FEATURES OF USING A LYMPHOCYTE TEST FOR BIOLOGICAL DOSIMETRY IN THE EARLY PERIOD AFTER EXPOSURE

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When eliminating the consequences of large-scale radiation accidents, primary triage of victims is of key importance during the early phase of medical evacuation. Information about lymphocyte counts (blood test) per unit of peripheral blood volume can be used for this purpose. The study was aimed to validate the method of using a lymphocyte test for prediction of acute radiation injury severity in the first days after the exposure associated with the radiation mass casualty incident, given peripheral blood was tested once. We performed correlation analysis of the data of laboratory studies focused on quantifying lymphocytes in peripheral blood of victims during the first days following the Chernobyl disaster and other radiation accidents on the territory of the countries of the former USSR (115 individuals), including radiation accidents with gamma neutron radiation (20 individuals). It was found that with the lymphocyte concentration of 0.2–1.0 × 10^9/L on day 2 after exposure, the absolute error of estimated dose was ±1.5 Gy in case of gamma exposure and ±1.3 Gy in case of exposure to gamma neutron radiation. When the lymphocyte concentration exceeds 1.0 × 10^9/L, mild acute radiation syndrome (ARS) is predicted, given the average dose is below 2.0 Gy; when the lymphocyte concentration is less than 0.2 × 10^9/L< the estimated average dose exceeds 4.0 Gy, which corresponds to severe or extremely severe ARS. Thanks to the lymphocyte test accessibility and simplicity, this biological dosimetry method can occupy a worthy position in the diagnosis of radiation injury associated with large-scale accidents, since the results of cytogenetic tests are not available within first days after the accident.

Keywords: radiation dose, lymphocytes, acute radiation syndrome, radiation accident, radiological accident, nuclear accident, biological dosimetry

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ОСОБЕННОСТИ ИСПОЛЬЗОВАНИЯ ЛИМФОЦИТАРНОГО ТЕСТА ДЛЯ БИОЛОГИЧЕСКОЙ ДОЗИМЕТРИИ В РАННИЕ СРОКИ ПОСЛЕ ОБЛУЧЕНИЯ

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При ликвидации последствий крупномасштабных радиационных аварий на ранних этапах медицинской эвакуации ключевое значение имеет первичная медицинская сортировка пострадавших. Для этой цели может быть использована информация о количестве лимфоцитов (анализ крови) в единице объема периферической крови. Целью исследования было провалидировать метод использования лимфоцитарного теста для прогнозирования степени тяжести острого лучевого поражения в первые дни после облучения при массовых радиационных поражениях при условии однократного анализа периферической крови. Проводили корреляционный анализ данных клинико-лабораторных исследований числа лимфоцитов в периферической крови пострадавших в первые дни после облучения при аварии на ЧАЭС и других радиационных инцидентах на территориях стран бывшего СССР (115 человек), в том числе в радиационных инцидентах с гамма-нейтронным облучением (20 человек). Установлено, что при концентрации лимфоцитов 0,2–1,0 × 10^9/л на 2-е сутки после облучения абсолютная погрешность оценки дозы составляет ±1,5 Гр при воздействии гамма-лучей и ±1,3 Гр — при воздействии гамма-нейтронного излучения. При концентрации лимфоцитов более 1,0 × 10^9/л в обоих случаях прогнозируется легкая степень острой лучевой болезни (ОЛБ) при средней дозе менее 2,0 Гр; при концентрации лимфоцитов менее 0,2 × 10^9/л оценка средней дозы составляет более 4,0 Гр, что соответствует тяжелой или крайне тяжелой степени ОЛБ. Благодаря доступности и простоте лимфоцитарного теста, этот метод биологической дозиметрии способен занять важное место в диагностике радиационных поражений при крупномасштабных авариях, в связи с тем что результаты цитогенетических тестов недоступны в течение первых дней после инцидента.

Ключевые слова: доза облучения, лимфоциты, острая лучевая болезнь, радиационная авария, радиологический инцидент, ядерный инцидент, биологическая дозиметрия

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When eliminating the consequences of large-scale radiation accidents, primary triage of victims is of key importance in the early phase of medical evacuation. Provided victims have no personal dosimeters, information about primary response to exposure considering personal data on the exposure conditions and/or blood testing can be used for this purpose. The results of summarizing information about clinical manifestations of primary response to exposure and their prognostic value for assessment of radiation injury severity are provided in many papers [1–6]. Thus, actual knowledge about the symptoms of primary response in victims of the Chernobyl disaster (1986) and other radiation accidents was analyzed [5, 6]. It has been shown that among all symptoms of primary response to exposure, the time to emesis following the exposure is the most informative one. However, in some cases these data can be of low prognostic value, for example, due to the fact that victims

could use antiemetics [2, 7] or other reasons (head injuries, psychoemotional disorders, etc.). In this regard, information about lymphocyte counts per unit of peripheral blood volume, i.e. the so-called lymphocyte test (white blood cell count), can be an additional source of information about the radiation injury severity.

Peripheral blood cell counts are an important biomarker of radiation exposure. The lymphocyte test, i.e. measuring absolute lymphocyte counts and the dynamics of their changes in victims' blood, is of special prognostic value. Measuring absolute lymphocyte counts is the fastest and easiest laboratory test for radiation dose estimation within 24 h after the exposure. Physicians initially used a nomogram developed by G.A. Andrews to predict the radiation injury severity. Detection of low absolute lymphocyte counts or a progressive decrease in lymphocyte counts within certain time

Table 1. Peripheral blood lymphocyte counts ($\times 10^9$/L) on day 2 after exposure in victims of the Chernobyl disaster (1986) and other radiation accidents (according to the data from the database on acute radiation injury in humans compiled by the State Research Center — Burnasyan Federal Medical Biophysical Center of Federal Medical Biological Agency), amended and reworked from the earlier published paper [13]. The cases of exposure to gamma neutron radiation are allocated separately

Unique patient identifier	Dose, Gy	Lymphocyte counts, $\times 10^9$/L	Unique patient identifier	Dose, Gy	Lymphocyte counts, $\times 10^9$/L	Unique patient identifier	Dose, Gy	Lymphocyte counts, $\times 10^9$/L
Gamma radiation (95 individuals)								
1001	7.5	0.117	1055	5.3	0.522	1096	3.7	0.342
1005	5.2	0.165	1056	3.6	0.41	1097	1	0.662
1007	5.5	0.325	1057	3	0.516	1098	2	0.63
1011	6.3	0.081	1058	3	0.437	1099	5.6	1.2
1013	6.3	0.108	1059	5.8	0.54	1100	2.6	0.48
1018	2.7	0.229	1060	6.1	0.432	1101	3.2	0.841
1019	4.6	0.216	1061	4.4	0.513	1102	1.2	1.597
1021	4.7	0.164	1062	7	0.483	1103	1.9	0.817
1022	7.1	0.162	1063	1.1	0.51	1105	1.5	0.557
1024	2.3	0.365	1065	3.1	1.008	1106	2.3	0.69
1025	6	0.637	1066	1	0.884	1107	0.7	1.128
1028	7.3	0.376	1067	2.6	0.75	1108	2.3	0.756
1030	6.4	0.189	1068	4.6	0.293	1140	0.3	1.092
1031	7.7	0.399	1070	1.2	0.56	3033	7.7	0.296
1032	4.2	0.754	1071	5.4	0.128	3034	4	0.285
1033	3.9	0.636	1072	3.6	0.45	3035	6	0.222
1035	4	0.532	1073	3.5	2.52	3038	1.3	0.98
1037	2.8	0.566	1075	1.4	1.162	3044	1.7	1.155
1039	4.3	0.852	1078	0.3	1.842	3048	2.6	0.405
1040	1.7	0.612	1079	0.6	2.12	3050	2.3	0.484
1041	3.1	0.344	1081	1.2	1.275	3051	3	0.438
1042	6.3	0.357	1082	1.2	2.352	3052	3	0.7
1043	4.7	0.281	1083	1.9	0.989	3053	3	0.335
1044	3.7	0.609	1084	1.1	1.058	3067	2.3	1.044
1047	3.2	0.744	1085	3.3	0.3	3068	3	0.728
1048	2	0.924	1087	3.5	0.378	3069	3.5	0.504
1049	2.1	0.235	1089	1.7	0.846	3077	0.85	1.107
1050	3.3	0.897	1090	1.2	0.74	3078	0.9	1.26
1051	1.8	0.943	1091	1.2	0.608	3082	2.1	0.697
1052	4.3	0.436	1092	2.7	0.72	3083	1.3	1.798
1053	2.8	0.594	1094	6.6	0.684	3084	2.1	0.91
1054	3.6	0.456	1095	2.2	0.923			
Gamma neutron radiation (20 individuals)								
3008	3.8	0.352	3036	3.3	0.067	3065	2.25	0.504
3010	0.9	1.564	3037	3.7	0.269	3071	3.7	0.93
3011	0.5	1.222	3040	5.8	0.204	3073	5	0.08
3020	4	0.396	3042	4.1	0.259	3079	2.1	1.147
3025	2.5	0.423	3043	3	0.551	3081	1.5	0.774
3027	1.1	1.071	3045	5.5	0.444	3086	1.9	0.769
3030	3.6	0.403	3046	7.4	0.072			

Table 2. Dose estimation based on the time between blood tests and the ratio of lymphocyte concentrations L_2/L_1 within 2–18 h after exposure

L_2/L_1	Time between measurements, h				
	4	6	8	10	12
0.8	8	5.4	4	3.2	2.7
0.7	>12	8.6	6.4	5.1	4.3
0.6	>12	>12	9.2	7.4	6.1
0.5	>12	>12	>12	10	8.3

period indicates probable exposure to a high radiation dose, which follows classical lymphocyte depletion curves [8].

Generally, the use of lymphocyte test is based on the fact that the average concentration of lymphocytes in peripheral blood remains more or less constant during the period between days 2 and 9 following a substantial decrease within the first 24 h after the exposure. The guidelines on the lymphocyte test practical use are based on these patterns. The correlation between peripheral blood lymphocyte counts/concentrations and the dose received was thoroughly investigated in the population of victims of the Chernobyl accident (1986) and other radiation accidents [9]. It has been shown that the highest correlation between the dose and the average peripheral blood lymphocyte concentration is observed on days 3–6 after exposure. However, the earlier period is not discussed in this paper.

However, in practice situations are possible when only one blood test performed in the first days after exposure is reported for the victim. According to the domestic literature, this time range is insufficiently studied. Further research is needed to raise the lymphocyte test informativity in this time period.

Dose estimation based on only one blood test performed within the first 24 h is not very informative, since there is a high degree of uncertainty. This issue is poorly understood in terms of statistics. The literature provides data on assessing injury severity within the first days or hours after the radiation accident [10, 11]. The radiation injury severity can be predicted based on the victim's absolute peripheral blood lymphocyte counts within the first two days after exposure in accordance with the guidelines issued by the IAEA and the WHO [12].

Currently, it is important to develop and improve the lymphocyte test as a biological dosimetry method in order to assess and predict the severity of injury in victims of radiation accidents in the first days after the accidents during the early phase of medical evacuation and subsequent echelon care.

The study was aimed to validate the method of using a lymphocyte test within two days after the exposure to predict the severity of injury associated with the radiation mass casualty incident, given peripheral blood was tested once, based on the use of laboratory data on victims of radiation incidents on taken from the database on ARS compiled by the State Research Center — Burnasyan Federal Medical Biophysical Center of Federal Medical Biological Agency.

METHODS

The study involved the use of clinical data from the database on ARS compiled by the State Research Center — Burnasyan Federal Medical Biophysical Center of Federal Medical Biological Agency as background information [13]. The populations of victims of the Chernobyl disaster (77 individuals) and other radiation accidents (38 individuals), who received the exposure doses not exceeding 8 Gy were considered (Table 1). Correlation analysis was used as a research method.

We assessed the relationship between the absorbed dose and the concentration of lymphocytes in peripheral blood of

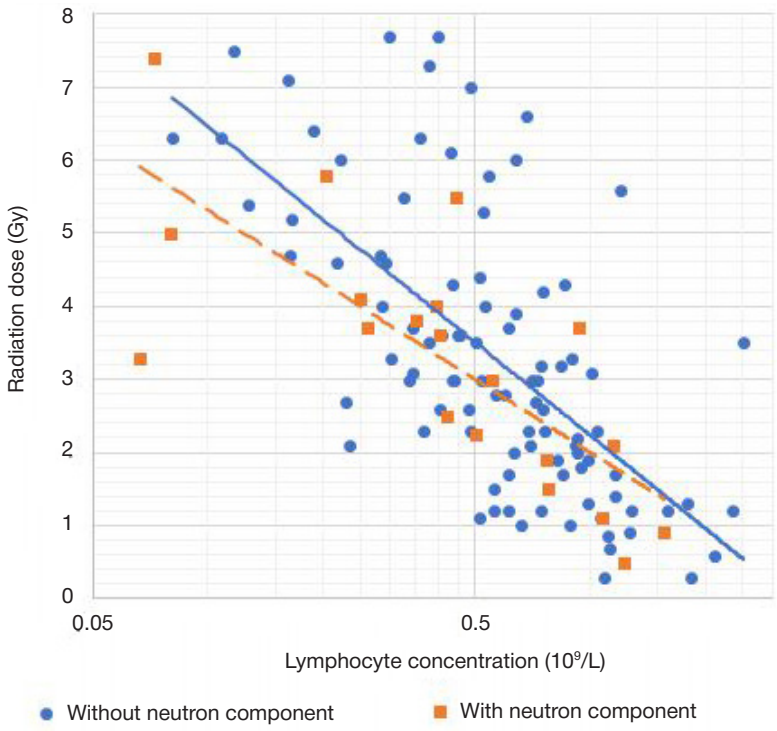

Fig. Radiation dose (Gy) as a function of lymphocyte counts (×10^9/L) on day 2 after exposure. Markers indicate baseline data (separately for groups exposed to gamma and gamma neutron radiation), and lines indicate the observed trends

Table 3. Estimated uncertainty of the received dose (Gy) and radiation injury severity based on peripheral blood lymphocyte concentration on day 2 and the average value on days 3–6 after exposure (based on the data from [9])

Lymphocyte concentrations, $\times 10^9$/L	Days after exposure			
	Gamma radiation		Gamma neutron radiation	
	2	3–6	2	3–6
< 0.2	II–IV	III–IV	II–IV	III–IV
0.3	4.4 (3–5.9) II–III	5.8 (4.2–7.4) III–IV	3.7 (2.6–4.9) II–III	4.5 (3.0–6.0) II–III
0.4	3.9 (2.5–5.4) II–III	5 (3.3–6.6) II–IV	3.3 (2.2–4.4) II–III	3.7 (2.2–5.2) II–III
0.5	3.5 (2.1–5) II–III	4.3 (2.7–5.9) II–III	3 (1.9–4.1) I–III	3.1 (1.7–4.6) I–III
0.6	3.2 (1.7–4.6) I–III	3.8 (2.2–5.4) II–III	2.7 (1.6–3.9) I–II	2.6 (1.3–4.0) I–II
0.8	2.6 (1.2–4.1) I–III	3 (1.4–4.6) I–III	2.3 (1.2–3.4) I–II	1.8 (0.7–3.0) I–II
1	2.2 (0.8–3.7) до II	2.3 (0.7–3.9) I–II	2 (0.9–3.1) до II	1.1 (0.2–2.0) до II

victims on day 2 after exposure for the data considered, we also determined the correlation between these parameters. The cases of combined exposure to gamma neutron radiation were considered separately. The results are provided in Figure and Table 3.

RESULTS

The earlier published report showed that the concentration of lymphocytes in peripheral blood decreased approximately exponentially during the first day after exposure to the clinically significant dose range [10]. The constant of the lymphocyte concentration decrease rate between hours 2 and 18 after exposure is correlated to the radiation dose D, which enables estimation of this dose based on two time points of blood testing [11]:

$$D = -(k/\Delta T) \times \ln(L_1/L_2) \quad (1)$$

where L_1 and L_2 — lymphocyte counts in blood samples collected at time points t_1 and t_2 after exposure ($t_2 > t_1$), $\Delta T = t_2 - t_1$ — time between blood samples, and the constant $k = 144$. Similar to the data provided in the paper [11], using the formula provided makes it possible to estimate absorbed dose according to two blood tests (Table 2).

The data provided in Table 2 were used to assess the correlation between the absorbed dose and the lymphocyte counts in peripheral blood of victims on day 2 after exposure (Figure). Statistical processing made it possible to estimate uncertainty of the radiation injury severity predicted based on one blood test performed on day 2 after exposure (Table 3). For comparison, the table also provides the values predicted based on the average lymphocyte counts on days 3–6 after exposure.

DUSCUSSION

It should be noted that in case of gamma neutron exposure the predicted dose is on average 10–15% lower than in case of gamma exposure.

Turning to the issue of the possibility of using one blood test performed within the first 24 h after exposure, the results of the study can be used that show that individual fluctuations of peripheral blood lymphocyte counts in healthy people constitute about + 20% of the average value at long-term follow-up [11]. That is why the data of previous blood testing cannot be considered as a reliable guide to refine the prediction. The ratio (1), where the data of first lymphocyte count measurement L1 are represented by the data of the victim's blood testing performed a few days before exposure and the $\Delta T = t - 2$ parameter, where t is an interval between the time of exposure and blood testing performed within the period between hours 2 and 18 after exposure, can be used for dose estimation.

The concentration parameters of blood can be of lower significance due to multiple clinical problems not related to radiation exposure and the spread of biological parameters: the fact of infusion and transfusion therapy, non-radiation-induced injuries, ethnicity, age, health status, and gender of the assessed victims, parameter reduction or elevation using drugs, etc. [14, 15]. That is why establishing preliminary diagnosis based on the lymphocyte test only, without taking into account other data and the listed above reasons, can result in biased estimated dose or radiation injury severity.

CONCLUSIONS

Validation of information about the peripheral blood lymphocyte counts on day 2 after exposure made it possible to adjust the predicted radiation injury severity: 1) when peripheral blood lymphocyte concentration is below 0.2 × 10^9/L, severe (grade III) or extremely severe (grade IV) ARS is predicted; 2) when the lymphocyte concentrations are within the range of 0.2–1.0 × 10^9/L, the estimated dose absolute error is ±1.5 Gy in case of gamma exposure and ±1.3 Gy in case of gamma neutron exposure. Victims are diagnosed with moderate (grade II) or severe (grade III) radiation injury, it is necessary to start treatment in a specialized hospital as soon as possible; 3) when the peripheral blood lymphocyte concentration exceeds 1.0 × 10^9/L, mild (grade I) to moderate (grade II) ARS can be predicted. As compared with the method reported in the paper [9], this test enables prediction of radiation dose based on the data of only one blood test performed on day 2 after exposure. This may be preferable in cases of large-scale radiation accidents and incidents, when the medical resources available are not enough for full-fledged diagnosis of the ARS severity. The lymphocyte test remains one of the most simple and accessible biological dosimetry methods, which defines its role in the diagnosis of radiation injury associated with large-scale accidents, when no cytogenetic test results are available in the first days after the incident. Prediction accuracy can be improved in the future with additional sources of information about the lymphocyte concentration in the first days after exposure.

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