https://doi.org/10.47183/mes.2025-413

BY 4.0

Check for updates

DEVELOPMENT OF CRITERIA FOR TRIAGE OF EXPOSED INDIVIDUALS BASED ON DICENTRIC CHROMOSOME ANALYSIS: A PILOT STUDY

Yulia R. Akhmadullina✉, Yana V. Krivoshchapova, Anastasiya V. Kupriyanova

Southern Urals Federal Research and Clinical Center for Medical Biophysics, Ozersk, Russia

Introduction. In the event of mass radiation incidents, medical triage of exposed individuals must enable rapid identification of individuals at risk of developing the hematopoietic form of acute radiation sickness (ARS).

Objective. Development of effective cytogenetic criteria for identifying individuals at increased risk of developing the hematopoietic form of ARS during mass radiation incidents.

Materials and methods. The study involved 12 donors aged 23–73 years. The study object was phytohemagglutinin-stimulated T-lymphocytes from peripheral blood. Blood samples were exposed to *in vitro* gamma-irradiation at doses of 1 Gy and 2 Gy using an IGUR-1M unit. Cytogenetic preparations were obtained according to a standardized cytogenetic protocol and stained with a 2% Giemsa solution. Images were digitized and analyzed using the Metafer/Ikaros (Metasystems, Germany) software package. The frequency of chromosomal aberrations per cell was assessed. Metaphases of T-lymphocytes with dicentric chromosomes were counted in the order of their occurrence during slide analysis. For statistical analysis, Past 4.01 and SPSS Statistics 21 software packages were used.

Results. The decision regarding the classification of a potentially irradiated sample as one falling into the dose range likely to cause ARS development should be based on an analysis of the number of T-lymphocyte metaphases required to identify five dicentric chromosomes. In the study, dicentric chromosomes were detected in samples without irradiation in 33% of the examined individuals. After *in vitro* irradiation of blood samples at a dose of 1 Gy, the average frequency of dicentric chromosomes was 0.073 ± 0.008 per cell, reaching 0.28 ± 0.02 per cell at a dose of 2 Gy.

Conclusions. A preliminary algorithm for differentiating *in vitro* irradiated cytogenetic samples into dose ranges has been developed. The identification of the fifth dicentric chromosome in order of analysis within the first 26 T-lymphocyte metaphases served as the basis for assigning the sample to a dose range of 2 Gy and above. In cases where the fifth dicentric chromosome was identified between the 27th and 85th metaphase, the sample was assigned to a dose range of 1 Gy and above. In cases where fewer than five dicentric chromosomes were detected upon analysis of 85 metaphases, the sample was assigned to a dose range below 1 Gy. Future research will be aimed at refining the algorithm and validating the results.

Keywords: dicentric chromosome; chromosomal aberrations; cytogenetic analysis; medical triage; gamma-irradiation; acute radiation sickness

For citation: Akhmadullina Yu.R., Krivoshchapova Ya.V., Kupriyanova A.V. Development of criteria for triage of exposed individuals based on dicentric chromosome analysis: A pilot study. *Extreme Medicine.* 2026;28(2):178–186. https://doi.org/10.47183/mes.2025-413

Funding: the study was carried out within the framework of State Contract No. 27.501.24.2 dated 17.06.2024 “Modernization of High-Tech Methods Aimed at Identifying Medical Consequences of Radiation Exposure on Personnel of PA Mayak and the Population of the Urals Region” (code “Medical Consequences-24”) under the Federal Target Program “Ensuring Nuclear and Radiation Safety for 2016–2020 and for the Period up to 2035”.

Compliance with the ethical principles: informed consent for blood sample collection and further cytogenetic studies was obtained from all participants. The study was approved by the Ethics Committee of the Southern Urals Federal Research and Clinical Center for Medical Biophysics (Minutes No. 9 dated 20.11.2023).

Acknowledgment: the authors express their gratitude to A.V. Vozilova, Cand. Sci. (Biology), for the research idea, and to senior laboratory assistants Z.I. Sychenko, I.A. Chikireva, N.F. Savkova, E.V. Velichutina, and N.E. Shelomentseva for their assistance in conducting the laboratory research.

Potential conflict of interest: the authors declare no conflict of interest.

✉ Yulia R. Akhmadullina akhmadullina.yul@yandex.ru

Received: 13 Oct. 2025 **Revised:** 4 Dec. 2025 **Accepted:** 11 Dec. 2025 **Online first:** 2 Feb. 2026

УДК 612.112.94:614.876:575.224.23

РАЗРАБОТКА КРИТЕРИЕВ ДЛЯ СОРТИРОВКИ ОБЛУЧЕННЫХ ЛИЦ НА ОСНОВЕ АНАЛИЗА ДИЦЕНТРИЧЕСКИХ ХРОМОСОМ: ПИЛОТНОЕ ИССЛЕДОВАНИЕ

Ю.Р. Ахмадуллина✉, Я.В. Кривощапова, А.В. Куприянова

Южно-Уральский федеральный научно-клинический центр медицинской биофизики, Озерск, Россия

Введение. При массовых радиационных инцидентах для медицинской сортировки облученных лиц необходимо иметь подход, позволяющий оперативно определить группу риска по развитию костномозговой формы острой лучевой болезни (ОЛБ).

Цель. Разработка эффективных цитогенетических критериев для выявления людей с повышенным риском развития костномозговой формы ОЛБ при массовых радиационных инцидентах.

Материалы и методы. В исследовании приняли участие 12 доноров в возрасте 23–73 лет. Объектом исследования были стимулированные фитогемагглютинином Т-лимфоциты периферической крови. Образцы крови подвергали *in vitro* гамма-облучению в дозах 1 и 2 Гр с использованием установки ИГУР-1М. Цитогенетические препараты получали по стандартизированному

© Yu.R. Akhmadullina, Ya.V. Krivoshchapova, A.V. Kupriyanova, 2026

цитогенетическому протоколу и окрашивали 2% раствором Гимзы. Изображения оцифровывали и анализировали с помощью Metafer/Ikaros (Metasystems, Германия). Оценивали частоту хромосомных аберраций на одну клетку. Проводили подсчет метафаз Т-лимфоцитов с дицентрическими хромосомами по порядку их встречаемости при анализе препарата. Для статистического анализа использовали программы Past 4.01 и SPSS Statistics 21.

Результаты. Для принятия решения об отнесении потенциально облученного образца к дозовому диапазону, при котором вероятно развитие ОЛБ, необходимо проанализировать то количество метафаз Т-лимфоцитов, в которых будет идентифицировано пять дицентрических хромосом. В ходе исследования в образцах без облучения дицентрические хромосомы были выявлены у 33% обследуемых лиц. После *in vitro* облучения образцов крови в дозе 1 Гр средняя частота встречаемости дицентрических хромосом составила 0,073 ± 0,008 на одну клетку, в дозе 2 Гр — 0,28 ± 0,02 на одну клетку.

Выводы. Разработан предварительный алгоритм разделения *in vitro* облученных цитогенетических образцов на дозовые диапазоны. Идентификация пятой дицентрической хромосомы по порядку анализа в первых 26 метафазах Т-лимфоцитов являлась основанием для отнесения исследуемого образца к дозовому диапазону от 2 Гр и выше. Если пятая дицентрическая хромосома была выявлена с 27-й по 85-ю метафазу, образец относили к дозовому диапазону от 1 Гр и выше. Если при анализе 85 метафаз было выявлено менее пяти дицентрических хромосом, образец относили к дозовому диапазону менее 1 Гр. Планируется продолжение исследований с доработкой алгоритма и валидацией результатов.

Ключевые слова: дицентрическая хромосома; хромосомные аберрации; цитогенетический анализ; медицинская сортировка; гамма-облучение; острая лучевая болезнь

Для цитирования: Ахмадуллина Ю.Р., Кривощапова Я.В., Куприянова А.В. Разработка критериев для сортировки облученных лиц на основе анализа дицентрических хромосом: пилотное исследование. *Экстремальная биомедицина*. 2026;28(2):178–186. https://doi.org/10.47183/mes.2025-413

Финансирование: работа выполнена в рамках государственного контракта № 27.501.24.2 от 17.06.2024 «Модернизация высокотехнологичных методов, направленных на выявление медицинских последствий радиационных воздействий на персонал ПО «Маяк» и население Уральского региона» (шифр «Медицинские последствия-24») в рамках ФЦП «Обеспечение ядерной и радиационной безопасности на 2016–2020 гг. и на период до 2035 г.».

Соответствие принципам этики: от всех участников получено информированное согласие на забор образцов крови и дальнейшие цитогенетические исследования. Исследование одобрено этическим комитетом ФГБУН УНПЦРМ ФМБА России (протокол № 9 от 20.11.2023).

Благодарности: авторы выражают благодарность канд. биол. наук Возиловой А.В. за идею исследования, старшим лаборантам Сыченко З.И., Чикиревой И.А., Савковой Н.Ф., Величутиной Е.В. и Шеломенцевой Н.Е. за помощь в проведении лабораторных исследований.

Потенциальный конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

✉ Ахмадуллина Юлия Рафисовна akhmadullina.yul@yandex.ru

Статья поступила: 13.10.2025 **После доработки:** 04.12.2025 **Принята к публикации:** 11.12.2025 **Online first:** 02.02.2026

INTRODUCTION

In the event of large-scale radiation incidents involving a large number of people, physical methods for dose reconstruction may be difficult or impossible to apply. In incidents involving the public, the lack of dose information complicates accurate assessment of the risks of medical and biological effects of radiation, thus impeding the decision-making process [1, 2].

Prompt identification of individuals with a high probability of developing moderate to severe acute radiation sickness (ARS) is essential for their timely referral to specialized facilities. The hematopoietic form of ARS is a treatable form, which occurs at radiation doses ranging 1–10 Gy, with varying degrees of disease severity classified within this range depending on the dose. According to the literature data, clinically recognizable signs of the hematopoietic syndrome of ARS are noted after exposure at doses above 1 Gy. In the exposure dose range of 1–2 Gy, mild ARS is observed, while moderate ARS is observed in the dose range of 2–4 Gy [3, 4].

As a rule, medical assessment includes taking a history (including location during the incident), physical examination, documenting clinical symptoms, and, when possible, determining the absolute lymphocyte and/or neutrophil count. Although hematological investigations are helpful in assigning exposed individuals into critical groups, they yield ambiguous results in cases of combined trauma or comorbid conditions [3, 5–7].

Assessment of radiation dose is critical for planning medical care. When physical dosimetry is impossible, cytogenetic biodosimetry based on the scoring of unstable chromosomal aberrations in metaphases of peripheral blood T-lymphocytes can be applied [6, 8]. Elevated frequencies of dicentric chromosomes and centric rings in the spectrum of observed aberrations indicate exposure to ionizing radiation. The analysis of these types of chromosomal aberrations is referred to as the “gold standard” in biological dosimetry, due to the established dose-effect relationship and the low spontaneous frequency of these aberration types [9]. The cytogenetic method requires specialized laboratory conditions, where cell culture is performed for approximately two days, followed by slide preparation and staining, microscopic analysis with image digitization, and dose calculation using calibration curves [10–13]. Manually, one researcher can analyze no more than ~500 cells per working day, rendering this method labor- and resource-intensive.

In mass casualty incidents, the goal of rapid medical triage requires approaches that significantly reduce resource intensity and increase laboratory throughput, rather than precise estimation of absorbed radiation doses. Therefore, in this study, we set out to create an approach for rapid cytogenetic examination of exposed individuals, who had undergone relatively uniform whole-body gamma-exposure, and identification of those at risk of developing the hematopoietic syndrome of ARS.

Our research is based on the hypothesis about the possibility of classifying *in vitro* gamma-irradiated blood samples into dose ranges corresponding to the development of mild and moderate severity ARS. This can be carried out by detecting unstable chromosomal aberrations during the analysis of a relatively small number of peripheral blood T-lymphocyte metaphases. It is assumed that an increase in the gamma-irradiation dose is associated with an increase in the probability of detecting dicentric chromosomes in the earliest analyzed T-lymphocyte metaphases. The proposed approach is based on automated slide digitization and data analysis using specialized software, which ensures higher quality of the primary material obtained, reproducibility of results, and allows researchers to verify the detected chromosomal aberrations.

In this work, we aim to develop effective cytogenetic criteria for identifying individuals at increased risk of developing the hematopoietic form of ARS in mass radiation incidents.

MATERIALS AND METHODS

The study involved 12 donors:

- 7 persons aged 57–73 years (mean age — 60 ± 2 years), 5 female and 2 male donors;
- 5 persons aged 23–38 years (mean age 27 ± 3 years), 4 female and 1 male donors.

At the time of blood sample collection, the donors had no history of treatment for diseases using ionizing radiation sources. There were also no severe forms of diabetes mellitus, autoimmune diseases, history of blood transfusion, and radiological examinations in the three months preceding the study.

To identify individuals meeting the above criteria, the potential participants underwent a preliminary interview. In the absence of exclusion criteria, a questionnaire was administered, and consent for the processing of personal data and participation in the study was obtained.

Obtaining metaphase chromosomes from human peripheral blood T-lymphocytes

Venous blood, 6 mL in volume, was collected from the cubital vein into sterile heparinized tubes. For each individual, three samples were prepared: two of them were irradiated *in vitro* on an IGUR-1M unit (Kvant, Russia) at doses of 1 Gy and 2 Gy, while the third sample was not irradiated. The IGUR-1M has the following characteristics: ^{137}Cs source, dose rate of 0.014 Gy/s, and irradiation non-uniformity of 5%.

Cytogenetic preparations from peripheral blood T-lymphocytes were obtained according to a protocol that included four sequential stages: cell culturing to the metaphase stage, hypotonic treatment, fixation of metaphase plates, and preparation of chromosome spreads, as described in [14].

T-cell culturing was conducted in sterile vacuum containers (Medpolymer, Russia). The culture consisted of 2 mL of blood, 5 mL of RPMI-1640 medium (PanEco, Russia), 0.5 mL of qualified fetal bovine serum (PanEco, Russia), and phytohemagglutinin (PHA) at a final concentration of 20 mg/mL (PanEco, Russia). Cells were cultured in a CO_2 incubator at 37.5°C for 54 h. Two hours before the end of culturing, a colchicine solution (PanEco, Russia) was added to the culture at a final concentration of 0.03 mg/mL.

For further processing, the cell suspension was transferred to centrifuge tubes and centrifuged at 1500 rpm for 10 min in a centrifuge (ThermoScientific, USA). The supernatant was removed, leaving the pellet. For hypotonic treatment, warm (37°C) 0.55% KCl solution was added, the pellet was resuspended, and the mixture was incubated in a thermostat at 37°C for 30 min. The mixture was then centrifuged for 10 min at 1100 rpm. The supernatant was carefully removed, the pellet was resuspended, and cold (4°C) freshly prepared fixative liquid (ethanol to glacial acetic acid in the ratio of 3:1) was added, bringing the total volume in the tube to 10 mL. The cells were left in the fixative medium for 10 min at 4°C, then centrifuged at 1500 rpm for 10 min. The supernatant was removed, and a fresh portion of fixative agent was added. Thus, the cells were passed through the fixative agent three times.

To obtain chromosome spreads, the total volume of the pellet in the tube was adjusted to 1.5 mL with a fresh fixative liquid, mixed well with a pipette, and the cell suspension was dropped onto a chilled slide (two drops). The slide was dried on a heating plate at 40°C. The quality of the preparation was checked using phase-contrast microscopy. Three slides were prepared per donor. The slides were stained with 2% Giemsa stain (PanEco, Russia) for 5–6 min.

Analysis of chromosome preparations was conducted using light microscopy. To that end, images of high-quality metaphases were obtained using the Metafer scanning and imaging platform (Metasystems, Germany) with a ×63 immersion objective. Image processing was performed using the Ikaros software (Metasystems, Germany).

For cytogenetic analysis, metaphase plates containing 45–47 chromosomes, with good spreading and no overlaps, were selected. During the study, a cytogenetic protocol was completed, recording the following chromosomal aberrations: dicentric chromosomes, ring chromosomes, paired fragments, and acentric rings. For each slide, 500–1000 cells were scored in unirradiated samples and 220–500 cells in irradiated samples. The figure presents images of T-lymphocyte metaphases with chromosomal aberrations.

The identified chromosomal aberrations were grouped as follows: dicentric chromosomes, unstable chromosomal exchanges (dicentric and ring chromosomes), paired fragments, acentric rings.

The work involved counting T-lymphocyte metaphases with dicentric chromosomes in the order of their occurrence among all metaphases included in the analysis on a digitized slide.

Methods for statistical data processing

The frequency of chromosomal aberrations was calculated per cell. Standard descriptive statistical methods were used: mean values and the standard error of the mean were calculated for chromosomal aberrations.

When calculating the number of T-lymphocyte metaphases in which dicentric chromosomes were identified in the order of their occurrence during slide analysis, the data are presented as the median (M_e) and the 95% confidence interval (CI). The CI for the median estimate was calculated using the bootstrap procedure with 1000 iterations. The 10th and 90th percentiles (%), as well as the minimum and maximum individual values for the number of T-lymphocytes with dicentric chromosomes in the order of their occurrence during slide analysis, were also assessed.

The expected number of cells in which dicentric chromosomes can potentially be identified (D_n) was calculated using formula (1):

$$X = \frac{D_n}{F}, \tag{1}$$

wherein X — the expected number of cells in which dicentric chromosomes can be identified in the order of their occurrence on the slide; D_n — sequence number of dicentric chromosomes during slide analysis (D_n = 1, 2, 3, 4, 5, 10, 15, 20, 25, 30); F — frequency of dicentric chromosomes, calculated per cell.

To calculate the probability of encountering dicentric chromosomes in unirradiated samples for each donor, the average number of dicentric chromosomes was analyzed using the moving average method with a window size of 10, 20, 30, 50, and 100 cells. Subsequently, based on these values, the probability of the frequency of dicentric chromosomes was calculated using formula (2):

$$P = \frac{m}{k} \times 100\%, \tag{2}$$

wherein P — probability of dicentric chromosome occurrence; m — number of identified dicentric chromosomes; k — number of analyzed cells (window size).

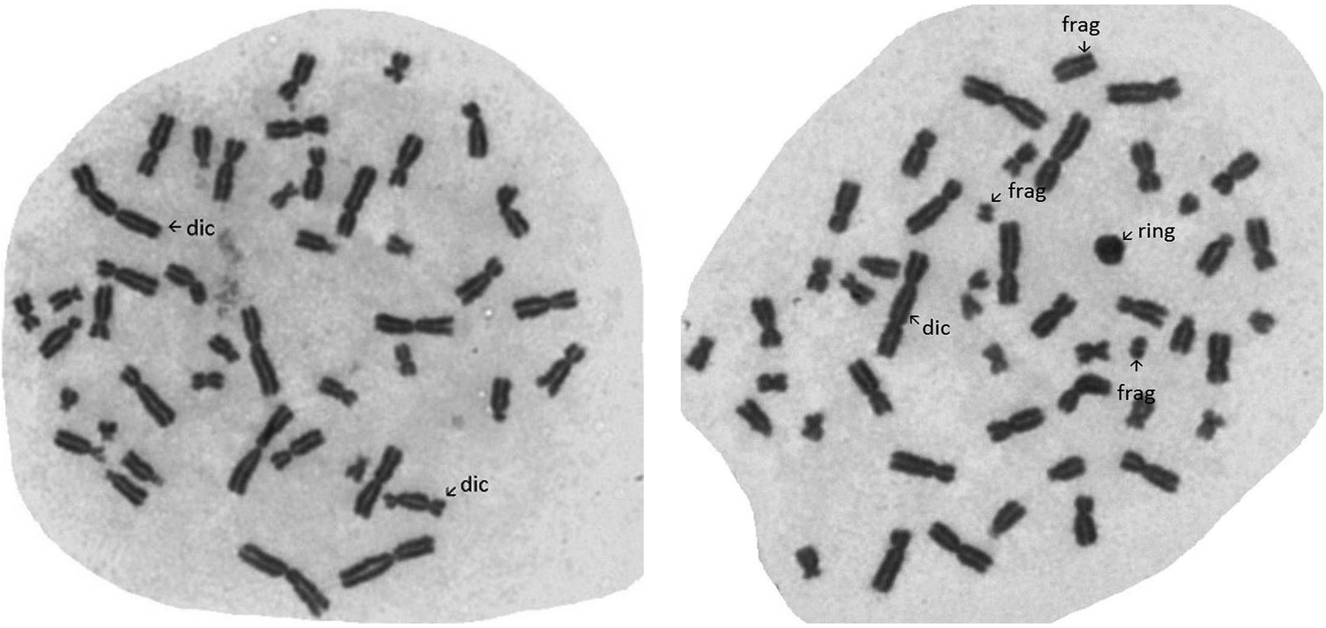

Figure prepared by the authors based on their own data

Fig. T-lymphocyte metaphases with chromosomal aberrations: dic — dicentric chromosome; frag — paired fragment; ring — ring chromosome; Images of T-lymphocyte metaphases were obtained using the Ikaros software (immersion objective, magn. × 63)

For statistical analysis, the Past 4.01 and SPSS Statistics 21 statistical software were used.

RESULTS AND DISCUSSION

During the study, dicentric chromosomes were detected in 33% of the examined individuals (4 out of 12) in unirradiated samples. The frequency of dicentric chromosomes ranged 0–1 abs. unit per 500 cells and 0–2 abs. units per 1000 cells. The mean frequency of dicentric chromosomes and unstable chromosomal exchanges was 0.0007 ± 0.0003 per cell, of paired fragments — 0.016 ± 0.003 per cell, and of acentric rings — 0.0003 ± 0.0002 per cell.

After *in vitro* irradiation of blood samples at a dose of 1 Gy, the mean frequency of chromosomal aberrations increased statistically significantly compared to unirradiated samples ($p < 0.0001$). The mean frequency of dicentric chromosomes was 0.073 ± 0.008 per cell, unstable chromosomal exchanges — 0.081 ± 0.009 per cell, paired fragments — 0.17 ± 0.01 per cell, and acentric rings — 0.009 ± 0.002 per cell.

For *in vitro* irradiation at a dose of 2 Gy, the mean frequency of dicentric chromosomes was 0.28 ± 0.02 per cell, unstable chromosomal exchanges — 0.3 ± 0.02 per cell, paired fragments — 0.64 ± 0.03 per cell, and acentric rings — 0.03 ± 0.005 per cell (Table 1).

In unirradiated samples (among those individuals in whom dicentric chromosomes were detected), the expected number of T-lymphocytes with one dicentric chromosome ranged 412–500 cells. The first dicentric chromosome was identified in T-lymphocytes with sequence numbers 77, 169, 341, and 314. The second dicentric chromosome was identified in two donors: in a T-lymphocyte with sequence number 341 (one cell contained two dicentric chromosomes) and in cell number 492.

Table 2 presents the expected number of cells in which dicentric chromosomes can be identified in the order of cell scoring following *in vitro* irradiation at doses of 1 Gy and 2 Gy. According to the presented data, after irradiation at a dose of 1 Gy, two dicentric chromosomes were detected in T-lymphocytes between the 17th and 69th cells scored, $M_e = 27$ [95% CI: 21; 41]. To detect 5 dicentric chromosomes, it was necessary to analyze 67 T-lymphocytes [95% CI: 41; 83]; for 10 dicentric chromosomes — 135 T-lymphocytes [95% CI: 103; 206]; for 20 dicentric chromosomes — 270 T-lymphocytes [95% CI: 206; 414]; and to identify 30 dicentric chromosomes — 405 T-lymphocytes [95% CI: 309; 620].

At an *in vitro* irradiation dose of 2 Gy, the expected number of T-lymphocytes that need to be analyzed to detect a comparable number of dicentric chromosomes was statistically significantly lower than that at an *in vitro* irradiation dose of 1 Gy ($p < 0.001$): 2 dicentric chromosomes were detected between the 5th and 12th cells; the median value for T-lymphocytes was 8 [95% CI: 6; 9]; 5 dicentric chromosomes were detected upon analysis of 19 T-lymphocytes [95% CI: 16; 22]; 10 dicentric chromosomes — upon analysis of 38 T-lymphocytes [95% CI: 31; 45]; 20 dicentric chromosomes — upon analysis of 77 T-lymphocytes [95% CI: 63; 90]; and 30 dicentric chromosomes — upon analysis of 115 T-lymphocytes [95% CI: 94; 134].

Table 3 presents data on the number of T-lymphocyte metaphases with detected dicentric chromosomes in the order of their analysis on digitized cytogenetic slides. Upon *in vitro* irradiation at a dose of 1 Gy, the median number of cells in which dicentric chromosomes were detected differed statistically significantly from the expected values only for the 1st, 25th, and 30th dicentric chromosomes in the order of their identification during analysis. For instance, the expected median value for T-lymphocytes was statistically significantly ($p = 0.002$)

Table 1. Mean frequency of chromosomal aberrations under *in vitro* irradiation (per cell)

Type of chromosomal aberrations	Mean frequency of chromosomal aberrations under *in vitro* irradiation, per cell		
	Irradiation dose		
	unirradiated	1 Gy	2 Gy
Dicentric chromosomes	0.0007 ± 0.0003*	0.073 ± 0.008**	0.28 ± 0.02
Ring chromosomes	0*	0.008 ± 0.002**	0.02 ± 0.006
Unstable chromosomal exchanges	0.0007 ± 0.0003*	0.081 ± 0.009**	0.3 ± 0.02
Paired fragments	0.016 ± 0.003*	0.17 ± 0.01**	0.64 ± 0.03
Acentric rings	0.0003 ± 0.0002*	0.009 ± 0.002**	0.03 ± 0.005

Table compiled by the authors based on their own data

Note: Data are presented as the mean value and standard error of the mean; * — statistically significant differences in the frequency of chromosomal aberrations between unirradiated and irradiated samples, $p < 0.0001$; ** — statistically significant differences in the frequency of chromosomal aberrations between samples with *in vitro* irradiation at a dose of 1 Gy and samples with *in vitro* irradiation at a dose of 2 Gy, $p < 0.05$.

Table 2. **Expected number of cells in which dicentric chromosomes can be identified**

Number of dicentric chromosomes	Irradiation dose					
	1 Gy			2 Gy		
	Number of T-lymphocyte metaphases			Number of T-lymphocyte metaphases		
	Median [CI]	10–90 percentiles	min–max	Median [CI]	10–90 percentiles	min–max
1	13* [10–21]	9–32	9–34	4 [3–4]	3–5	3–6
2	27* [21–41]	18–64	17–69	8 [6–9]	5–11	5–12
3	40* [31–62]	27–96	26–103	12 [9–13]	8–16	8–17
4	54* [41–83]	36–129	34–138	15 [13–18]	10–22	10–23
5	67* [52–103]	45–161	43–172	19 [16–22]	13–27	13–29
10	135* [103–206]	91–321	86–344	38 [31–45]	25–56	25–58
15	202* [155–310]	136–482	129–517	58 [47–67]	38–82	38–87
20	270* [206–414]	182–643	172–689	77 [63–90]	50–110	50–116
25	337* [258–517]	227–803	216–861	96 [79–112]	63–137	63–145
30	405* [309–620]	272–963	259–1033	115 [94–134]	76–165	75–174

Table compiled by the authors based on their own data

Note: CI — confidence interval; * — statistically significant differences for the median number of cells upon *in vitro* gamma-irradiation at doses of 1 Gy and 2 Gy, $p < 0.0001$.

Table 3. **Number of metaphases of T-lymphocytes with dicentric chromosomes (in accordance with the sequence number of analyzed cell)**

Sequence number of dicentric chromosomes	Irradiation dose					
	1 Gy			2 Gy		
	Number of T-lymphocyte			Number of T-lymphocyte		
	Median [CI]	10–90 percentiles	min–max	Median [CI]	10–90 percentiles	min–max
1	4* [1–26]	1–25	1–26	2 [2–4]	1–6	1–6
2	28* [8–42]	8–70	8–81	8 [5–10]	2–13	2–14
3	35* [9–72]	10–97	9–107	11 [7–16]	4–28	4–31
4	43* [14–74]	19–112	14–123	14 [10–19]	6–30	5–32
5	56* [27–85]	30–127	27–138	16 [12–25]	6–31	5–32
10	103* [60–115]	62–182	60–192	40 [6–24]	12–70	11–71
15	146* [120–182]	133–425	120–486	49 [44–78]	29–90	24–91
20	177* [157–217]	162–419	157–457	67 [59–94]	41–113	34–116
25	237* [195–289]	195–426	195–426	87 [77–112]	51–125	42–128
30	278* [225–349]	225–405	225–405	105 [96–129]	72–147	70–151

Table compiled by the authors based on their own data

Note: CI — confidence interval; * — statistically significant differences for the median number of T-lymphocytes upon irradiation at doses of 1 Gy and 2 Gy, $p < 0.001$.

higher than the experimentally obtained value, i.e., 0.032 and 0.019 cells, respectively.

Upon *in vitro* irradiation at a dose of 2 Gy, the median expected number of T-lymphocytes with one dicentric chromosome was statistically significantly higher than that obtained from direct slide analysis (4 vs. 2, $p = 0.033$). In all other cases, no statistically significant differences were found.

The analysis showed that identifying the first dicentric chromosomes in *in vitro* gamma-irradiated T-lymphocytes at doses of 1 and 2 Gy requires an analysis of a relatively small number of cells. According to our and literature data, the frequency of dicentric chromosomes in unirradiated samples ranged 0–2 per 1000 analyzed cells [15].

Table 4 shows the probability of detecting the first dicentric chromosome in unirradiated samples. Thus, when analyzing 50 cells, it was 4.9%; when analyzing 100 cells, it was 9.4%. The probability of detecting two dicentric chromosomes in 100 T-lymphocyte metaphases was 2%.

Thus, given the probability of detecting two dicentric chromosomes upon analyzing the first 100 T-lymphocyte metaphases in unirradiated samples, it is necessary to increase the number of identified dicentric chromosomes to indicate radiation exposure. Based on the data presented in Table 3, to identify radiation exposure and classify a sample into a respective dose range, it is proposed to evaluate the number of T-lymphocyte metaphases containing the fifth dicentric chromosome in the order of cytogenetic slide analysis.

For instance, the median number of T-lymphocyte metaphases with the fifth dicentric chromosome was 56 [95% CI: 27; 85] for *in vitro* gamma-irradiation at a dose of 1 Gy and 16 [95% CI: 12; 25] for *in vitro* gamma-irradiation at a dose of 2 Gy ($p < 0.001$). The choice of the median and its corresponding confidence interval as the criterion for dividing exposed individuals into dose ranges is based on its robustness against the influence of outliers, which are characteristic of distributions containing inter-individual differences in radiosensitivity. Although the accuracy of the median estimate is clearly linked to sample size, increasing the latter will only partially reduce the uncertainty in estimating the central tendency. We believe that the reduction in the confidence interval will be insignificant due to individual responses to radiation. Therefore, the use of median values and the confidence interval appear to be an optimal solution at this stage of the research.

To make a decision regarding the classification of a potentially irradiated sample into one of the dose ranges, it is necessary to analyze the number of cells in which five dicentric chromosomes are identified. Based on the preliminary results, the following algorithm for analyzing T-lymphocyte metaphases is proposed:

1. Determine the sequence number of the analyzed T-lymphocyte metaphase containing the fifth dicentric chromosome in the order of analysis.
2. When the fifth dicentric chromosome in the order of analysis is identified within the first 26 cells, then the irradiated sample should be classified into the radiation exposure dose range of 2 Gy or higher.
3. When the fifth dicentric chromosome is identified between the 27th and 85th T-lymphocyte metaphase during analysis, the irradiated sample should be classified into the radiation exposure dose range of 1 Gy or higher.
4. When fewer than five dicentric chromosomes are identified upon analysis of 85 T-lymphocyte metaphases, the irradiated sample should be classified into the exposure dose range below 1 Gy.
5. When no dicentric chromosomes are detected upon analysis of 50 T-lymphocyte metaphases, there is a 94% probability that the absorbed gamma-irradiation dose of the sample is close to zero.

Table 5 presents the proportion of examined individuals for whom the sequence number of the T-lymphocyte containing the fifth dicentric chromosome falls within the range calculated according to the algorithm described above.

All non-irradiated samples were classified into the "< 1 Gy" group; 100% of them were assigned to the group where the radiation dose is close to zero. At the same time, according to the algorithm, two (16.7%) samples irradiated at a dose of 1 Gy were classified into the "< 1 Gy" group, and two (16.7%) samples irradiated

Table 4. Probability of dicentric chromosome occurrence depending on the number of analyzed cells (in unirradiated samples), %

Number of dicentric chromosomes	Number of analyzed T-lymphocyte metaphases, abs. units				
	10	20	30	50	100
	Probability, %				
0	98.9	97.6	96.4	94.1	88.6
1	0.9	2	3	4.9	9.4
2	0.2	0.4	0.6	1.0	2

Table compiled by the authors based on their own data

Table 5. Distribution of the studied blood samples into dose groups according to the algorithm criteria

Parameter	Dose range			
	≥ 2 Gy	≥ 1 Gy	< 1 Gy	Dose close to 0 Gy
Proportion of individuals, % (abs. units)	83.3 (10)	83.3 (10)	100 (12)	100 (12)
of which:				
False-negative result, % (abs. units)	16.7 (2)	16.7 (2)	–	–

Table compiled by the authors based on their own data

Note: "–" — not identified.

at a dose of 2 Gy were classified into the "≥ 1 Gy or higher" group.

The preliminary results of our study revealed a potential issue of false-negative identification, associated with a possible underestimation of the calculated dose range for a certain proportion of irradiated samples. For medical triage, however, a conservative approach is preferable, where an exposed individual is assigned to a dose group with higher exposure values. A conservative approach reduces the risk of underestimating ionizing radiation exposure and ensures that all potentially exposed individuals receive the necessary medical care. At the next stage of the research, it will be necessary to refine the criteria for assigning an irradiated sample to a particular dose group, taking into account the requirements of conservatism.

According to the literature, the lower limit of detection for the method of scoring unstable chromosomal exchanges depends on the number of analyzed cells and averages 100–250 mGy for sparsely ionizing radiation. At higher radiation doses (5–6 Gy and above), significant impairment of cellular proliferation occurs, resulting in fewer cells reaching metaphase and being included in the cytogenetic analysis [16–18]. Medical triage of exposed individuals based on the frequency of dicentric chromosomes or unstable chromosomal exchanges in T-lymphocytes should be conducted by reducing the number of analyzed T-lymphocyte metaphases to 50 per person, which will significantly increase laboratory throughput. The use of 20–50 cells for analysis increases the confidence intervals for estimating radiation doses assessed using a calibration curve. However, the dose intervals of 0–0.75 Gy and 2–3 Gy are clearly distinguishable when analyzing 50 cells [19, 20], which is consistent with the results obtained in this study.

It is important to note that the present study did not aim to accurately determine accidental exposure doses based on the dose-effect relationship. Under conditions of limited equipment and human resources, it is necessary to develop criteria that, by reducing the number of analyzed cells, will enable timely assignment of the exposed population into groups at risk of developing the hematopoietic form of ARS. This will facilitate the planning and adjustment of further medical follow-up. Following cytogenetic triage, it is possible to increase the number of analyzed cells for biodosimetry purposes.

In future studies, we aim to validate the developed method on other sample sets, conduct inter-laboratory comparisons, and, based on the data obtained, optimize the algorithm for assessing dose intervals.

CONCLUSION

One of the tasks in providing emergency medical care to exposed individuals during mass radiation incidents is to conduct medical triage aimed at identifying persons at high risk of developing ARS. In this work, we have developed a preliminary cytogenetic triage algorithm, based on determining the number of analyzed T-lymphocyte metaphases containing the fifth dicentric chromosome in the order of their analysis.

It was established that when the fifth dicentric chromosome is identified within the first 26 analyzed T-lymphocyte metaphases, the radiation dose falls within the range of 2 Gy or higher. When the fifth dicentric chromosome is identified between the 27th and 85th analyzed T-lymphocyte metaphase, the radiation dose falls within the range of 1 Gy or higher. In the case of analyzing 85 T-lymphocyte metaphases with fewer than 5 dicentric chromosomes identified, the radiation dose falls within the range below 1 Gy. In cases where no dicentric chromosomes are detected upon analysis of 50 T-lymphocyte metaphases, the radiation dose falls within the range below 1 Gy and/or is close to zero values. This algorithm is preliminary and requires further refinement, taking into account a conservative approach to reduce the risk of underestimating ionizing radiation exposure. Continuation of the research is planned to refine the algorithm and validate the results obtained.

References

1. Kutkov VA. Substantive provisions of the IAEA guidelines for criteria for protecting the public and workers in radiation emergency. *Radiation and Risk*. 2006;15(3–4):133–56 (In Russ.). EDN: JWSDRF
2. Guskova AK, Krasnyuk VI, Galstyan IA, Nadejina NM. 30 years of Chernobyl NPP accident: experience of mitigation of medical consequences. *Medical Radiology and Radiation Safety*. 2016;61(3):30–5 (In Russ.). EDN: VWDLJD
3. Samoylov AS, Konchalovsky MV, Bushmanov AYu, Galstyan IA, Nugis VYu, Davtyan AA, et al. Recommendations for the diagnosis and treatment of bone marrow form of acute radiation syndrome. *Russian Journal of Hematology and Transfusiology.* 2023;68(1):98–128 (In Russ.). https://doi.org/10.35754/0234-5730-2023-68-1-98-128
4. Bushmanov AYu, Galstian IA, Kashirina OG, Konchalovsky MV, Lisunov VYu, Metlyaeva NA, et al. Results of long-term medical observation of direct participants of the accident at the Chernobyl NPP in the Russian Federation. *A.I. Burnasyan Federal Medical Biophysical Center Clinical Bulletin*. 2024;4:51–9 (In Russ.). https://doi.org/10.33266/2782-6430-2024-4-51-59
5. Sedankin MK, Gudkov EA, Soloviev VYu, Mershin LYu. Features of using a lymphocyte test for biological dosimetry in the early period after exposure. *Extreme Medicine*. 2023;25(3):65–70 (In Russ.). https://doi.org/10.47183/mes.2023.034
6. Sullivan JM, Prasanna PG, Grace MB, Wathen LK, Wallace RL, Koerner JF, et al. Assessment of biodosimetry methods for a mass-casualty radiological incident: medical response and management considerations. *Health Physics*. 2013;105(6):540–54. https://doi.org/10.1097/hp.0b013e31829cf221
7. Coleman CN, Sullivan JM, Bader JL, Murrain-Hill P, Koerner JF, Garrett AL, et al. Public health and medical preparedness for a nuclear detonation: the nuclear incident medical enterprise. *Health Physics*. 2015;108(2):149–60. https://doi.org/10.1097/hp.0000000000000249
8. International Atomic Energy Agency. *Cytogenetic analysis for radiation dose assessment: a manual.* Vienna: IAEA; 2001.
9. Neronova EG. Calibration curves development for biological cytogenetic dosimetry and experience with medical exposure. *Medico-Biological and Socio-Psychological Problems of Safety in Emergency Situations*. 2021;4:85–93 (In Russ.). EDN: JAWXRM
10. Kuznetsova TV, Shilova NV, Tvorogova MG, Kharchenko TV, Lebedev IN, Antonenko VG. Practical recommendations to ensure quality and safety of cytogenetic research. *Medical Genetics.* 2019;18(5):3–27 (In Russ.). https://doi.org/10.25557/2073-7998.2019.05.3-27
11. Port M, Barquinero JF, Endesfelder D, Moquet J, Oestreicher U, Terzoudi G, et al. RENEB Inter-Laboratory Comparison 2021: Inter-Assay Comparison of Eight Dosimetry Assays. *Radiation Research*. 2023;199(6):535–55. https://doi.org/10.1667/rade-22-00207.1
12. Pinto MM, Santos NF, Amaral A. Current status of biodosimetry based on standard cytogenetic methods. *Radiation and Environmental Biophysics*. 2010;49(4):567–81. https://doi.org/10.1007/s00411-010-0311-3
13. Vijayalakshmi J, Chaurasia RK, Srinivas KS, Vijayalakshmi K, Paul SFD, Bhat NN, et al. Establishment of ex vivo calibration curve for X-ray induced “dicentric + ring” and micronuclei in human peripheral lymphocytes for biodosimetry during radiological emergencies, and validation with dose blinded samples. *Heliyon.* 2023;9(6):e17068. https://doi.org/10.1016/j.heliyon.2023.e17068
14. Vozilova AV, Akhmadullina YR. Study of the individual radiosensitivity in humans based on the assessment of the frequency of chromosome aberrations and micronuclei in peripheral blood T lymphocytes. *Russian Journal of Genetics*. 2019;55:1234–41. https://doi.org/10.1134/S1022795419100156
15. Ainsbury E, Barquienero JF, Beinke C, Blakely WF, Braselmann H, Carr Z, et al. *Cytogenetic dosimetry: applications in preparedness for and response to radiation emergencies*. Vienna: IAEA; 2011. EDN: VPPDFP
16. Bauchinger M. *Chromosome painting and biological dosimetry of absorbed radiation.* Proceedings of the Tenth International Congress of Radiation Research. Würzburg; 1995.
17. Flegal FN, Devantier Y, Marro L, Wilkins RC. Validation of QuickScan dicentric chromosome analysis for high throughput radiation biological dosimetry. *Health Physics.*2012;102:143–153. https://doi.org/10.1097/hp.0b013e3182307758
18. Testa A, Palma V, Patrono C. Dicentric Chromosome Assay (DCA) and Cytokinesis-Block Micronucleus (CBMN) Assay in the Field of Biological Dosimetry. In: Dhawan A, Bajpayee M (eds). *Genotoxicity Assessment. Methods in Molecular Biology*, vol 2031. New York: Humana; 2019:105–19. https://doi.org/10.1007/978-1-4939-9646-9_5
19. Romm H, Wilkins RC, Coleman CN, Lillis-Hearne PK, Pellmar TC, Livingston GK, et al. Biological dosimetry by the triage dicentric chromosome assay: potential implications for treatment of acute radiation syndrome in radiological mass casualties. *Radiation Research.* 2011;175(3):397–404. https://doi.org/10.1667/RR2321.1
20. Lloyd DC, Edwards AA, Moquet JE, Guerrero-Carbajal YC. The role of cytogenetics in early triage of radiation casualties. *Applied Radiation and Isotopes*. 2000;52(5):1107–12. https://doi.org/10.1016/s0969-8043(00)00054-3

Authors’ contributions. All the authors confirm that they meet the ICMJE criteria for authorship. The most significant contributions were as follows: Yulia R. Akhmadullina — research planning, statistical analysis of results, results interpretation, draft writing, editing and final manuscript preparation; Yana V. Krivoshchapova — conducting laboratory research, obtaining primary data, statistical processing of primary data, draft writing; Anastasiya V. Kupriyanova — conducting laboratory research, obtaining primary data, draft editing.

Authors:

Yulia R. Akhmadullina, Cand. Sci. (Biol.), ORCID: https://orcid.org/0000-0003-4394-2228
Yana V. Krivoshchapova, ORCID: https://orcid.org/0000-0002-2555-2616
Anastasiya V. Kupriyanova, ORCID: https://orcid.org/0009-0005-8699-2745