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DIAGNOSIS AND TREATMENT OF ACUTE RADIATION SICKNESS IN VICTIMS OF THE CHORNOBYL DISASTER

(brief medical and biological analysis)

On the 40th anniversary of the Chornobyl disaster. In loving memory of Professor L.P. Kindzelsky — with respect, gratitude, and a deep bow.

Aim: *to summarize (including our own research) the experience of clinical diagnosis, cytogenetic assessment, and treatment of acute radiation sickness in victims of the Chornobyl nuclear power plant accident, as well as to determine the significance of the combined use of hematological and cytogenetic methods in biological dosimetry and prognosis of radiation injuries.* ***Results*** *of clinical, hematological, and cytogenetic examinations were performed in individuals exposed to external and internal irradiation following the Chornobyl accident and diagnosed with grade I–III acute radiation sickness were analyzed. It has been demonstrated that combined clinical, hematological, and cytogenetic approaches are effective for diagnosis and retrospective assessment of acute radiation sickness severity. Cytogenetic dosimetry remains one of the most informative methods for biological assessment of radiation exposure.*

Keywords: *Chornobyl disaster, acute radiation sickness, cytogenetic dosimetry, chromosomal aberrations, bone marrow, radiation injury.*

Every major radiation disaster is unique in its own way, and therefore its experience can only be partially, and not mechanically, applied to post-Chernobyl events. Data from long-term studies of the consequences of the Chernobyl radiation disaster have revealed both similarities and differences between these significant tragic events of the past century [1–3].

The explosion of the fourth unit of the Chernobyl Nuclear Power Plant (ChNPP) on April 26, 1996, occurred as a result of the process of "reactor acceleration by fission neutrons", which is identical to the process of a nuclear explosion. The ground-level explosion of the Chernobyl reactor, which used low-enriched fuel, differs fundamentally from the high-altitude explosion of an atomic bomb containing highly enriched uranium-235 in Hiroshima. It was precisely the physical processes that occurred inside the destroyed reactor that caused a plume of radioactive noble gases and fine aerosols to rise to a great height, subsequently spreading across virtually the entire Northern Hemisphere.

The unique nature of the Chernobyl accident is due to a number of its specific features. It is the largest nuclear reactor accident that resulted in casualties among plant workers and firefighters. The accident was accompanied by a massive release of radionuclides into the environment — approximately 2×10^{18} Bq, which caused radioactive contamination of territories in various countries. Numerous segments of the population — several million people, including hundreds of thousands of participants in the accident cleanup (ULPA) — were exposed to radiation at levels exceeding safety standards.

The greatest danger to human life and health was, of course, in the immediate vicinity of the damaged reactor. As a result of the Chernobyl accident, relatively limited groups of people were exposed to significant external radiation (gamma and beta radiation): the shift on duty at the nuclear power plant (about 200 people) and 300 construction workers on the reactor units within 1 km of the accident site, as well as firefighters and members of emergency response teams. In the first 12 hours after the accident, 237 individuals were diagnosed with acute radiation syndrome (ARS) of grades I–IV (radiation doses ranged from

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1 to 6 Gy and higher), of whom 2 died at different times.

Thus, a distinctive feature of the Chernobyl disaster was that large segments of the population, including high-risk groups and the descendants of irradiated parents, were forced to remain constantly in environments with radiation levels above background levels, which were caused not only by the external gamma component but also by radionuclides entering the body via inhalation.

In the first days following the accident, the primary diagnostic task was to assess the severity of bone marrow syndrome based on the dose of external total radiation exposure, as determined by the number of lymphocytes and the frequency of chromosomal aberrations in peripheral blood lymphocyte cultures [4]. Almost every victim of the Chernobyl accident with suspected BMD underwent cytogenetic assessment of the absorbed dose based on the level of chromosomal aberrations, which was based on an *in vitro* model previously developed to assess changes in the chromosomal apparatus following total (whole-body) irradiation. Determining the nature of the dose-response relationship based on these data and constructing empirical curves for the levels of neutrophils, lymphocytes, and platelets was used for dosimetric assessments of the victims.

In the first days, weeks, and months following the accident at the Chernobyl NPP, the full attention of medical specialists was focused on victims diagnosed with ARS of grades I–IV who were present at the NPP site in the immediate vicinity of the accident zone: operators of Unit 4 of the nuclear power plant, on-duty shift workers and support staff, firefighters, and turbine hall workers. Under psychological stress, a significant number of firefighters and nuclear power plant workers continued working until they lost consciousness. The second, no less significant factor was the impact on the body of aerosols from burning bitumen, rubber, plastics, and other materials, as well as the elevated ambient temperature. The lack of individual dosimetric monitoring prevented the prompt medical triage of affected individuals based on the radiation dose received and the formulation of an individual prognosis for the development of radiation-induced pathology. This resulted in delays in seeking medical care, ranging from a few minutes to several days, and in some cases even up to 2–3 weeks, by which time symptoms of ARS or progressive radiation damage to the skin and mucous membranes had begun to manifest [4].

The victims were exposed to four factors of the radiation accident: short-term, relatively uniform external irradiation (gamma + beta) from the gas cloud of the release; decreasing external gamma and beta irradiation from fragments of the damaged reactor core scattered across the industrial site; inhalation of gases and aerosol particles containing a mixture of radionuclides, and the deposition of these particles on the skin and mucous membranes. The primary exposure was general, relatively uniform external gamma irradiation of the entire body and beta irradiation of large body surfaces.

Diagnosis of acute radiation syndrome. In the first days following the accident, the primary diagnostic task was to assess the severity of bone marrow syndrome based on the dose of external whole-body radiation. This was made possible by methods developed prior to the accident, specifically by counting lymphocytes and the frequency of chromosomal aberrations in peripheral blood lymphocyte cultures, or by counting aberrations in bone marrow cells [5, 6]. Later, these data were used to predict the overall progression of the blood picture. Almost every victim of the Chernobyl accident with suspected acute radiation syndrome underwent a cytogenetic assessment of the absorbed dose based on the level of chromosomal aberrations, which was based on an in vivo model previously developed to assess changes in the chromosomal apparatus during total (wide-field) therapeutic irradiation. However, it should be noted that the blood response to therapeutic irradiation can vary significantly under the influence of the pathological process for which radiation therapy is prescribed. Many years of experience in conducting radiation cytogenetic studies show that adhering to certain requirements when selecting metaphase slides allows for increased accuracy in recording structural chromosomal abnormalities and reduces the subjective factor in their identification. The degree of chromosome coiling must ensure clear identification of the chromatids and centromeres of all chromosomes without exception. The optimal level of chromosome coiling should be within the following limits: at the maximum, small acrocentrics are clearly visible as chromosomes; at the minimum, chromosomes are separated into two chromatids and lie apart from one another. Therefore, the determination of the nature of the dose-response relationship based on these data and the construction of empirical curves for the content of neutrophils, lymphocytes, and platelets were used for dosimetric assessments of radiation exposure in the victims.

The total number of personnel affected who were working at the Chernobyl Nuclear Power Plant on April 26, 1986, was 203 (as reported at the IAEA meeting in August 1986); of these, 115 were treated at a specialized hospital beginning on the second day. Differential diagnosis between Grade I acute radiation syndrome and radiation reactions in other patients was conducted according to generally accepted criteria throughout 1986.

Health damage caused by acute radiation syndrome is determined primarily by its severity, which, on the one hand, reflects the extent of destructive processes and, on the other, compensatory and restorative processes in the body during the acute phase and recovery stages. Structural and functional disturbances in the activity of organs and systems that persist in the long

term are eventually compensated for in some individuals who have undergone Grade I acute radiation syndrome, and their overall health status is assessed as stable. However, over the past period, various non-stochastic (deterministic) and stochastic effects of radiation exposure have developed in the majority of patients. The biological age of the affected individuals (an integral indicator of health) exceeded the so-called population standard by an average of 6.5 years.

Given the importance of using the experience of the Chernobyl disaster to deepen our understanding of the diagnosis of radiation damage across a wide range of doses, we present the clinical and hematological indicators of radiation damage to the body, which were studied using the example of 53 patients with acute leukemia who were treated at the clinic of the Kyiv Scientific Research Institute of Radiology and Oncology (now the National Cancer Institute of the Ministry of Health of Ukraine).

When seeking medical care 1–3 days after participating in the cleanup of the accident's aftermath, patients who subsequently developed acute leukemia complained of weakness, irritability, abdominal pain, and diarrhea, with a broader range of symptoms than immediately after exposure. The basis for the initial diagnosis and determination of the severity of acute leukemia was a decrease in the total white blood cell count, as well as the percentage and absolute number of lymphocytes in the blood, combined with the initial symptoms of systemic intoxication resulting from acute radiation exposure.

The final diagnosis of acute leukopenia and its severity were established based on the dynamics of blood parameters, bone marrow examination data, and cytogenetic dosimetry, in combination with clinical symptoms of radiation-induced intoxication in the affected individuals.

The clinical course and the periods of development of acute leukemia of grades II–IV differed from traditional concepts of this disease in that the development of bone marrow syndrome and blood cytopenia was delayed due to the delayed effects of incorporated radionuclides. Certain difficulties arose in diagnosing grade I acute leukemia due to the unclear transition from subclinical to clinical signs of the body's reaction to radiation exposure, including individual radiosensitivity [5–7].

Among the victims diagnosed with Grade I acute leukopenia based on hematological parameters, three groups were identified. Group I consisted of individuals with leukopenia, with a white blood cell count of $(2.1–3.0)\times10^9$/L. In this case, the percentage of lymphocytes corresponded to a normal blood count, while the absolute count was below normal $(0.38–0.93)\times10^9$/L. Group II, based on hematological parameters, consisted of individuals with a normal white blood cell count. Lymphopenia was observed (4–12%), with absolute values of $(0.408–0.576)\times10^9$/L, which persisted for 5–7 days. Subsequently, a wave-like pattern of changes in blood cell composition was recorded — a decrease in the total white blood cell count to $(3.7–2.7)\times10^9$/L with lymphocyte percentages and absolute counts close to normal. A wave-like decrease in white blood cell counts was observed at intervals of 12–14 days. Group III included patients in whom leukopenia was not detected against a background of normal, reduced, or slightly elevated peripheral blood leukocyte counts. After 20 days, the lymphocyte count was within the range of $(0.8–1.05)\times10^9$/L. At the same time, the wave-like pattern in the decrease in leukocytes persisted.

Analysis of dynamic blood parameters in patients with a final diagnosis of Grade I acute leukopenia indicates heterogeneity in changes, particularly during the first 5–7 days following irradiation. With the exception of a possible contribution from the body's individual radiosensitivity, other mechanisms for these effects can also be considered. They may be caused by both external and internal irradiation due to incorporated radionuclides. In the first case, the classic form of acute leukopenia as a bone marrow syndrome with changes in blood cell composition is more clearly observed. In the second case, when radioactive isotopes account for the majority of the total dose, changes in blood parameters appear with a delay, since the integral radiation dose itself accumulates over a certain period of time. Under these conditions, "timely excretion of radionuclides can and should contribute to reducing the extent of changes in hematological parameters and the potential severity of acute leukemia during its manifestation."

In patients with grade II acute leukemia, the white blood cell count was within the range of $(2.0–3.2)\times10^9$/L. Such low white blood cell counts persisted for 12–15 days after irradiation, followed by a temporary rise to subnormal levels. The percentage of lymphocytes varied from 10 to 70%. Regardless of the percentage, the absolute lymphocyte count was reduced in all patients and ranged from $(0.42–0.65)\times10^9$/L. In cases of severe leukopenia, granulocytopenia was observed with a preserved lymphocyte count 0.5×10^9/L.

In patients with stage III acute lymphoblastic leukemia (ALL), marked changes in blood parameters were observed, caused by bone marrow pancytopenia. Restoration of blood parameters at the critical moment was possible only through donor bone marrow, which temporarily took over the function of hematopoiesis.

Thus, the initial response of hematological blood parameters, based on quantitative and temporal criteria, was inadequate relative to the subsequent level of hematopoietic dysfunction. As a rule, leukopenia and lymphocytopenia developed later compared to the classical form of acute leukemia. Blood cytopenia exhibited a wave-like pattern.

In cases of acute radiation injury, the need to identify a set of informative biological diagnostic methods

primarily concerns the early (latent) stage of the disease, since in the advanced stage of the disease, the clinical picture of acute leukemia is informative in itself. For the early diagnosis of the degree of radiation damage in the event of radiation incidents, it is most effective to use a combination of hematological methods in conjunction with cytogenetic dosimetry.

In the context of the Chernobyl accident, the use of a method for counting chromosomal aberrations in blood lymphocytes, along with hematological indicators (cytopenias), was decisive for verifying absorbed doses.

The diagnosis of ARS and its severity were established based on primary clinical symptoms (nausea, vomiting, headache, fever, damage to the skin and mucous membranes, etc.) manifesting over time following exposure; based on the curves showing changes in the number of leukocytes, lymphocytes, and granulocytes during the first month after irradiation; based on the cellularity of sternal bone marrow aspirates, myelograms, and the percentage of degenerating cell forms obtained during the first week and by the end of the first month after irradiation, as well as on the assessment of these parameters at the end of treatment (upon discharge from the hospital).

Cytogenetic assessment of the absorbed dose of ionizing radiation was performed in the first weeks following the accident based on metaphase analysis of chromosomal aberrations in peripheral blood lymphocyte (PBL) cultures from patients. All chromosomal aberrations detectable by conventional staining of cytogenetic preparations were taken into account. On average, 300 metaphases were analyzed for each patient. Absorbed dose values for patients were determined using the obtained cytogenetic curves, dose calculation tables, and calibrated dose curves

It must be assumed that as the time elapsed since irradiation increased, the proportion of irradiated lymphocytes in peripheral blood, which carry potential radiation-induced changes in the chromosomal apparatus, decreased.

Intensive therapy administered during the latent phase of the disease reduced the potential radiation dose to the body, as evidenced by a decrease in the number of chromosomal aberrations in patients' lymphocytes. Taking into account the dynamics of clinical symptoms and hematological parameters, the potential severity of acute leukemia during its active phase decreases. If, however, active pathogenetic therapy was not administered during the latent phase of OLB, cytogenetic changes in the cells largely persist, and the symptoms of OLB during the active phase are more pronounced.

In this study, cytogenetic examinations were conducted on patients who underwent active therapy aimed at treating ARS (entero- and hemosorption, blood transfusion, bone marrow transplantation).

Based on the cytogenetic data obtained, methodological recommendations titled "Cytogenetic Assessment of the Severity of Acute Radiation Sickness" were developed. The main group consisted of 30 radiation-exposed individuals with a verified diagnosis of acute radiation sickness (ARS) of grades I–III severity and unknown absorbed dose values, who underwent cytogenetic examination at the clinic during the course of intensive detoxification therapy [4, 8].

For the retrospective assessment of the severity of OLB, we propose for the first time a multiple linear regression model using the classical least squares method (LSM) [4]:

$$y = c_1 \cdot x_1 + c_2 \cdot x_2 + ... + c_n \cdot x_n + c_0,$$

where c_0, c_1, c_2, ..., c_n are the OLS estimates of the linear regression coefficients; x_1, x_2, ..., x_n are explanatory variables (cytogenetic indicators); and y is the dependent variable (measure of the degree of OLB), whose values are determined by x_1, x_2, ..., x_n. The following indicators of chromosome damage were considered: frequency of damaged cells (x_1), frequency of chromosome aberrations (x_2), frequency of paired fragments (x_3), dots (x_4), centromeric rings (x_5), dicentrics (x_6), translocations (x_7), fragments (x_8), and chromatid-type exchanges (x_9).

For most cytogenetic parameters, correlation coefficients ($R = 0.5–0.7$) indicate a moderate correlation with the degree of radiation damage.

Analysis of the results showed that, for the retrospective assessment of the degree of OLB, multiple linear regression—which takes into account a set of cytogenetic markers — is preferable to univariate linear or linear-quadratic regression based on individual markers, as it yields a lower number (percentage) of errors. The highest accuracy (minimum number of errors) in determining the degree of OLB is achieved with a set of cytogenetic markers x_1, x_3, x_5, $x_{(7)}$, and x_9. Such accuracy is already achieved with just 3 variables—$x_{(1)}$, x_3, and $x_{(7)}$ (frequency of damaged cells, paired fragments, and abnormal monocentric cells), and further improvements in accuracy were minimal upon adding additional indicators. The adequacy of the proposed method is confirmed by the preservation of group differences in the estimates of the degree of acute radiation sickness for individuals with a verified diagnosis (*Table*) [4].

In all patients with OLB presenting with marked leukopenia, lymphopenia, thrombocytopenia, or pancytopenia upon admission to specialized clinics, bone marrow examination via sternal puncture was performed.

The manifestation of primary lesions in patients with OLB did not always correspond to the severity of the radiation pathology. Thus, the initial response of blood parameters in terms of magnitude and timing did not fully correspond to the subsequent level of hematopoietic dysfunction. As a rule, a delay in the development of leukopenia and lymphopenia was observed compared to the classical form of OLB. Blood cytopenias exhibited a wave-like pattern.

Table

Results of a retrospective assessment of the severity of acute radiation sickness using multiple linear regression [4]

Indicators	Number of errors	Percentage of errors	s2
x_1, x_2	11	36.667	6.54992
x_1, x_3, x_7	5	16.667	4.45414
x_1, x_3, x_5, x_7	4	13.333	3.81872
x_1, x_3, x_5, x_7, x_9	3	10,000	3.09612
$x_1, x_2, x_3, x_5, x_7, x_9$	4	13.333	2.95482
$x_1, x_2, x_3, x_4, x_6, x_7, x_9$	4	13.333	2.89499
$x_1, x_2, x_3, x_4, x_5, x_6, x_7, x_9$	4	13.333	2.89398
$x_1, x_2, x_3, x_4, x_5, x_6, x_7, x_8, x_9$	4	13.333	2.89012

The dynamics of cytogenetic surveillance in individuals with OLB were investigated based on natural killer cell counts. It was established that ionizing radiation suppresses and destroys the killer cell system in irradiated individuals. A parallel relationship was identified between the radiation dose, the severity of radiation pathology (degree of OLB), and the level of disruption of cytogenetic surveillance.

Bone marrow parameters regarding cellular composition and morphological features varied significantly depending on the severity of radiation pathology and the time elapsed since irradiation.

Those who developed radiation-induced leukemia (RIL) in the long term suffered from multiple chronic diseases of internal organs and systems (ranging from 5–7 to 10–12 concurrent diagnoses). In the first 5 years following the Chernobyl accident, this group of victims experienced a sharp increase in diseases of the cardiovascular, digestive, nervous, and hepatobiliary systems. Twenty-five years after the accident, the proportion of individuals with somatic diseases was 85% or higher. In the long term, OLB convalescents developed radiation-induced cataracts and various hematological syndromes associated with a decrease in the number of mature cells in peripheral blood. The age of those who died from malignant neoplasms and cardiovascular diseases was below the average life expectancy of the Ukrainian population.

Thus, in cases of radiation incidents, to assess the severity and prognosis of radiation-induced pathology and to improve biological dosimetry, it is advisable to conduct a comprehensive, in-depth examination of irradiated individuals: hematological (analysis of peripheral blood parameters), cytogenetic (analysis of the level and spectrum of radiation-induced chromosomal aberrations in blood lymphocytes), and analysis of quantitative and morphological bone marrow parameters in comparison with clinical radiation manifestations [9, 10].

Bone marrow transplantation. Despite the comprehensive therapy administered, a significant number of patients with acute lymphoblastic leukemia (ALL) developed severe bone marrow syndrome. The indicators of bone marrow blood cell composition were life-threatening for the patients. The use of freshly collected donor blood and its components proved ineffective in a number of cases. Therefore, 11 patients underwent transplantation of freshly collected or cryopreserved bone marrow. In all of these patients, blood parameters returned to normal. No immediate or long-term complications were observed following bone marrow transplantation. It has been clinically proven that bone marrow transplantation is a highly effective treatment for life-threatening damage to hematopoietic tissue. The goal of transplantation is to temporarily replace hematopoietic function. The study was conducted by a team at the clinic under the leadership of professor L.P. Kindzelsky. The recommended method ensured a satisfactory diagnostic level (correspondence with the initial clinical and laboratory diagnoses), that is, it allowed for the clarification or confirmation of the degree of acute leukemia against the background of therapeutic measures [1, 8].

Thus, bone marrow transplantation in patients with ARS made it possible to save patients with life-threatening hematopoietic damage. The scientifically grounded set of diagnostic and therapeutic-preventive measures implemented made it possible to achieve a cure in all patients treated for acute radiation sickness; in 24 cases, to reduce the severity of the condition during the active phase; and in 19 cases, to prevent its development. Nevertheless, convalescents from acute radiation sickness remain a priority group for medical and biological monitoring.

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ДІАГНОСТИКА ТА ЛІКУВАННЯ ГОСТРОЇ ПРОМЕНЕВОЇ ХВОРОБИ У ПОСТРАЖДАЛИХ ВНАСЛІДОК ЧОРНОБИЛЬСЬКОЇ КАТАСТРОФИ (короткий медико-біологічний аналіз)

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Резюме. ***Мета:*** *узагальнити(в тому числі, і за результатами власних досліджень) досвід клінічної діагностики, цитогенетичної оцінки та лікування гострої променевої хвороби у постраждалих внаслідок аварії на Чорнобильській атомній електростанції, а також визначити значення комплексного використання гематологічних і цитогенетичних методів у системі біологічної дозиметрії та прогнозуванні перебігу радіаційних уражень. Проаналізовано (в тому числі, і за результатами власних досліджень) дані клініко-гематологічного та цитогенетичного обстеження осіб, які зазнали зовнішнього і внутрішнього опромінення внаслідок аварії на Чорнобильській АЕС та мали верифікований діагноз гострої променевої хвороби I–III ступеня тяжкості. Продемонстровано, що комплексне використання клінічних, гематологічних та цитогенетичних методів є ефективним підходом для діагностики та ретроспективної оцінки тяжкості гострої променевої хвороби. Цитогенетична дозиметрія залишається одним із найбільш інформативних методів біологічної оцінки радіаційного впливу.*

Ключові слова: Чорнобильська катастрофа, гостра променева хвороба, цитогенетична дозиметрія, хромосомні аберації, кістковий мозок, радіаційне ураження.

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