

PATTERNS OF HEMATOLOGICAL MALIGNANCIES IN CHERNOBYL CLEAN-UP WORKERS (1996–2005)

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Aim: The question as to whether the incidence of leukemias and malignant lymphomas among the Chernobyl clean-up workers increased in 20 years after the catastrophe is still a point of much controversy. Precise diagnosis of the main forms of hematopoietic malignancies according to FAB classification and new WHO classification and comparison of these data with that in the general population will be helpful in estimating the relative contribution of the radiation factor to the overall incidence of such pathologies. **Patients and methods:** The data on 218 consecutive cases of malignant diseases of hematopoietic and lymphoid tissues in Chernobyl clean-up workers diagnosed in 1996–2005 are given in comparison with the data of 2697 consecutive patients of general population of the same age group. The morphology and cytochemistry of bone marrow and peripheral blood cells were studied. Immunocytochemical techniques (APAAP, LSAB-AP) and the broad panel of monoclonal antibodies to lineage specific and differentiation antigens of leukocytes were employed for immunophenotyping leukemic cells. **Results:** Various types of oncohematological diseases developing 10–20 years after Chernobyl accident were registered in a group of clean-up workers under study including myelodysplastic syndromes (MDS), acute leukemias (ALL and AML), chronic myelogenous leukemia (CML) and other chronic myeloproliferative diseases, chronic lymphocytic leukemia (B-CLL) and other chronic lymphoproliferative diseases of B and T cell origin. MDS percentage among patients of clean-up workers group tended to exceed MDS percentage in the group of patients representing the general population examined at the same period (4.58 vs. 3.70%). Among 34 AML cases, leukemia was preceded by MDS in seven patients. The relative contribution of CML to the total number of clean-up workers with leukemia was higher than the corresponding percentage value in general population examined at the same period (9.17 vs. 6.59%). B-CLL was a predominant form of hematopoietic malignancies in clean-up workers under study (25.68%). Nevertheless, B-CLL percentage in patients of clean-up workers group did not differ significantly from that in the patients of general population. The multiple myeloma percentage (7.79%) in the group of patients belonging to clean-up workers in our study turned out to be twice as much as in the patients of general population (4.0%). **Conclusion:** The verified diagnosis of tumors of hematopoietic and lymphoid tissue according to modern classification (EGIL, WHO) could be the prerequisite for further molecular genetic and analytical epidemiology study of leukemias that may be related to Chernobyl NPP accident consequences.

Key Words: Chernobyl clean-up workers, leukemias, cytochemistry, immunophenotyping.

The development of leukemias and cancer seem to represent one of the most serious stochastic effects of the exposure to ionizing radiation. Meanwhile, the question as to whether the incidence of leukemias and malignant lymphomas among 101, 427 Ukrainian clean-up workers of 1986 and 43,366 clean-up workers of 1987 (average doses of 18.3 cGy and 10.8 cGy respectively) has increased in 20 years after the catastrophe is still a point of much controversy [1–7].

Unfortunately, previously the actual incidence of hematopoietic malignancies as well as the risks of increasing incidence of leukemias were assessed according to the primary data categorized on the basis of the obsolete ICD-9 (1975) classification where the acute leukemias have been registered in total without delineation of myeloid and lymphoid forms as well as the immunophenotypic variants. Moreover, until recently only cytomorphology and several cytochemical techniques were routinely used for the diagnostic purposes in oncohematology in the vast

majority of the hematological clinics in Ukraine. In 1993, the Reference Laboratory was set up as a public service in R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine with the aim of the precise diagnosis of the haematopoietic malignancies based on cytomorphology, cytochemistry, immunophenotyping in accordance with FAB, WHO, EGIL, ICD-10 and ICD-O-2 classifications.

The aim of the study is to present the data on the various forms and cytological variants of leukemia and lymphoma verified by Western standards in the consecutive group of Ukrainian Chernobyl clean-up workers developed in 10–19 years after Chernobyl accident, diagnosed in the Reference Laboratory in 1996–2005 and categorized according to the up-to-date classification schemes.

MATERIALS AND METHODS

Patients. In all, 218 clean-up workers admitted to oncohematological departments of Kyiv Regional Hospital No. 2, Kyiv Regional Hospital No. 1, Kyiv City Hospital No. 9 and clinics of Research Center for Radiation Medicine, Academy of Medical Sciences of Ukraine from February 1996 to December 2005 were examined in the Reference Laboratory (Immunocytochemistry Department of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of

Received: February 11, 2006.

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Abbreviations used: ALL – acute lymphoblastic leukemia; AML – acute myeloid leukemia; CLL – chronic lymphocytic leukemia; CML – chronic myelogenous leukemia; LGL-L – large granular lymphocytic leukemia; MDS – myelodysplastic syndromes; NHL – non-Hodgkin's lymphomas.

Ukraine). All the clean-up workers referred to the Reference Laboratory in the period stated above were examined consecutively without any previous selection of the cases. The radiation dose load of the clean-up workers under study varied from 7.5 to 25 cGy.

2697 consecutive patients of general population aged over 30, mainly the residents of Kyiv city and district (hereinafter referred as 'general population'), diagnosed in the Reference Laboratory at the same period comprised the group of comparison, again presented without any selection.

Methods. Bone marrow and peripheral blood smears stained by May-Grunwald-Giemsa were studied morphologically. Activities of myeloperoxidase, acid phosphatase (tartrate-sensitive and tartrate-resistant), non-specific esterase (sodium fluoride-sensitive), naphthol-AS-D-chloracetate esterase, alkaline phosphatase were analyzed cytochemically. Glycogen was assayed cytochemically by PAS-reaction. Immunocytochemical techniques (APAAP, LSAB-AP) and a broad panel of monoclonal antibodies (MoAbs) against lineage specific, differentiation and activation antigens of leukocytes were employed for immunophenotyping pathological cells [8].

The following antigens have been assayed immunocytochemically: the markers of hematopoietic stem cells and the markers of commitment (CD34, CD38, CD45RA, HLA-DR), the antigens of myeloid cells (CD33, CD13, CD15, CD64, CD16, MPO), the antigens of erythroid and megakaryocytic cells (CD71, CD61, CD62, CD41, CD42, glycophorin A), the antigens of T-cells (CD7, CD5, CD3, CD2, CD1a, CD4, CD8, CD45RO, γ TTCR) and B-cells (CD19, CD20, CD22, CD23, CD10, κ , λ and μ chains of immunoglobulins).

The main biological forms and cytological variants of hematopoietic malignancies were diagnosed according to FAB-classification, immunological classification of acute leukemias proposed by EGIL group, and REAL classification schemes. In 2000–2005 the diagnostic findings were revised in accordance with recently published new WHO classification [9].

RESULTS

The various types of oncohematological diseases developing in 10–20 years after Chernobyl accident were registered in a group of Chernobyl clean-up workers under study including myelodysplastic syndromes (MDS), acute leukemias (ALL and AML), chronic myelogenous leukemia (CML) and other forms of chronic myeloproliferative diseases, B-CLL and other forms of chronic lymphoproliferative diseases of B- and T-cell origin (Table).

Various forms of MDS have been observed in Chernobyl clean-up workers, namely refractory anemia (RA) and RA with the excess of blasts (RAEB-1 and RAEB-2). MDS percentage among patients of clean-up workers group tended to exceed MDS percentage in the group of patients representing the general population examined at the same period (4.68 vs. 3.70%).

The peculiar feature of AML in Chernobyl clean-up workers under study consisted in the development of leukemia in the setting of preceding MDS in 22.5% of

all AML cases studied. In some of these patients a *p53* gene mutation has been found [10, 11].

Table. Summary of leukaemia cases diagnosed in patients belonging to Chernobyl clean-up workers and general population in 1996–2005

Type of leukemia	Absolute number of patients (percentage is given in the brackets)	
	Chernobyl clean-up workers	General population
Myelodysplastic syndromes	10 (4.58%)	107 (3.70%)
Acute myeloid leukemia	34 (15.59%)	732 (27.14%)
Acute lymphoblastic leukemia	10 (4.58%)	214 (7.93%)
Chronic myelogenous leukemia	20 (9.17%)	178 (6.59%)
Polycythemia vera	5 (2.29%)	3 (0.11%)
Essential thrombocythemia	7 (3.21%)	–
Chronic eosinophilic leukemia/ Hypereosinophilic syndrome	2 (0.91%)	–
Chronic idiopathic myelofibrosis	2 (0.91%)	2 (0.07%)
Chronic myelomonocytic leukemia	6 (2.75%)	84 (3.11%)
Chronic lymphocytic leukemia	56 (25.68%)	791 (29.32%)
B-cell prolymphocytic leukemia	3 (1.37%)	23 (0.85%)
Hairy cell leukemia	8 (3.67%)	118 (4.37%)
Multiple myeloma	17 (7.79%)	108 (4.00%)
Non-Hodgkin's lymphoma (leukemic change)	28 (12.84%)	296 (10.97%)
Sezary syndrome	3 (1.37%)	8 (0.29%)
T-cell prolymphocytic leukemia	2 (0.91%)	3 (0.11%)
Large granular lymphocytic leukemia	5 (2.29%)	3 (0.11%)

ALL to AML ratio in the group of Chernobyl clean-up workers was typical of that in adult patients from general populations not exposed to ionizing radiation. Five subvariants of ALL of B-cell origin (ALL with phenotype of stem hematopoietic cell; pre-pre-B-ALL; common ALL; pre-B-ALL; B-ALL) and four subvariants of ALL of T-cell origin (blast cells with phenotype of subcortical, cortical and medullar thymocytes, and leukemic cells with γ T-cell receptor) were delineated upon immunophenotyping.

The relative frequencies of selected types of leukemias in clean-up workers and in the general population are given in Fig. 1. Several patterns concerning the relative contribution of particular leukemias forms into the total number of the leukemias having been studied are worthwhile to be noticed.

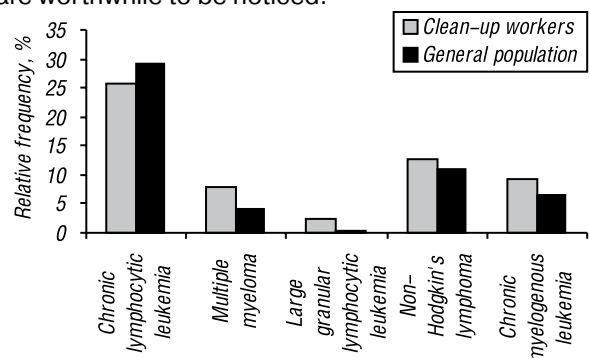


Fig. 1. Percentage of selected forms of hematopoietic malignancies in clean-up workers and in general population

First, the relative contribution of CML to the total number of clean-up workers with leukemia was higher than the corresponding percentage value in general population examined at the same period (9.17 vs. 6.59%). The multiple myeloma percentage (7.79%) in the group of patients belonging to clean-up workers in our study turned out to be about twice as much as in the patients of general population (4.00%).

In contrast, while B-CLL was a predominant form of hematopoietic malignancies in clean-up workers under study (25.68%), its percentage in the patients

of clean-up workers group did not differ significantly from that in the patients of general population.

The different forms of B-cell non-Hodgkin's lymphomas (NHL) in leukemization phase were diagnosed (follicular lymphoma, lymphoplasmacytic lymphoma, mantle cell lymphoma, splenic marginal zone B-cell lymphoma, diffuse large B cell lymphoma, extranodal marginal zone B-cell lymphoma of MALT type). The overall average percentage of NHL of B-cell origin among the patients of clean-up workers group tended to exceed that in the patients of general population.

In five patients working in Chernobyl in 1986–1987 the unusual chronic lymphoproliferative disorder, large granular lymphocytic leukemia (LGL-L) was diagnosed. Three of LGL-L cases belonged to T-cell subvariant (CD3⁺, CD5⁺, CD2⁺, CD7^{low}, CD4⁻, CD8⁺, CD56^{low}, CD57^{+/-}, CD16⁺, HLA-DR⁻) and two – to NK-cell subvariant (CD3⁻, CD5⁻, CD2⁺, CD7⁺, CD4⁻, CD8^{+/-}, CD56^{low}, CD57⁺, CD16⁺, HLA-DR^{low}). Recently Imamura [12, 13] described three cases of chronic NK-cell leukemia upon studying 52 patients with chronic neutropenia in Hiroshima A-bomb survivors.

Cytochemical study of the smears of bone marrow in clean-up workers suffering from malignant diseases of hematopoietic and lymphoid tissue as well as clean-up workers without overt malignant diseases revealed the villous cells which are strongly alkaline phosphatase-positive (Fig. 2). Such features were not evident in samples of bone marrow of the patients observed in pre-Chernobyl period.

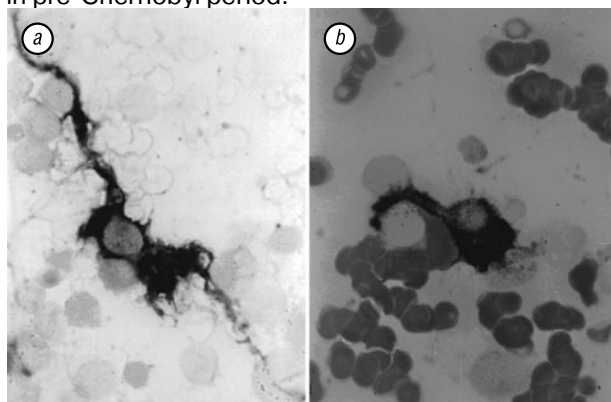


Fig. 2. Reactive response in bone marrow microenvironment in clean-up workers (a, b). Strongly alkaline phosphatase positive dendritic-like cells (endothelium or blood vessels? precursors of osteoblasts?), x 900

DISCUSSION

The long-term study performed by the Reference Laboratory in R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology in collaboration with the clinical facilities is one of the attempts to characterize in details the major forms and cytological variants of the oncohematological diseases in Chernobyl clean-up workers that became evident in 10–19 years after their exposure to radiation. The data on the relative frequency of the various forms of hematopoietic malignancies in the Chernobyl clean-up workers (218 consecutive cases) have been compared with the groups of the patients of the same age who have not been exposed to the ionizing radiation (2697 patients). The leukemias have been di-

agnosed according to FAB-classification as well as new WHO classification taking into account morphology and cytochemistry of bone marrow and peripheral blood cells and immunophenotype of leukemic cells assessed with the aid of the broad panel of MAbs to lineage-specific and differentiation antigens of leukocytes.

As it is seen from the data presented above, practically all the major forms of malignant diseases of hematopoietic and lymphoid tissues were diagnosed in the group of Chernobyl clean-up workers under study. The comparison of the relative distribution of the specified forms of hematopoietic and lymphoid malignancies in the patients diagnosed among Chernobyl clean-up workers demonstrates the increasing multiple myeloma rate, and the tendency to the increasing NHL (in leukemization phase) and CML rates as compared to the group of general population. The significant excess of LGL-L, which is extremely rare in the general population, is also worthwhile to mention.

B-CLL was shown to be a predominant form of hematopoietic malignancies in clean-up workers under study (25.68%). While in our study B-CLL percentage in patients of clean-up workers group did not differ significantly from that in the patients of general population, the question of B-CLL association with exposure to ionizing radiation seems to be worth of further study. Several authors in fact believe that there is not a persuasive basis for the accepted view that B-CLL is nonradiogenic form of cancer [14]. The delineation of specific B-CLL subtypes with different somatic mutations contributing to the genesis of the disease followed by the analysis of their incidence will undoubtedly clarify this subject.

The reactive responses in bone marrow stroma in clean-up workers suffering from malignant diseases of hematopoietic and lymphoid tissue exhibiting the strongly alkaline phosphatase-positive villous cells (endothelium of sinuses or blood vessels? cells precursors of osteoblasts?) does not seem to be incidental. The appearance of these cells in bone marrow of clean-up workers (both leukemia patients and patients with non-malignant diseases of hematopoietic and lymphoid tissue) could be regarded as a response to incorporation of the osteotropic heavy metals including radionuclides in endostal areas. It is highly probable that such cells that were not evident in the bone marrow of the patients observed in pre-Chernobyl period could serve as the markers of radiogenic leukemias. This problem deserves further studying.

Our data to some extent are comparable with the findings of the increased leukemia incidence (primarily acute and chronic myeloid leukemias) in the cohort of the Cheliabinsk region residents subjected to 5–20 years of the protracted radiation exposure due to Kyshtym accident [15].

In Japan in the cohort of 86,572 A-bomb survivors followed up in 1950–1990 upon the reclassification of hematopoietic malignancies according to FAB system accounting new dosimetry systems DS86 and DS02 the differential effects of radiation on major subtypes of human leukemias were shown. In particular, the incidence of multiple myeloma, ALL and CML was affected more than the incidence of other biological subtypes of leukemia [16].

At present, it is evident that only verified diagnosis could be the prerequisite for the advanced studies in analytical epidemiology of leukemias aimed at elucidating the role of the radiogenic factor in the pathogenesis of the malignant diseases of hematopoietic and lymphoid tissue [17, 18]. In this context, joint research efforts are of utmost importance to resolve this challenging issue.

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СТРУКТУРА ОПУХОЛЕВЫХ ЗАБОЛЕВАНИЙ СИСТЕМЫ КРОВИ У ЛИКВИДАТОРОВ АВАРИИ НА ЧАЭС (1996–2005)

Цель: вопрос о количественных показателях заболеваемости лейкозами и злокачественных лимфомах у ликвидаторов аварии на ЧАЭС, спустя 20 лет после Чернобыльской катастрофы, остается невыясненным. Диагностика этих заболеваний в соответствии с ФАБ- классификацией и новой классификацией ВОЗ, сравнение полученных данных с данными лиц, не подвергавшимся воздействию ионизирующего излучения, является необходимой предпосылкой для разрешения этого вопроса и выяснения роли радиационного фактора в развитии злокачественных заболеваний кроветворной и лимфоидной тканей. **Методы:** проанализированы 218 последовательных случаев лейкозов, диагностированных в 1996–2005 гг. у ликвидаторов аварии на ЧАЭС, по сравнению с группой лиц того же возраста, которые не подвергались воздействию излучения (2697 больных). Диагностику лейкозов осуществляли на основе морфологического и цитохимического изучения мазков крови и костного мозга, иммунофенотипирования лейкоэмических клеток с использованием широкой панели моноклональных антител к линейноспецифическим и дифференцировочным антигенам лейкоцитов. **Результаты:** спустя 10–20 лет после Чернобыльской катастрофы у ликвидаторов аварии на ЧАЭС выявлены различные формы онкогематологических заболеваний, включая миелодиспластические синдромы (МДС), острые лейкозы (ОЛЛ и ОМЛ), хронический миелолейкоз (ХМЛ) и другие миелолипролиферативные заболевания, В-клеточный хронический лимфолейкоз (В-ХЛЛ) и другие лимфолипролиферативные процессы В- и Т-клеточной природы. Установлена более высокая частота МДС в группе ликвидаторов по сравнению с группой лиц, не подвергавшихся воздействию излучения (4,58 и 3,70% соответственно). У 7 из 34 больных с ОМЛ в группе ликвидаторов заболевание развилось на фоне предшествующего МДС. Более высокой в этой группе была и заболеваемость ХМЛ (9,17 в сравнении с 6,59% в контрольной группе). В-ХЛЛ был преобладающей формой гемобластозов у ликвидаторов в целом, однако не было установлено существенных различий в частоте развития этого заболевания в сравниваемых группах. У ликвидаторов аварии на ЧАЭС почти в два раза чаще, чем у населения в целом, диагностировалась множественная миелома (7,79 в сравнении с 4,0%). **Выводы:** представленные данные могут служить основой для проведения последующих молекулярно-генетических и эпидемиологических исследований при основных формах гемобластозов, индуцируемых при действии ионизирующей радиации.

Ключевые слова: ликвидаторы аварии на ЧАЭС, лейкозы, цитохимия, иммунофенотипирование.