doses. The alteration of collagen caused by high doses of radiation was evident in the decrease of the Amide I content. The splitting of the Amid II absorption band and the appearance of the Amide III were observed at low doses of radiation. Rebuilding of the collagen matrix can be explained by the changes of amino acids by radiation (proline and oxyproline), which are parts of collagen.

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AFTERWORD

This book is arriving twenty-five after Chernobyl and continues a line of studies and international publications of the last period. Among them are the Report of the UN Chernobyl Forum (2005) and its recommendations to the governments that have induced a lot of neglect in the suffered countries. The 2006 UNSCEAR report has a synopsis on the epidemiological studies of radiation and cancer (Annex A), epidemiological evaluation of cardiovascular disease and other non-tumour diseases after radiation exposure (Annex B). The report has provided an international evaluation of a new data explaining the difference between an amount of radiation dose and effect by such entities as non-targeted and delayed effects of exposure to ionizing radiation (Annex C), and effects on the immune system (Annex D). Of special interest is the 2008 Report focusing on effects due to ionizing radiation. The important message of the Committee is that with regard to health effects of Chernobyl, there have been dramatic improvements in the understanding of acute radiation effects and their treatment, and of the long-term local radiation injuries due to irradiation of the skin and lens of the eye. With respect to the incidence of stochastic effects other than thyroid cancer, the UNSCEAR suggests that there have been few observations that have challenged pre-existing understanding derived from the studies of other exposed groups, such as the survivors of the atomic bombings in Japan and other studies of radiation exposed populations.

The experimental research in parallel to Chernobyl studies, funded by the DOE Low Dose Radiation Research Program has provided new information suggesting that basic radiation paradigms may need re-evaluation (Brooks, Couch, 2007). This ambitious program was focused on biological mechanisms involved in response to low doses of both low and high-LET radiation at doses below 0.1Gy. A linear type of dose-effect response was demonstrated for molecular changes induction while a non-linearity was typical for response and repair reactions.

The ongoing in Chernobyl studies of the last period bridge the existing and new experience on the health effects with knowledge obtained at experiments. The fact that the object of research is a human has its scientific strength and its limitations. Studies on cohorts of the acute radiation syndrome survivors, emergency and clean-up workers, evacuees and exposed in childhood have provided information invaluable for the scientific understanding of radiation effects and for radiation protection as well. From the other hand, radiation effects in humans have to be separated from a set of health effects of a non-radiation origin. The understanding strengthens that current knowledge on the health effects due to ionizing radiation is based mainly on an epidemiology approach.

and there exists a gap in identifying the specific markers of a radiation-induced disease as well as of biological markers of radiation exposure effective at the late period.

ARS survivors follow-up study has confirmed the previously described dependencies on cancer and radiation cataracts rates due to dose of exposure and stage of disease. The main causes of late deaths are cancers and cardiovascular diseases. An intriguing point is an absence of differences between the general mortality rates in ARS cases confirmed and non-confirmed by the 1989 expertise and higher cancer mortality rates at the last sub-group. The research on the mechanisms of late effects in ARS survivors is in process. It is necessary to expand research of long-term effects of the acute radiation sickness taking into account the organ doses, and also to support survivors' follow-up and medical care. This will provide an unique possibility to understand the biological relationship between cancer and non-cancer diseases and ionizing radiation. Biological specimens from the ARS survivors and possibly their offspring should be collected and stored for future research using the appropriate technologies of cell immortalization etc.

Studies in Chernobyl clean-up workers ("liquidators") have been directed to get data on the health effects at low dose. This cohort is the biggest cohort of exposed at low dose range to its size (approximately 600,000) and collective dose and should be most informative, with great statistical power, for the study of cancer and non-cancer effects in adults at exposure. The biggest part of this cohort is represented by clean-up workers from Ukraine with mean doses of 17 mSv exceeding the doses in Russian clean-up workers by 1.5-fold and Belorussian — by factor of 2. The Ukrainian cohort is characterized also by a high percentage of a first year of recovery workers including those of the early emergency period. Subsequently, a higher is proportion of clean-up workers exposed to radiiodine and other short-living isotopes harmful to thyroid. However, all clean-up workers cohorts have an important limitation due to the insufficient individual dosimetry of the first most important period.

To solve a problem of dose assessment several approaches have been proposed and implemented after the accident. It was not possible to provide biological dosimetry to all exposed due to technological limitations of the chromosome aberrations test, FISH WCP-1,2,4 analysis or EPR-dosimetry of tooth enamel as the assays are time-consuming and show valuability at doses above 250 mSv. That is why the questionnaires based on the combined estimates of staff routes and tasks inside the radiation zone, and radiation fields were introduced for retrospective dosimetry. The U.S.-Ukrainian cataract study and the RADRUE dose reconstruction for leukemia risks study are the examples of successful retrospective dosimetry implementation. Of course, the value of the questionnaire approach decreases with time and still there is an urgent need in development of new biological marker assays.

Several results important for radiation epidemiology were obtained in clean-up workers cohort. An increased risk of radiation-induced leukemia among Chernobyl clean-up workers has been established during last 5 years in the U.S. NCI-RCRM and the IARC international studies. Risk levels for the first 15-years after the exposure are similar to those previously noted following the nuclear bombing in Japan. Contradictory to the findings in the A-bomb survivors, the studies conducted on Russian, Baltic and Ukrainian clean-up workers suggest a substantial increase in radiation risks of the

chronic lymphocytic leukemia. Some additional confirmation arrives to the last data from the professional exposure studies. The ongoing research in Ukraine is aimed to obtain updated information on the risks of the CLL at a longer time period.

The studies of a nature of leukemia after radiation exposure have also gained some new information. The changes in bone marrow microenvironment, surface phenotype changes are described accompanied by a loss of genetic material in myeloid leukemia, and decreased percentage of the heavy immunoglobulin chains rearrangements in CLL. In this book the results of a study are presented of the myelodysplastic syndrome pathology, immunology and cytogenetics in complex with its clinical characteristics. Features of this syndrome after radiation exposure also need some clarification as time trends of its after Chernobyl onset are not similar to those in A-bomb survivors. Up-to-date the studies have failed to the moment to encircle a specific radiogenic type of leukemia, however part of the pathological changes could be explained by the radiogenic effects of the early period.

Radiation risks of thyroid cancer in adults are the subject of discussion. The book contains last update on the elevated thyroid cancer risks in Ukrainian clean-up workers; however information on dose-effect dependency is not available yet. Some information on the increased radiation risks of thyroid cancer arrived from the 2010 IARC study on Belarusian, Baltic and Russian clean-up workers, who were exposed to iodine radioisotopes and external radiation. Radiation risks for each of these factors have to be studied in future. The increased incidence of thyroid cancer among persons exposed to 131-I in adulthood has been also reported in Ukraine and Belarus and needs further research.

The information on the incidence of solid cancers is more controversial. For some types of solid cancers among the clean-up workers the incidence is increased. Increased risks of radiation induced breast cancer are demonstrated among female clean-up workers exposed in 1986–1987 as well as among the females, inhabiting in the most contaminated areas. According to the experience of A-bomb survivors study, the excess of radiation induced cancers appears after a latent period and lasts for a different time due to cancer type and possibly dose of exposure. Therefore, radiation risks of solid cancers in such groups as clean-up workers and evacuees have to be further studied and evaluated.

Evidence from epidemiological studies suggests an increase in the incidence of cataracts among clean-up workers exposed at lower doses starting from 250–300 mSv. Still there remains uncertainty about the stochastic or deterministic nature of radiogenic cataracts. Results of the studies indicating the contributive role of irradiation to the early development of senile cataracts and macular degeneration have to be confirmed in future.

Recent advance in analysis of low-dose effects in human immune cells has greatly expanded our knowledge on the effects due to ionizing radiation. Follow-up studies of more than 160,000 humans exposed after Chernobyl and clarification of dose-effect dependencies were in need to overcome the uncertainties and dualism of estimates due to the action of confounding factors — psychoemotional stress, chemicals etc. demonstrated by the UNSCEAR-2006 and the EGH (2005) reports. The early and late changes of cell subsets at different stages of differentiation, proliferative activity and, later, the molecular effects have been demonstrated in clean-up workers exposed at a wide range of doses, including a special follow-up group of the ARS survivors. The study reported

NPP, scientists and fireman, drivers, desactivators, foresters etc., who are exposed to a protracted low-dose irradiation. The last addition to this group is presented by construction workers of the new safe confinement upon the Chornobyl "Shelter".

A reader can refer the studies of health effects in staff of Chornobyl NPP and the exclusion zone as well as of children inhabiting in Slavutych in such books as "ChNPP, "Shelter", Slavutych: health effects 10 years later" (1996), "Chornobyl: the Exclusion zone" (2001), "Health effects of the accident at the Chornobyl nuclear power plant" (2007). Making the estimates of the radiation risks it's also important to encounter the confounders like working and social conditions, the duration of work experience in the Zone, as well as the degree of contamination by hazardous chemicals at the industrial sites. During 1991–1994 a comprehensive study of radiation-hygienic assessment of the environment in Chornobyl exclusion zone, working conditions and health of shift staff was performed by the Institute of Medical Ecology and Hygiene and the Institute of Labour Medicine. Based on the radiation-ecological situation in the exclusion zone the staff have been identified at risk, especially those working outdoors, and subjected to additional risk of penetration of radionuclides and chemicals in the body (the alpha-beta emitters and heavy metals) through inhalation of dust. Such critical groups include the operators of heavy construction machinery, heavy vehicle drivers, welders, drillers, concrete workers and staff working on dosimetry and decontamination.

The study on drivers of heavy vehicles and operators of heavy construction equipment (bulldozers, excavators, loaders, scrapers, etc.) was focused on the work points to the disposal of radioactive waste, as well as the destruction of worn-out and cleaning the "dirty" buildings in Chornobyl. Additionally to the exposure to elevated levels of ionizing radiation workers were influenced by a traditional complex of environment factors (noise, vibration, dust, climate, etc.). As a control group served dosimetrists and other professionals working on the same production site. Exposure dose of gamma-irradiation at reference points ranged from 0.6 to 1.4 mR/h. In the hygienic evaluation of industrial sites in environmental samples, except for chemical substances (heavy metals, nitrogen dioxide, carbon monoxide, etc.), radioactive elements were determined: ^{137}Cs , ^{134}Cs , ^{90}Sr , ^{238}Pu , ^{239}Pu , ^{240}Pu , measuring the parameters of microclimate, noise and vibration levels (overall and local) in the workplaces. The dust samples have been taken to determine the level of specific activity of ^{137}Cs . Conditions of survey of professionals working in open areas and on heavy equipment were modeled using the anthropomorphic heterogeneous body phantom. Physiological studies of persons surveyed and control groups were performed before and after working for 2–3 days at the beginning, middle and end two weeks of the watch.

The main pollutant of air at the Exclusion zone is the dust factor. The highest concentration of suspended solids was determined in the field excavations in the heavy construction equipment, where the dust content in the air exceeded the limits from 3 to 12 times. At these sites the carbon monoxide and nitrogen dioxide concentrations approach the higher limits. Surface aerosol concentration of heavy metals in ambient air study points did not exceed sanitary norms. The content of ^{137}Cs , ^{90}Sr , ^{238}Pu , ^{239}Pu , ^{240}Pu in the air of the surveyed production areas was recorded as three or more fold below the acceptable values for the B category staff. The contamination of surface soil at the Chornobyl exclusion zone was characterized by a mosaic and heterogeneity of heavy metals and radionuclides contamination. However, analysis of results showed

more. During last 5 years the new studies in Ukraine provide support to a possibility of increased risks of non-cancer diseases including autoimmune thyroiditis in liquidators with external doses over 250 mGy and groups of adults with doses to thyroid over 300 mGy. The highest risks were demonstrated in patients with thyroid doses exceeding 2 Gy. This evidence needs further support.

Among the general population exposed to fallout from the Chernobyl accident, the main cancer consequence of radiation exposure demonstrated to date is an increased risk of thyroid cancer. Since 4–5 years after the accident, a dramatic increase of thyroid cancer rates has been demonstrated among exposed as children to radionuclides of iodine, mainly iodine-131. The UN Chernobyl Forum has reported in three suffered countries more than 5,000 thyroid cancer cases in exposed as children. According to the later data from the V.Komissarenko Institute of Endocrinology and Metabolism 6,049 thyroid cancer patients exposed at age of 0–18 years underwent surgery during 1986–2008. Among them 74.1% at the moment of accident were at age group of 0–14 years. The relation between the increase in thyroid cancer incidence in those exposed as children and adolescents at the time of the accident and absorbed dose in the thyroid gland has been clearly demonstrated. The increased incidence continues to occur and further changes are likely.

Childhood leukemia was one of the most important expected health effects of Chernobyl. Over the past 25 years the incidence increase of leukemia among those exposed in childhood has not been identified. The reports on an increase in the incidence of leukemia in Ukrainian children exposed *in utero* are few, do not coincide with statistics from the other countries and need thoroughful validation. In general population exposed to Chernobyl fallout the assessments for solid cancers other than thyroid haven't demonstrated till nowadays a detectable increase. Our reader will have the possibility to estimate figures of solid cancer morbidity at the specific parts of this book. To our opinion solid cancer relationship with irradiation among clean-up workers and evacuees has to be proven and it's not a time to make final decisions.

Still there is controversy over many health consequences of the accident to date, and considerable variability in the assessment of the potential consequences in the future which merits careful, well designed studies of various health outcomes. Of special potential importance is a cohort of offspring of clean-up workers so that transgenerational effects can be studied. A family study performed jointly by the RCRM and the Institute of Labour Medicine together with British scientists has demonstrated present of immune function changes. Preliminary data from the later study in Narodichi district by the RCRM and the University of South Carolina also demonstrate an increase of non-tumor diseases rates in a progeny of exposed. Of importance for studies of radioiodine influence on cancers other than thyroid and non-cancer effects are the UkrAm and BelAm cohorts enumerating 25,000 exposed as children with established doses on thyroid.

The Chernobyl accident resulted in widespread radioactive contamination of populated areas of Ukraine and exacerbated the problem of the combined effects of low doses of radiation in combination with other man-made environment and industrial environment. Particular problem is worst in the Chernobyl exclusion zone, the area which has undergone the most powerful radioactive contamination, and where a substantial staff number works by a shift method. Staff groups include personnel of the Chernobyl

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no relationship of soil contamination with heavy metals and radionuclides, indicating the differences of their entry sources. The highest concentrations of heavy metals, particularly lead and zinc were determined along the highways. This suggests that in this case, the main source of heavy metals in the soil was the leaded gasoline and motor vehicles. In all the surveyed industrial sectors of the exclusion zone a maximum content of heavy metals in soil exceeded background values for this region from 1.5 to 7.9 times. At some study points the minimum concentrations of some metals were also higher than background values (lead, nickel, zinc). At the virgin lands of the 30 km zone the radionuclides have been mainly located in the upper layer of soil. In areas where decontamination was conducted and other works a deeper penetration was found. At the areas with an intensive excavation the activity of ^{238}Pu , ^{239}Pu , ^{240}Pu was recorded in the range of 2.1 to 4.0 Bq/kg, which does not preclude its penetration into the respiratory system with the dust.

The results of studies on the hygienic characteristics of working conditions of operators of heavy vehicles and drivers of heavy vehicles demonstrate that by the severity of impact factors of production of non-radiation nature in the most unfavorable conditions were bulldozer drivers. The intensity of the exposures depended on the type of machinery, their technical preparedness for work in radiation area and the type of work performed. From the data presented in the table that the working conditions at the bulldozers were characterized by excess noise on the remote control of 3–20 dB. Levels were higher for overall vibration for 5–12 dB, local — 2–5 dB. Dustiness of air as the working area was the highest in-cab tractors, exceeding the MPC by 3–12 times, and in some samples of samples — up to 17 times. In some cases, in the working area of bulldozers the petroleum hydrocarbons and carbon monoxide were noticed at an increased levels (1.5–2.0 times).

The content of dust in the air cab trucks and trucks exceeded the limits from 2 to 10 times; in the cabins of excavators and scrapers the dust levels were close to normal values. A dispersed dust composition was as follows: the dust particles less than 1 micron were 10%, from 1 to 5 microns — about 70% and from 5 to 10 microns — 20–25%. The air dust samples in the surveyed working areas contained ^{137}Cs at the average concentration of 0.033 Bq/l. Given that at 6-hour shift ventilation volume in human is about 9 m³, and in the working area there is up to 70% of dust particles, with the most deeply penetration ability into the respiratory tract, the dust factor can substantially increase the effective dose in employees.

The obtained data are of importance for planning radiation protection strategies for the staff working at the Chernobyl exclusion zone. Such stage has started in 2003 when the recruitment of the personnel was started for construction works in the conversion of the Chernobyl "Shelter" into a radioecologically safe object. Strategies of radiation protection have been elaborated with the experience of studies of health effects after Chernobyl and implementation of the medical and biophysical control system of the work activities on the Shelter Implementation Plan (SIP) for the period of 2004–2009 have demonstrated its efficiency. A substantial set of data was collected on the changes of various parameters after low-dose and low-dose rate exposure starting from molecular to system levels. Efforts basing on the current levels of analysis are in need to assess this data and transform it to a knowledge for use in radiobiology and radiation protection.

The scale of Chernobyl and its consequences to health and environment have accumulated most of the research and practical health care workforce and resources. The tasks for radiation biology, medicine and protection are not limited to Chernobyl. Nuclear energy sector in Ukraine includes 50,000 occupationally exposed workers of the 15 power and research units, uranium mines and radioactive waste and spent fuel storages; radiation sources are widely used in industry, science and medicine. Additional groups include population of the zones surrounding the other nuclear sector objects. Creation of the unified register including dosimetry and health data is essentially actual. No centralized health registry for nuclear workers is supported in Ukraine, but there exist local data sources that could be used for the creation of such system. The individual data exist on the local levels of the medical-sanitary departments and the dosimetry shops of the nuclear objects. Regional registries with individual data are supported for radiologists. National cancer registration system includes local and centralized registry with sufficient quality of patient identification and clinico-pathological data, but lacking information on the professional radiation exposure status. A registry with the extended health and dosimetry data including doses of external irradiation and transuranium elements incorporation is supported by the RCRM for staff of the "Shelter" object. Aggregated information is available for the Ministry of Health Care and Energoatom company from the local statistical reports.

The understanding exists of the improvement of the health care system for nuclear workers and population of the zones of control inside the government and the local authorities of Ukraine. The task of establishment of the national dose registry in Ukraine is formulated in a number of legislative acts (State Laws, Decrees of the Cabinet of Ministers, radiation safety regulations), however, *de facto* this work was not initiated so far due to lack of budgetary funding. The existing experience French and other international registries as well as of the State registry of exposed after Chernobyl, the Clinical-epidemiological registry, and dosimetry databases gained in the Research Center for Radiation Medicine from the prospective follow-up studies could help for practical implementation of such united registry.

Quarter of century after Chernobyl is not only the time to время собирать камни, время разбрасывать камни. Several projects including the EU ARCH present analysis and strategies of future research. The priorities in the field of radiation cancers risks include:

- (a) to assess if there is a risk of thyroid cancer in those who were adults at exposure, and first of all clean-up workers and evacuees of the first period with the maximal release of radiiodine and other short-living isotopes incorporating to the thyroid. Last studies show the possibility of thyroid dose reconstruction from radiiodine incorporation;
- (b) for thyroid cancer in those exposed in childhood the change in risk with time since exposure, the role of dietary iodine and the role of inherited factors and other subsidiary tasks have to be evaluated;
- (c) radiation risks of a non-CLL leukemia show a tendency to decrease. The studies have to be concentrated on CLL, lymphomas and multiple myeloma;
- (d) breast cancer studies with the priority to female liquidators and females from the highly contaminated territories;

- (e) Tumors with a longer latency, where registry data suggest a possible radiation related increase.

In the field of radiation risks of non-cancer effects, the priority has to be given to analytical studies of cardiovascular and cerebrovascular diseases, cognitive effects and cataracts, among clean-up workers exposed to radiation in the range of low doses.

The development of retrospective dose reconstruction techniques on the thyroid gland from incorporated radioiodine, as well as restoration of doses to critical organs from ^{137}Cs , ^{90}Sr and transuranium elements should be considered as priorities in the field of dosimetry.

It is necessary to expand research on biological markers of radiation, radiosensitivity, and radiation induced disease to use them for the biological dosimetry and validation of the diagnoses in analytical studies. An epidemiological approach is needed to address the role of genetic and epigenetic factors as in radiation effects at cellular level, as well as a modifying role of age, gender and environmental factors. Tissue banking has to be expanded to store of biological specimens from exposed to low dose, family samples, immortalized normal and cancer cells from exposed.

It was not possible to include to this book all the spectra of health data and opinions that arrived after the Chernobyl accident. Along with the results of the known international studies that are performed in Ukraine the editors tried to present assessments of the last period or those national studies that escaped the attention of the international scientific community. It has been our profound feeling and the last world events only provide confirm for it, that Chernobyl experience is of invaluable importance not only in radiation research and security but in planning the early response strategies, providing care to radiation exposed and assessment of late effects.

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