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Case Report Open Access

Myelodysplastic Syndrome in Patients with Acute Radiation Syndrome Following the Chornobyl Nuclear Power Plant Accident

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Abstract

The MDS cases (n=3) among acute radiation syndrome (ARS) survivors after the ChNPP accident were analyzed. MDS diagnoses were based on FAB (1982) and WHO classifications and included the refractory anemia (RA) (patient D. with ARS grade III), refractory anemia with ringed sideroblasts (RARS) (patient B. with ARS grade III), and unclassified MDS (MDS-U) (patient S. with ARS grade I). Clinical management of MDS in ARS patients was analyzed taking into consideration the morphological, immunological, and cytochemical peculiarities of haemopoietic cells. The ARS diagnoses were confirmed in a survey on a regular basis using standard cytogenetic method. Described MDS cases in ARS patients may be the secondary MDS variants taking into consideration possible radiation-induced injuries of hematopoietic cell genome due to the high dose of IR (1.7-5.5 Sv). The possible role of previous irradiation in a range of occupational dose limits before the ChNPP catastrophe for patients D. and B. with ARS grade III, both with possible role of such a confounding factor as petroleum exposure for patient S. (ARS grade I) for the development of MDS cannot be excluded.

Keywords: MDS; ARS; IR; ChNPP accident; PB; BM; Biodosimetry

Abbreviations:

MDS: Myelodysplastic Syndrome; ARS: Acute Radiation Syndrome; IR: Ionizing Radiation; ChNPP: Chornobyl Nuclear Power Plant; NRCRM: National Research Centre for Radiation Medicine; PB: Peripheral Blood; BM: Bone Marrow; MCV: Mean Corpuscular Volume or Mean Cell Volume

Introduction

Secondary Myelodysplastic Syndrome (MDS) is known to be associated with an impact of different negative factors including ionizing radiation of different origin (occupational, medical, accidental, etc.) [1,2]. The risk of MDS depends on magnitude of the absorbed radiation dose. A retrospective cohort study of atomic bomb survivors [3] revealed 151 patients with MDS in the Nagasaki University Atomic-Bomb Disease Institute cohort and 47 patients with MDS in the Radiation Effects Research Foundation Life Span Study cohort. The MDS risk existed in atomic bomb survivors from 40 to 60 years after the radiation exposure and showed a significant linear response to exposure dose level ($p<0.001$) with an ERR of 4.3 per Gy (95% CI: 1.6 to 9.5; $p<0.001$). The incidence of MDS among the ChNPP accident clean-up workers tended to exceed a respective value among population of Ukraine examined at the same period (4.58 vs. 3.70%) [4]. Monitoring of the cohort of acute radiation syndrome (ARS) survivors in the post-accidental period of the Chornobyl accident at the National Research Centre for Radiation Medicine (NRCRM) was performed since 1986 [5]. Three cases of MDS were diagnosed whereupon among the ARS patients. This case report hereby suggests a possible link between irradiation and development of MDS in ARS patients after the Chornobyl and allows considering these cases as the secondary MDS.

Case Presentation

The MDS cases (n=3) among ARS survivors after the ChNPP accident were analyzed. MDS diagnoses were based on FAB [6] and WHO [7] classifications and included the refractory anemia (RA) (patient D. with ARS grade III), refractory anemia with ringed sideroblasts (RARS) (patient B. with ARS grade III), and unclassified MDS (MDS-U) (patient S. with ARS grade I). Clinical management of MDS in ARS patients was analyzed taking in consideration the morphological, immunological, and cytochemical peculiarities of haemopoietic cells.

Biodosimetry data *vs.* the official dose records were compared. To assess the severity of radiation-induced chromosome damages in somatic cells both with its time pattern as well as to verify the radiation doses by cytogenetic criteria, an obligatory cytogenetic examination in different terms following the Chornobyl accident was required in all cases. Radiation doses were verified using a widely accepted method of biological dosimetry, namely by the frequency of cytogenetic radiogenic markers (dicentrics and centric rings) in uniformly stained metaphases of peripheral blood lymphocytes (PBL) following standard 48-hour cell cultivation [8]. From 200 to 400 metaphases corresponding to necessary requirements were analyzed in all cases, except one when conducting the cytogenetic analysis [9].

Patient D., born in 1931, was 64 years old at the time of RA diagnosis (ARS grade III). Being a professional worker at the ChNPP he was on duty at the 4th nuclear reactor overnight into the accident, April, 26, 1986. He was under a health survey in the NRCRM clinic since 1993. According to cytogenetic examination performed at the Institute of Biophysics (Moscow) his received radiation dose was 5.5

Sv. The latter confirmed a severity of radiation injury in the patient (ARS grade III). The organ dose to thyroid gland was 400 mSv.

For the first time a cytogenetic examination of patient D. was performed at the NRCRM in 1987 i.e. 22 months upon the exposure and was repeated in 1990 and 1995 afterwards. Results are shown in Table 1. Frequency of aberrant metaphases and especially the level of chromosome aberrations in all points of examination significantly exceeded the spontaneous value (~0-3 per 100 cells) [10]. Only 24 metaphases had been analyzed under first cytogenetic examination because of inhibition of cell division in the culture induced by intense irradiation and resulting low mitotic index. There were 46 aberrations in 75% of them (1.92 chromosome damages per cell) mainly of chromosome type, which is typical for human radiation exposure in high doses. Frequency of unstable radiogenic markers (i.e. markers of radiation exposure), namely the sum of dicentrics and centric rings being 33.3 per 100 metaphases significantly ($p<0.001$) exceeded the spontaneous level (0.1-0.2 per 100 metaphases) and at the time of examination corresponded to the absorbed radiation dose of ~350 cSv which was slightly lower vs. one established in 1986 (390 cSv) immediately after the Chornobyl accident due to gradual elimination of cells with unstable chromosome aberrations. In patient D. the grade III of ARS was confirmed by cytogenetic criteria.

Year of examination	Aberrant cells, % (M ± m)	Chromosome aberrations, per 100 cells (M ± m)	Frequency of chromosome aberrations, per 100 cells								
			Chromatid type			Chromosome type					
			single fragments	exchanges	Total	double fragments	dicentrics	centric rings	abnormal monocentrics	acentric rings	Total
1987	75.00 ± 8.84	46/24	2/24	0	2/24	36/24	6/24 25/100	2/24 8.30/100	0	0	44/24
1990	16.50 ± 2.62	37.00 ± 3.39	1.50	0	1.50	12.50	12.50	2.00	8.00	0.50	35.50
1995	17.71 ± 1.74	24.80 ± 1.97	1.25	0	1.25	5.63	2.50	1.88	12.71	0.83	23.55

Table 1: Results of cytogenetic examination of patient D. in due course

Further reduction of chromosome aberrations frequency mainly due to unstable cytogenetic markers of radiation exposure had been established under the cytogenetic examination conducted in 1990 and 1995 (200 and 480 metaphases analyzed, respectively). Thus, frequency of free double fragments decreased to 12.50 and 2.50 per 100 metaphases in each session, and total frequency of dicentrics and centric rings to 14.50 and 4.38 per 100 metaphases, respectively. However, the spectrum of aberrations changed essentially, as the frequency of stable cytogenetic markers of radiation exposure (abnormal monocentrics, appeared through the translocations and inversions and not eliminated and therefore accumulated with time) increased from 0.0 in 1987 up to 8.00 and 12.71 per 100 metaphases in 1990 and 1995, respectively [11]. Permanent increase in frequency of stable chromosome rearrangements had been indicative of damage not only in leucocytes, but also in hematopoietic stem cells and probably of formation of clones with identical chromosome aberrations resulting in their function breakdown. All that could play a certain role in MDS realization in patient D.

Patient was admitted to the NRCRM clinic on October, 10, 1995. Anemia was the main complaint. Patient's skin was pale. Weight loss and cough were of concern too. Physical examination at admission revealed no lymphadenopathy and normal size of spleen and liver. The chest examination revealed a lot of dry wheezing in both lungs.

Complete peripheral blood (PB) count at an admission showed the erythrocyte count of 2.53 T/L, 91 g/L Hb level, 104.3 fL erythrocyte MCV, platelet count of 112 G/L, white cell count of 6.17 G/L, 2% of myelocytes, 2% of metamyelocytes, 2% of band neutrophilic granulocytes, 31% of segmented neutrophilic granulocytes, 1% of basophilic granulocytes, 3% of eosinophylic granulocytes, 31% of lymphocytes, 28% of monocytes, and normoblast ratio of 10:100. Qualitative changes of leukocyte lineage manifested with presence of toxic granules, vacuolization, degranulation of granulocyte cytoplasm, binuclear cells, fragmentation of nuclei, vacuolization and basophilia of lymphocyte cytoplasm, nuclei fragmentation, decreased nuclear/cytoplasmic ratio, vacuolization and granularity of monocyte cytoplasm. Macroforms were registered in nearly 50% of platelets, and about 25% of them were giant cells. Biochemical parameters were normal. Immunophenotyping of PB cells showed the 44% of CD45+14 lymphocytes, 44.8% of CD3+22- lymphocytes, 38.5% of CD5+20- cells, 44.8% of CD7+33- cells, 23% of CD4+8- cells, 21% of CD4-8+ cells, CD4+/CD8+ ratio of 1.1, 7.7% of CD10-19+ cells, 0.2% of immature CD10+19+ B-cells, 0.5% of CD3-22+ cells, 6.5% of CD5-20+ B-cells, 2.3% of CD5+20+B- cells, 18.5% of activated T- and B-lymphocytes (CD13-HLADR+). Granulocytes showed normal CD10+33-13+DR- cell percentage and antigen expression, and monocytes featured low expression of HLADR-antigen.

Bone marrow investigation wasn't performed due to the patient's refusal. He was diagnosed as MDS, RA and symptomatic treatment was provided. Because of deterioration of patient's health due to anemia he was administered corticosteroids 30 mg per day orally on November 04. Patient had periodically received red cell infusions because of low red cell count. The platelet count normalized (280 G/L), and white cell count reached 13 G/L with gradual increase of lymphocytes up to 56.6% and monocytes to 28%.

In January 1996 the patient was transferred to Dermatology clinic of Munich University where the MDS diagnosis was confirmed by BM biopsy. There was a manifested MDS with domination of megacaryocytopoiesis, severe atrophy of the red BM combined with interstitial edema that confirmed a toxic damage of BM cells. The patient died at home 2 weeks later, so the duration of disease was 4 months (October, 1995-January, 1996).

Patient S., born in 1951, was 45 years old at the time of U-MDS diagnosis (ARS grade I, 1.7 Sv radiation dose). He had worked as a driver near the 4th nuclear reactor on April, 26, 1986 for about 20

minutes. Diagnosis of ARS was verified at Kyiv Regional ("Oblast") Hospital #1. Later on he was surveyed at Kyiv City Hospital #25 up to 1990. Cytogenetic examination was carried out annually since 1987 till 1992 due to the uncertainties in estimation of "biological" radiation dose, but yet before the manifestation of MDS (1996). Results of cytogenetic analysis are summarized in Table 2.

Year of Examination	Aberrant cells, % (M+m)	Chromosome aberrations, per 100 cells (M+m)	Frequency of chromosome aberrations, per 100 cells								
			Chromatid type			Chromosome type					
			single fragments	excha-nges	total	double fragments	dicentrics	centric rings	abnormal monocentrics	acentri c rings	total
1987	8.00+1.92	8.00+1.92	3	0	3	3	1	1	0	0	5
1988	8.00+1.92	8.00+1.92	2.5	0	2.5	4.5	0	0	0	1	5.5
1990	5.50+1.61	6.50+ 1.74	4	0	4	1.5	0.5	0.5	0	0.5	2.5
1991	6.50+1.74	6.50+1.74	2	0	2	1.5	2	0.5	0	0.5	4.5
1992	3.50+1.30	3.50+1.30	0	0	0	0.5	0	1	0	2	3.5

Table 2: Results of cytogenetic examination of patient S. in due course

As can be seen from the data in Table 2 the cytogenetic effect in all cases exceeded the average population norm but mostly due to simple aberrations, namely single and free double fragments, both with acentric rings. Frequency of unstable radiogenic cytogenetic markers (sum of dicentrics and ring chromosomes) was also slightly elevated over their spontaneous value (0.1-0.2 per 100 metaphases) and ranged from 0 to 2.50 per 100 metaphases, which corresponded to absorbed radiation dose no more than ~25 cSv (even under the first cytogenetic examination in 18 months following the accident). Such dose value is significantly different from that established in 1986 (170 cSv). Stable aberrations (chromosome exchanges) had not been registered in any examination point, probably because of their low spontaneous and radiation induced frequency and modest overall cytogenetic effect.

Year of examination	Aberrant cells, % (M ± m)	Chromosome aberrations, per 100 cells (M ± m)	Frequency of chromosome aberrations, per 100 cells								
			Chromatid type			Chromosome type					
			single fragments	exchanges	total	double fragments	dicentrics	centric rings	abnormal monocentrics	acentric rings	total
1987	10.50 ± 2.17	11.00 ± 2.21	1.00	0	1.00	0.50	3.50	0.00	6.00	0	10.00
1990	5.00 ± 1.54	5.00 ± 1.54	3.50	0	3.50	0.50	0.00	0.00	1.00	0	1.50

Table 3: Results of cytogenetic examination of patient B. in due course

The patient was surveyed at the NRCRM Clinic since 1991. In September, 1996 the PB count showed 71-86 G/L of platelets. The patient was admitted to the NRCRM Clinic on October 10, 1996. Main complaints were caused by hemorrhagic syndrome (petechiae, occasional gum bleeding) and anemia. The patient's skin was pale. Physical examination at admission revealed no lymphadenopathy, normal size of spleen and moderate hepatomegalia.

A complete PB count at admission showed the erythrocyte count of 3.43 T/L, Hb level of 87 g/L, 84.0 fL erythrocyte MCV, 81 G/L platelet count, 5.2 G/L white cell count, normal leukocyte formula, and 4:100 normoblast fraction. The BM biopsy done on October, 1 showed the myelokariocyte content of 20 G/L, 1.5% of blasts, and 27.25% of lymphocytes. Megakaryocytes were absent. The erythrocyte lineage was restricted; leuko/erythro ratio was 6:1, so the BM was hypoplastic. The 5:400 ratio of mitosis, 15:400 ratio of macrophages, and 4:400 ratio of reticular cells were revealed. Quality changes of haemopoietic cells include presence of toxic granules, vacuolization of granulocyte nuclei and cytoplasm, degranulation of cytoplasm of eosinophilic granulocytes, and vacuolization of lymphocyte cytoplasm.

The patient was diagnosed with U-MDS. Survey in 1996-1997 revealed a platelet count of 70-80 G/L followed by permanent decrease with reaching a minimal count of 1-2 G/L and presentation of hemorrhagic syndrome. There was a severe anemia with minimal erythrocyte count of 0.7 T/L, and 32 g/L Hb level. Red cell infusions were periodically administered to the patient. In October, 1998 the white cell count dropped reaching level of 0.7 G/L with gradual increase of lymphocyte count. Along the 6 last months of life (beginning from October, 2001) single myelocytes and metamyelocytes were serially revealed.

Occasionally (on December 16, 1996; on April 8, 1997; and on August 8, 2000) the BM biopsy revealed low count of myelokaryocytes (6-20 G/L), normal blast count (1.5-2.75%), moderately increased lymphocyte count (27.25-33.5%), both with restricted megakaryocyte and erythrocyte lineage. Leuko/erythro ratio was 8:1 (1997) and 6:1 (2000). There were such dysplastic abnormalities of haemopoietic cells, as presence of toxic granules, vacuolization and fragmentation of nuclei and cytoplasm of granulocytes, degranulation of cytoplasm of eosinophilic granulocytes, vacuolization of lymphocyte cytoplasm and

basophilic cytoplasm of lymphocytes, single cells were looking like hairy ones. There were also single macroforms and groups of 8-15 platelets.

Immunophenotyping of the PB and BM cells was provided. BM immunophenotyping (August, 08, 2000) revealed the 65.88% of CD45+14 lymphocytes, 48.86% of T-lymphocytes CD3+22-, 21.47% of T-helpers/inductors CD4+8-, 23.78% of T-supressor/cytotoxic lymphocytes CD4-8+, CD4+/CD8+ coefficient value of 0.9, 0.86% of B-lymphocytes CD10-19+, and 29.94% of activated T- and B-lymphocytes CD13-HLADR+. The last PB immunophenotyping (January 01, 2001) showed a reduced T-supressor count despite ratio of lymphocyte subpopulations was normal. B-lymphocytes activation was revealed at that. There were 83.43% of CD45+14 lymphocytes, 49.19% of T-lymphocytes CD3+22-, 47.49% of T-lymphocytes CD5+20-, 50.52% of T-lymphocytes CD7+33-, 29.05% of T-helpers/inductors CD4+8-, 17.72% of T-supressor/cytotoxic lymphocytes CD4-8+, 30.59% of B-lymphocytes CD10-19+, 0.08% of immature B-lymphocytes CD10+19+, 32.70% of B-lymphocytes CD3-22+, 27.97% of B-lymphocytes CD5-20+, 3.89% of B-lymphocytes CD5+20+, and 33.10% of activated T- and B-lymphocytes CD13-HLADR+. Immunophenotyping showed normal CD45+14- granulocytes in 14.11%, and normal monocytes CD45+14+ in 2.46%. There were no changes of immunoglobuline A, G, M content in PB.

Treatment was symptomatic with low effectiveness, a gradual exacerbation of hepato- and splenomegalia was going on. Finally the bilateral pneumonia and transmural myocardial infarction caused the death on May 19, 2002.

Patient B., born in 1941, was 42 years old at the time of RARS diagnosis (ARS grade III). Being a professional worker of the CHNPP he was on duty at the 4th nuclear reactor overnight into the accident on April, 26, 1986. He was surveyed at the NRCRM Clinic since 1991. According to cytogenetic examination at the Institute of Biophysics (Moscow) his incorporated radiation dose was 390 cSv.

Cytogenetic examination of patient B. had been conducted twice (1987, 1990), even before the diagnosis of hematological disease. Results are shown in Table 3. As can be seen from the data, in 1987 (17 months after the accident) the frequency of aberrant cells and chromosome aberrations significantly exceeded their spontaneous level with a significant excess of chromosome type aberrations being unstable (dicentrics) as well as stable (abnormal monocentrics) markers of human radiation exposure (3.50 and 6.00 per 100 metaphases, respectively) [11]. By reference to stable aberrations frequency the absorbed radiation dose is not to be less than ~100 cSv at the time of survey. This value is significantly lower than dose established at the Institute of Biophysics in 1986 (390 cSv). Under a repeated examination (1990) the cytogenetic effect decreased significantly due to complete disappearance of dicentrics and significant reduction of abnormal monocentrics (1.00 per 100 metaphases). All that indicate an extensive elimination of aberrant lymphocytes not only from PB, but also from irradiated hematopoietic stem cells of the BM.

PB monitoring of patient B. revealed some moderate thrombocytopenia (100 G/L) and leukopenia (3.7 G/L) on December 07, 1992. One week later after an acute respiratory viral infection the PB count showed 60 G/L of platelets. The patient was admitted to the NRCRM Clinic on March 02, 1993. Low-grade fever (up to 37.4 °C), nose and gum bleedings, cough with moderate hemoptysis, and dark feces were the main complaints. Moderate pallescence, petechia on lips, and bleeding gums were revealed at physical examination. Physical examination at admission revealed no lymphadenopathy and normal size of spleen and liver. The chest examination revealed a lot of dry wheezes in both lungs.

Complete PB count at admission showed the 2.66 T/L of erythrocytes, 68 g/L Hb level, 46 G/L of platelets, 2.95 G/L of white cells, 2% of myelocytes, 10% of band neutrophilic granulocytes, 31% of segmented neutrophilic granulocytes, 1% of eosinophilic granulocytes, 48% of lymphocytes, 1% of monocytes, 16 mm/hour erythrocyte sedimentation rate, pronounced anisocytosis (++) and poikilocytosis (++), and normoblast ratio of 10:100. The biopsy on March 03, 1993 showed hypoplastic BM (13.0 G/L of myelokaryocytes) with features of dyserythropoiesis such as karyorhexis in normoblasts, normoblasts with 2 nuclei, chromatin "bridges" between the fragmented nuclei, vacuolization of nucleus and cytoplasm of normoblasts. There were 30.0% of ring sideroblasts. Immunophenotyping of PB and BM cells showed the 33.0% of CD45+14 lymphocytes, 22.0% of T-lymphocytes CD3+22-, 18.8% of T-helpers/inductors CD4+8-, 11.5% of T-supressor/cytotoxic lymphocytes CD4-8+, CD4+/CD8+ coefficient 1.64, and 15.6% of B-lymphocytes CD3-22+. BM histological study (March 03, 1993) revealed the enhancement of fatty bone marrow and hypoplasia of red cell lineage, small groups of dysplastic normoblasts, and BM fibrosis. Osmotic resistance of erythrocytes was reduced, and 36 mcmol/L iron content could be a result of erythrocyte hemolysis. Immunoglobulin content was the following: IgA – 0.43 g/L, IgG – 14.73 g/L, IgM – 1.87 g/L.

The patient was diagnosed with MDS, RARS. A symptomatic treatment was provided (orally prednisone 120 mg daily, red cell and platelet infusions). One week later the prednisone dose was increased to 240 mg daily because of exacerbation of hemorrhagic syndrome. Next BM biopsy samples (March 03 and April 16, 1993) featured a progressive depression of hemopoiesis with enhance of red cell lineage up to 59.5% and dyserythropoiesis. The ring sideroblast count was 70.0-80.0%. Megakaryocyte lineage was depressed and dysplastic. Percentage of blast cells was normal. Repeated cytochemical investigation revealed a high increase of nonspecific esterase coefficient (0.7-1.65) and glycogen coefficient count of 0.24-0.52. PAS-positive lymphocytes increased to 66% and reduced to 22% during the period of survey being therefore above the normal rate.

Severe hemorrhagic syndrome, failure of diabetes compensation, hepatitis exacerbation and acute pneumonia complicated with pulmonary edema caused the patient's death on April 27, 1993.

Discussion

The ARS diagnosis and its severity degree in each MDS case mentioned above were confirmed in a survey on a regular basis using standard cytogenetic method. Frequency of unstable and stable cytogenetic radiogenic markers (dicentrics, ring chromosomes, and abnormal monocentrics, respectively) was assayed in peripheral blood lymphocytes uniformly stained in metaphase after a standard 48-hour cell culturing. In patient D. the absorbed radiation doses identified by cytogenetic markers at the Institute of Biophysics (Moscow) in 1986 and at the NRCRM in 1988 differed slightly (550 and 350 cSv, respectively), but nevertheless confirmed the severity of radiation injury. Results of our repeated cytogenetic examinations showed slow elimination of cells with unstable chromosome aberrations and relatively long-term storage of stable chromosome damages in somatic cells, just not only in lymphocytes, but probably in BM stem cells. In

patient S. (170 cSv documented incorporated dose in 1986) the cytogenetic effect under all surveys conducted at the NRCRM significantly exceeded a population norm of chromosome aberrations, but mostly due to simple aberrations i.e. single and free double fragments. Frequency of unstable radiogenic cytogenetic markers was also slightly higher vs. spontaneous values with fluctuation from 0 to 2.50 per 100 metaphases which corresponded to the absorbed radiation dose of no more than ~ 25 cSv. Stabilization of increased cytogenetic effects under the repeated examinations may indicate an efficient repair of radiation-induced chromosome damages in somatic cells of the patient S. In patient B. the 390 cSv incorporated dose was established at the Institute of Biophysics (Moscow) in the first months following the Chornobyl accident. After 17 months following the accident we found that the frequency of aberrant cells and chromosome aberrations significantly exceeded their spontaneous level with essential advantage of chromosome-type aberrations, namely the unstable (dicentrics) and stable (abnormal monocentrics) markers of IR exposure in human. By reference to the frequency of these markers an absorbed radiation dose could be about 100 cSv at the time of the survey. Under a repeated cytogenetic examination (1990) the cytogenetic effect decreased significantly due to the complete disappearance of dicentrics and significant reduction in the rate of abnormal monocentrics, which confirms intense elimination of not only aberrant lymphocytes from PB, but possibly exposed BM stem cells with chromosome abnormalities. It was evident not only in the intensity and specificity of radiation-induced damage of chromosomes, but also in due course pattern, namely in elimination rate of cells with unstable chromosome aberrations, possibility and duration of cell survival with stable chromosome aberrations and formation of abnormal clones. Inefficiency of genome reparation at the chromosome level was confirmed. Such individual characteristics of radiation-induced cytogenetic effects could make a certain contribution to realization of somatic diseases (including MDS) as well as to specificity of its duration and disease course in remote terms following the Chornobyl accident.

Health and dosimetry survey of ARS persons at the NRCRM was launched in 1986. It was officially stated that 237 patients had got an ARS of different severity as a result of the Chornobyl accident. Until 1989 the ARS diagnosis was confirmed for 134 persons, including those 28 people, who had died within 11 to 96 days [5]. Three mentioned above MDS cases in ARS patients were diagnosed in remote period after the irradiation due to CHNPP accident, namely the RA after 113 months (9.4 years), the U-MDS after 125 months (10.4 years), and the RARS after 49 months (4.1 years). These time periods correspond to the data [1] on MDS cases that developed from 1 to 41 years upon radiation exposure of various kind and to the results [12] testifying that cases of therapy-related myeloid neoplasms arisen following the IR have a relatively long latency period (5-10 years) after a primary exposure. Risk of MDS is known to increase after cancer treatments including radiotherapy. Review of secondary cancer cases on the results of SEER study (Surveillance, Epidemiology, and End Results) [13] confirmed that persons with prostate cancer receiving a radiation therapy are under an increased relative risk (RR) of acute myeloid leukemia (AML) and MDS with peak incidence in 1.5–2.5 years; in turn the persons with non-Hodgkin lymphoma, lung and breast primary cancers have the highest RR for AML and MDS over the next 1–12 years.

Results of epidemiological retrospective cohort study of atomic bomb survivors [3] also showed that MDS risk existed in atomic bomb survivors 40 to 60 years after radiation exposure. Epidemiological study "The Ukrainian-American study of leukemia and related disorders among Chornobyl cleanup workers" was held on a cohort of 110,645 male cleanup workers from Ukraine. Leukemia, MDS, and multiple myeloma cases occurring during the period 1986 to 2000 were identified [14]. The cases of MDS were initially identified using the FAB system [6], later in 2007 they were reclassified according to the WHO system [7]. MDS was confirmed in 7 cases out of 139 ones initially referred to the expert panel, and one more case of MDS was reclassified using the WHO system as acute myelogenous leukemia, bringing the total for MDS to six [14].

It is necessary to emphasize a possible role of different confounding factors (i.e. chemical agents such as petroleum, cigarette smoking, alcohol, and others) in development of MDS. Relatively low-level exposure to benzene experienced by the petroleum distribution workers has also been associated with an increased risk of MDS, some types of which are recognized as precursors to AML [15]. Several studies have reported an excessive morbidity from leukemia or MDS in population groups either residentially or occupationally exposed to petroleum or its products [15-18].

In the frame of mentioned above epidemiological study "The Ukrainian-American study of leukemia and related disorders among Chornobyl cleanup workers" a set of data on a range of non-radiation exposures was collected [19]. Potential for additional effects of occupational (including exposure to petroleum) and lifestyle (cigarette smoking and alcohol consumption) factors on leukemia risk in this radiation-exposed cohort was investigated. After adjusting for radiation, there was found no clear association of leukemia risk with smoking or alcohol use but a two-fold elevated risk was identified for a non-CLL leukemia upon occupational exposure to petroleum (OR=2.28; 95% Confidence Interval 1.13,6.79). Risk was particularly high for the myeloid leukemia [19]. So, it can be suggested that exposure to similar confound factors has to be analyzed for MDS cases. No analysis was made however because of insufficient number of the MDS cases.

We have reviewed medical records on the described above ARS patients diagnosed as MDS cases and only one of them (patient S., ARS grade I) worked as a driver and was exposed to petroleum. All three ARS patients did not smoke and their consumption of alcohol was minimal.

We also want to emphasize that among our MDS cases the two of three patients (patient D. with ARS grade III and patient B. with ARS grade III) were the professional workers (i.e. staff members) of the Chornobyl NPP and were exposed to ionizing radiation before the ChNPP catastrophe in the range of occupational limits.

Analyzing the clinical course of MDS a survival period in RA case was 4 months, in RARS case it was 84 months with rapid progression within last 4 months, and in U-MDS case it was 47 months. All MDS cases in ARS patients discussed here are considered as indolent form of MDS. Thus, the life period in RA patient was very short comparing with literature data, whereas in other cases the survival periods were typical. In RARS patient along the last 4.5 months of life the disease was complicated by severe thrombocytopenia that is unusual with this type of MDS. It is known that indolent form of MDS including RARS is associated with a prolonged median survival i.e. for about 5-10 years [20-22]. But RARS usually is characterized by multifactorial prognostic heterogeneity, and patients with additional peripheral cytopenia show a shortened survival [22,23]. Life period of MDS-U cases usually depends on the degree of cytopenia.

Development of transfusion dependency significantly worsens the survival of patients with MDS, and this fact corresponds with our evidence here. Although most clinical features at diagnosis did not differ significantly between the transfusion-dependent and transfusion-independent MDS patients within WHO subgroups, however the development of secondary iron overload had significantly worsened a survival [24]. Effect of iron overload was noticeable mainly among the patients with RA. In contrast, secondary iron overload did not affect the survival of patients with refractory cytopenia, who have had a median survival of about 50 months.

Results of immunophenotyping in MDS patients with ARS were different. Immunophenotyping of PB cells of the RA patient revealed no any significant deviations i.e. the percentage and ratio of lymphocyte subpopulations were as common. Immunophenotyping also showed normal CD10+33-13+DR- granulocytes and monocytes with low expression of HLADR-antigen. Monitoring of immunoglobulin concentration in PB had not revealed any significant changes. Immunological monitoring in the MDS-U case (immunophenotyping of PB and BM cells, immunoglobulin concentration in PB) revealed the increased percentage of PB lymphocytes, changes in lymphocyte subpopulations ratio, and activation of B-cells. There were not significant immunological changes in the RARS cases.

In conclusion the provided above case reports suggest that described MDS cases in ARS patients may be the secondary MDS variants taking into consideration possible radiation-induced injuries of hematopoietic cell genome due to the high dose of IR (1.7-5.5 Sv). We also cannot exclude the possible role of previous irradiation in a range of occupational dose limits before the ChNPP catastrophe for patients D. and B. with ARS grade III, both with possible role of such a confounding factor as petroleum exposure for patient S. (ARS grade I) for the development of MDS.

References

1. Moloney WC (1987) Radiogenic leukemia revisited. Blood 70: 905-908.
2. Vozianov A, Bebeshko V, Bazyka (2003) Health effects of Chernobyl accident: Monograf in 4 parts. DIA: Kyiv pp: 512.
3. Iwanaga M, Hsu WL, Soda M, Takasaki Y, Tawara M, et al. (2011) Risk of myelodysplastic syndromes in people exposed to ionizing radiation: a retrospective cohort study of Nagasaki atomic bomb survivors. J Clin Oncol 29: 428-434.
4. Gluzman D, Imamura N, Sklyarenko L, Nadgornaya V, Zavelevich M, et al. (2006) Patterns of hematological malignancies in Chernobyl clean-up workers (1996-2005). Exp Oncol 28: 60-63.
5. Belyi D, Kovalenko A, Bazyka D (2013) Acute Radiation Syndrome Survivors after Chornobyl Accident: History of Irradiation, Diagnostic Mistakes and Death Reasons in Long-term Period. Radiation Emergency Medicine 2: 5-12.
6. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, et al. (1982) Proposals for the classification of the myelodysplastic syndromes. Br J Haematol 51: 189-199.
7. Harris NL, Jaffe ES, Diebold J, Flandrin G, Muller-Hermelink HK, et al. (2000) The World Health Organization classification of neoplasms of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting--Airlie House, Virginia, November, 1997. Hematol J 1: 53-66.
8. IAEA (2011) Cytogenetic dosimetry: applications in preparedness for and response to radiation emergencies. International Atomic Energy Agency, Vienna.
9. Zakharov AF, Benyush VA, Kuleshov NP, Baranovskaya LI (1982) Human chromosomes (Atlas) AMS of USSR. Moscow, Medicina p:264.
10. Bochkov NP, Chebotarev AN, Katosova LD, Platonova VI (2001) Data base for analysis of quantitative characteristics of chromosomal aberrations frequency in human peripheral blood lymphocytes culture. Vestnik of Russian Academy of Medical Sciencies 2: 21-29.
11. Pilinska MA, Schemetun AM, Redko DV (1991) Dynamic cytogenetic effect in repeatedly investigated persons participated in liquidation of Chornobyl accident in different terms after irradiation. Cytology and Genetic 25: 3-9.
12. Fedoriw Yu Pathology of Therapy-Related Myeloid Neoplasms. Overview.
13. Radivoyevitch T, Sachs RK, Gale RP, Molenaar RJ, Brenner DJ, et al. (2015) Defining AML and MDS second cancer risk dynamics after diagnoses of first cancers treated or not with radiation. Leukemia.
14. Romanenko A, Bebeshko V, Hatch M, Bazyka D, Finch S, et al. (2008) The Ukrainian-American study of leukemia and related disorders among Chornobyl cleanup workers from Ukraine: I. Study methods. Radiat Res 170: 691-697.
15. Schnatter AR, Glass DC, Tang G, Irons RD, Rushton L (2012) Myelodysplastic syndrome and benzene exposure among petroleum workers: an international pooled analysis. J Natl Cancer Inst 104: 1724-1737.
16. Barregard L, Holmberg E, Sallsten G (2009) Leukaemia incidence in people living close to an oil refinery. Environ Res 109: 985-990.
17. Talbott EO, Xu X, Youk AO, Rager JR, Stragand JA, et al. (2011) Risk of leukemia as a result of community exposure to gasoline vapors: a follow-up study. Environ Res 111: 597-602.
18. Stenehjem JS, Kjærheim K, Rabanal KS, Grimsrud TK (2014) Cancer incidence among 41,000 offshore oil industry workers. Occup Med (Lond) 64: 539-545.
19. Gudzenko N, Hatch M, Bazyka D, Dyagil I, Reiss RF, et al. (2015) Non-radiation risk factors for leukemia: A case-control study among chornobyl cleanup workers in Ukraine. Environ Res 142: 72-76.
20. Germing U, Strupp C, Kuendgen A, Isa S, Knipp S, et al. (2006) Prospective validation of the WHO proposals for the classification of myelodysplastic syndromes. Haematologica 91: 1596-1604.
21. Germing U, Gattermann N, Strupp C, Aivado M, Aul C (2000) Validation of the WHO proposals for a new classification of primary myelodysplastic syndromes: a retrospective analysis of 1600 patients. Leuk Res 24: 983-992.
22. Verburgh E, Achten R, Louw VJ, Brusselmans C, Delforge M, et al. (2007) A new disease categorization of low-grade myelodysplastic syndromes based on the expression of cytopenia and dysplasia in one versus more than one lineage improves on the WHO classification. Leukemia 21: 668-677.
23. Palmer SR, Tefferi A, Hanson CA, Steensma DP (2008) Platelet count is an IPSS-independent risk factor predicting survival in refractory anaemia with ringed sideroblasts. Br J Haematol 140: 722-725.
24. Malcovati L, Porta GMD, Pascutto C, Invernizzi R, Boni M, et al. (2005) Prognostic Factors and Life Expectancy in Myelodysplastic Syndromes Classified According to WHO Criteria: A Basis for Clinical Decision Making J of Clinical Oncology 23: 7594-7603.