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Research Article

Lymphocytes with "Tailed" Nuclei (LTN) in Blood Smears as the Easiest Biomarker of Radiation Exposure That is Acceptable in Emergencies

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Abstract

Lymphocytes with "tailed" nuclei (LTN) which are easily observed in routine smears of peripheral blood are a biological response to radiation. In this article, we describe LTN as a biomarker that has the same origin as dicentric chromosome. In emergency situations, we recommend to use this simple biomarker in conjunction with hematological tests of the blood smears of the exposed persons.

Introduction

Sources of ionizing radiation are now widely used in all areas of human life, which dramatically increases the likelihood of radiological emergency and the possibility of radiation injury to people. According to IAEA data (2003), apart from the Chernobyl disaster, more than 430 major radiation incidents have occurred globally, resulting in at least 3,000 people suffering significant irradiation, 133 of these cases fatal. The most significant radiation accidents were Chernobyl disaster (Ukraine, 1986), the accident in Goiânia (Brazil, 1987), San Salvador (El Salvador, 1989), Tammiku (Estonia, 1994), Tokai-mura (Japan, 1999). Unfortunately, this sad trend continues in this century: the accident in the Samara Region (Russia, 2000), Lja (Georgia, 2001), Bialystok (Poland, 2001), etc. The accident at Fukushima-1 Nuclear Power Plant, Japan, in March 2011 also has had tragic and nasty consequences.

Within a relatively short time upon such radiation accidents, many victims and alleged victims seek medical care, and such a situation results in dramatic difficulties in the operation of various medical institutions. The most important method used to confirm the fact of irradiation and measure the dose of radiation in a person is dosimetry, which in the initial stages of radiation accidents and disasters actually limits opportunities to provide health care.

The quantitative assessment of radiation dose received by the human body is conducted by physical dosimetry (with the use of dosimeters and radiometers) or biological dosimetry. Physical dosimetry shows the precise dose of ionizing radiation absorbed by the human body; however, the method has several disadvantages: high margin of error of equipment used to measure the radiation dose, measurements limited to the area where the dosimeter is attached to clothing, measurement of only gamma radiation, etc. In addition, in case of radiation emergency or other situations of uncontrolled exposure to radiation, physical dosimetry data can be absolutely unavailable.

In emergency situations it becomes particularly important to use clinical and laboratory parameters that can, with varying confidence level, confirm the fact of radiation exposure and determine the degree of its severity. Such indicators are called biomarkers of radiation exposure. The methods of biological dosimetry are based on dose-dependent reactions of body tissues to the effects of different types of ionizing radiation. A wide range of biodosimetry methods (mainly cytogenetic methods requiring cultivation of lymphocytes in vitro or the use of special equipment) has been developed and is currently used [1-5], but these are hardly feasible in emergency situations with a large number of people affected.

Among the earliest clinical manifestations of acute radiation exposure, the most diagnostically valuable clinical symptoms of the primary reaction to irradiation are: dyspeptic (anorexia, nausea, vomiting, diarrhea, intestinal dyskinesia), neuromotor (fatigue, lethargy, weakness), neurovascular (sweating, hyperthermia, headache, hypotension, tachycardia) and local reactions (redness of the skin and mucous membranes, dry mouth, swelling of the parotid glands). Apart from the clinical manifestations of the primary reaction to irradiation, a crucial role in the diagnosis of the severity of radiation injury belongs to the early postradiation changes in hematological parameters. The symptom with the best diagnostic value is early lymphopenia due to post-radiation cell death: a lymphocyte count within 48–72 hours after irradiation makes it possible to determine the dose of radiation absorbed, predict the degree of lymphocyte count fall and the period when agranulocytosis can be expected to develop. The most obvious dependence of the degree of leukopenia on the radiation dose is established only 7–9 days after the exposure to radiation.

In the inaugural issue of "Emergency Medicine" of this year, we present the simplest cytogenetic biomarker of radiation exposure, which we found in routine blood smears in persons irradiated as a result of the accident at the Chernobyl atomic power station in 1986. It is possible to conduct analysis to detect such biomarker of radiation, which we call a "lymphocytes with a "tailed" nucleus" (LTN), in any country, in any medical institution laboratory where differential leukocyte count in blood smears can be determined. We introduce the tailed nuclei lymphocytes found in the peripheral blood smears of irradiated patients (Chernobyl nuclear accident liquidators) and show the radiation-caused nature of the "tail" of the nuclei, namely its origin in dicentric chromosomes, which are already regarded as undisputed classic markers of radiation effects.

Materials and Methods

The study group of irradiated subjects comprised 207 males aged from 30 to 72 years. 203 of them had been irradiated 6±11 years previously while carrying out duties as Chernobyl liquidators. According to their medical histories, 200 received low irradiation doses (below 0.25 Gy), while three experienced high doses (above 1.5 Gy). In addition, four other subjects who were not Chernobyl liquidators were also sampled: patient A, accidental irradiation about 0.6 Gy, sampled 6 years after irradiation; patient B, accidental irradiation of skin with up to 20 Gy, sampled 6 months after irradiation; patient C, part body radiotherapy to a total dose of 6 Gy, sampled 3 days after irradiation; and patient D, accidental irradiation with a dose about 1.5 Gy, sampled 7 years after irradiation.

Control groups included 114 males aged from 19 to 60 years, 68 females aged from 19 to 60 years, and 37 children aged from 3 to 6 years; none of them, according to questionnaires, had ever been irradiated.

Smears of peripheral blood taken from a finger prick were prepared by a routine method, air dried, fixed with 96% ethanol, and stained with Giemsa. Frequencies of lymphocytes with "tailed nuclei" (LTN) were determined in samples of 500 cells. Cells with signs of degeneration or mechanical damage due to the spreading procedure and cells in thick parts of the smear were not analyzed.

Preparations of human metaphase chromosomes were obtained by routine 48 hour culture of PHA-stimulated lymphocytes. From 100 to 300 metaphases per subject were analyzed in 44 of the Chernobyl liquidators and patients A, B and D.

The bicolour hybridization in situ was performed on the peripheral blood smears by the method [6]. As DNA probes for the FISH, there were used the plasmide Bluescript KS that is biotinized using the nick-translation and contains the telomere-specific sequence (TTAGGG)n of the 181 n.b. length, as well as the alphoid DNA probe specific for the centromere regions of all human chromosomes (Cambio). To detect hybridizational signals, the standard system was used. FITC and TRITC served as detectors of the biotinized probe and of the probe labeled with digoxigenine, respectively.

The data were subject to statistical testing using the Wilcoxon-Mann-Whitney non-parametric u-test and Student t criteria. Coefficients of correlation were calculated according to Spearman's method. Standard errors are only quoted for parametric tests and where the error is less than half the mean.

Results and Discussion

The lymphocyte nuclear tails in our observations represent morphological anomalies of interphase nuclei. They consisted of a thin protrusion or outgrowth of the nucleus into the cytoplasmic space. Such tails often have a terminal enlargement in the form of an oval or round micronucleus. The lengths of these tails varied from 2 to 7 mm. The color, chromatin struc-

ture, and staining intensity of the tails generally corresponded to those of the nucleus of the lymphocyte in which it was observed. It should be noted that sometimes chromatin of the tails was more condensed at the periphery of the terminal enlargement than chromatin of the nucleus, while the terminal enlargement had a clear centre. Sometimes, the cytoplasm of the LTN also contained small separate micronuclei.

The most frequently detected types of lymphocyte "tails" are presented in Fig. 1. (The photomicrographs shown were obtained by us in 1994 from the archive of hematological samples taken from Chernobyl liquidators.)

Results of observation of more than 1000 LTN has permitted a classification of tails into 16 different morphological types in human lymphocytes and, in data not presented here, these types have also been observed in peripheral lymphocytes of other species. They are presented schematically in Fig. 2. A brief description of the main types is as follows:

• Types 1-3 have relatively thick tails with a terminal enlarge ment. Chromatin both of the tails and of the nuclei is slightly condensed, without aggregations or signs of pycnosis. The types 1-3 differ from each other only in length (1-long, 2-medium, 3-short).

• Type 4 is an elongated tail without constriction at the nucleus end and with no terminal enlargement.

• Type 5 is similar in size and shape to type 4, except for the presence of a constriction where it emerges from the nucleus.

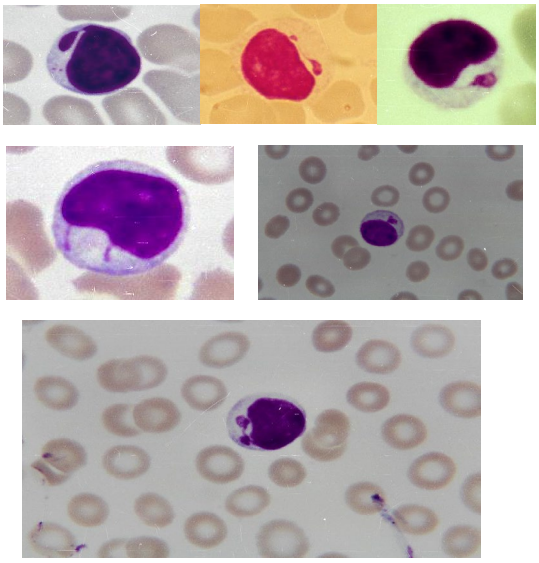


Fig. 1. Photomicrographs of lymphocytes with «tailed nuclei». Romanowsky stained peripheral blood smears. Magnification 1000x.

• Types 6-8 have a thin stalk of different lengths and a terminal enlargement in the form of a drop-like or round micronucleus, with chromatin more condensed at the periphery.

• Types 6-8, as with types 1-3, differ from each other only in the length of the stalk.

• Type 9 differs from types 6-8 only by the presence of breaks or discontinuities of the chromatin strand forming the stalk.

• Type 10 is a relatively thin and long tail without a terminal enlargement.

• Type 11 emerges from the nucleus at a point where the nucleus has a convex protuberance.

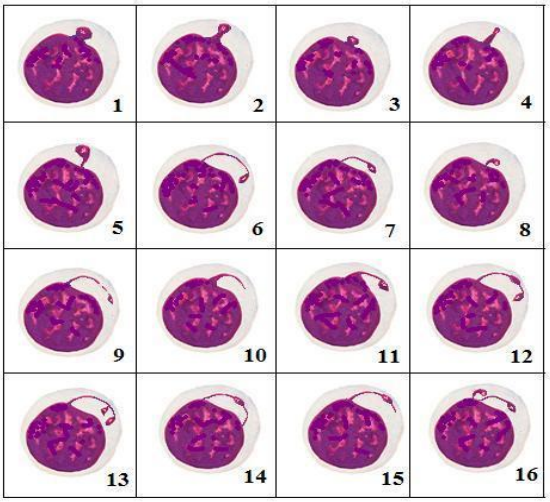


Fig. 2. Drawings of the 16 types of "tailed" nuclei observed in human lymphocytes.

• Type 12 has two consecutively located chromatin enlargements.

• Type 13 is a tail with a thin bifurcated stem with terminal enlargements.

• Type 14 has one enlargement in the form of a drop-like or round micronucleus connected to the nucleus by two stems.

• Type 15 has a thin distal chromatin strand extending out of the enlargement.

• Type 16 has two nuclear tails which could be all of the above types.

It should be noted that separate micronuclei can be present in

cells with all types of tails. It should be noted that in all cases, the nuclear tails consist of one or two strands; in more than 1000 LTN observed, the presence of more than two chromatin strands in the tail has never been detected.

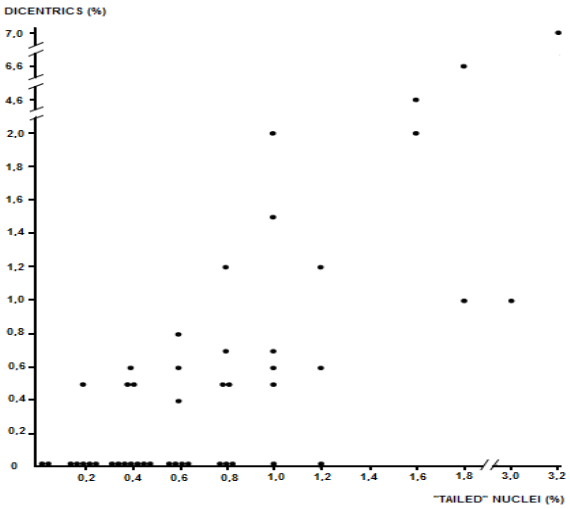


Fig. 3. Scatter diagram showing a correlation for 47 individuals whose lymphocytes were examined for both dicentrics and tailed nuclei.

In 47 of the irradiated subjects (44 Chernobyl liquidators and patients A, B and D) with differing frequencies of LTN the frequencies of lymphocytes with dicentric chromosomes was also scored. A positive, statistically significant correlation ($p < 0.001$) was found between the presence of tails and dicentrics in cells from the same individuals. The correlation coefficient by the Spearman test was 0.73 (Fig. 3). Elevated frequencies of dicentric chromosomes in lymphocytes were found in 17 out of 22 subjects in whom the frequency of LTN was 0.8 or higher. Thus, increased incidence of LTN and higher number of dicentrics were observed in all irradiated patients and liquidators, as well as in patients A, B and D. Such biomarker as the emergence of LTN is, like dicentric chromosomes, a predictable and universal manifestation of irradiation. In cases of radiation exposure, one can observe "tailed" nuclei in vivo not only in human lymphocytes, but even in nucleated erythrocytes of fish [7].

To study origin of the "tailed" nuclei, we used method of the bicolor FISH to locate the near-centromere and telomere heterochromatin in the nuclear "tails".

The bicolour fluorescent hybridization in situ revealed that centromeres were located predominantly in the base and/or in the region of the chromatin enlargement at the end of the "tails", while telomeres, only in the latter region (Fig.4 scheme and Fig.5 photomicrographs).

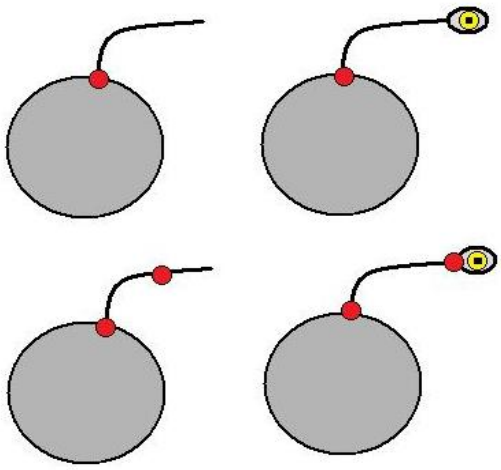
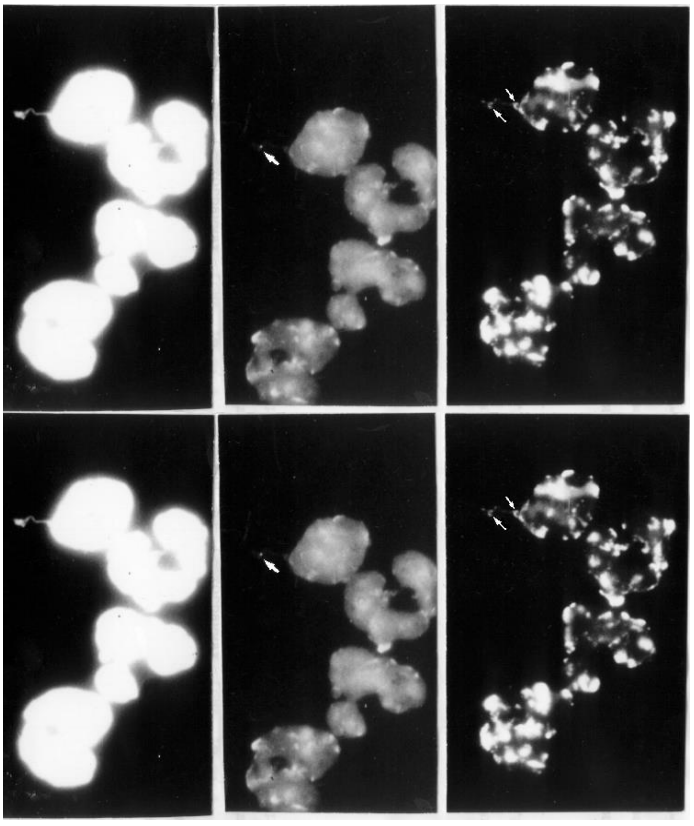


Fig. 4. Scheme of localization of the centromere signals revealed by TRITC (•) and telomere signals revealed by FITC (x) in the "tails" of lymphocytic nuclei.



(DAPI) (FITC) (TRITC)

Fig. 5. Centromere TRITC, telomere FITC signals in the "tale" of lymphocyte DAPI (a). Peripheral blood smear. Magnification 1000x.

Among 46 LTN examined there were no "tails" with more than two centromere signals and one telomere signal. This indicates the nuclear "tails" to be probably formed by one and/or two

chromosomes. It is also to be noted that sometimes in the lymphocyte culture in vitro there were revealed the "tailed" cells at the stage of prophase. In such cells, the "tails" always represented two parallel chromatin bands.

The data obtained seem to allow the following conclusions to be made. In the liquidators irradiated with small doses 6-11 years ago, the frequency of the incidence of LTN is increased. Most likely, this is due to the chromosomal cycles "breakdown —> fusion —> bridge" occuring in the lymphocytic population. The formation of the anomaly of interphase nuclei of the "tail" type seems to be due to appearance of dicentric chromosomes, chromosomal bridges with their subsequent breakdown. The proposed scheme of the formation of LTN is presented in Fig.6.

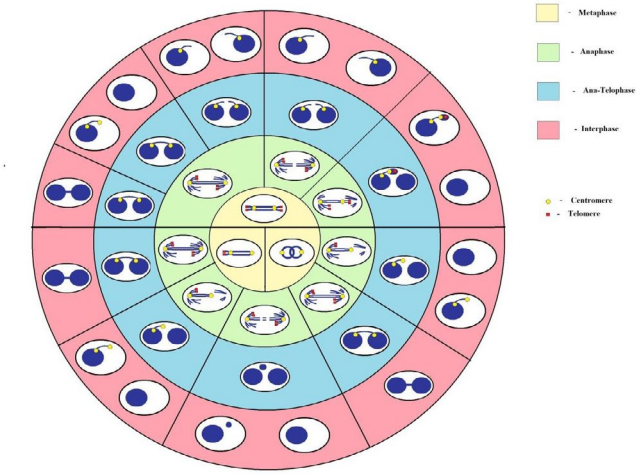


Fig. 6. The proposed scheme of formation of "tailed" nuclei from dicentrics and rings chromosomes.

It cannot be ruled out that the nuclear "tails" can include chromatin of the entire dicentric chromosome rather than a half of the broken dicentric only; this is confirmed by the data of study on the near-centromere and telomere regions of the chromosomes in the "tails". For this reason, to designate the nuclear anomalies considered in the current work, the term ""tailed" nuclei" rather than "semi-bridge" was chosen.

The same nuclear abnormalities in malignant cells are formed also as a result of pathologic mitoses with chromosomal bridges by dicentric and centric ring chromosomes, a mechanism shown in a series of studies using fluorescence in situ hybridization (FISH) by a wide spectrum of probes on whole chromosomes and their loci [8,9].

The distribution of healthy donors and irradiated subjects on parameter"frequency of lymphocytes with "tailed" nuclei" is shown in Fig. 7. In control groups, the frequency varied from 0 to 1.0%, so that in the vast majority of control persons no LTN were observed in their lymphocytes. We have arbitrarily assessed the upper limit of normal for the frequency of peripheral blood LTN as 0.6%. The mean frequency of LTN in the control men was 0.14%, in control women 0.17%, and in control children 0.05%. By contrast, most irradiated subjects had LTN. The maximum value in this group was 3.2%, with the mean 0.50%. The differences between the irradiated persons and the control groups were statistically significant ($p < 0,001$, in all cases).

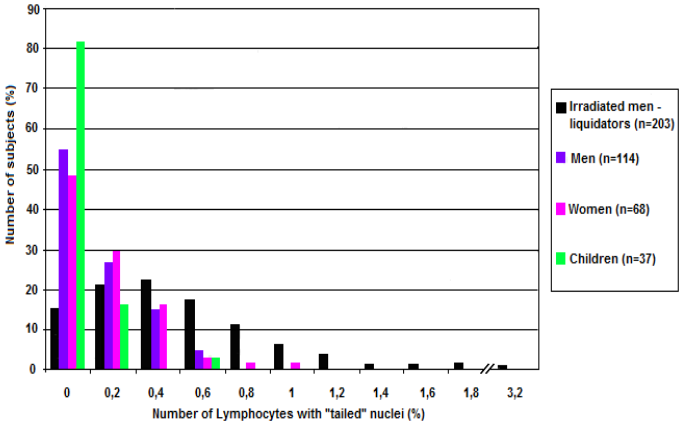


Fig. 7. Frequencies of blood lymphocytes with "tailed" nuclei observed in Chernobyl liquidators and control subjects.

The histograms above give an idea of the average spontaneous incidence, as well as variation of the incidence of lymphocytes with tailed nuclei in populations unexposed to radiation. Apparently, the upper normal level for this parameter is 0.6%. In patients A, B, C and D, these figures were much higher - 1.8%, 3.2%, 8.6% and 3.0%, correspondently.

The frequency of LTN and the degree of their morphological diversity could be used to judge the magnitude of the radiation dose and this judgment may become more accurate at some time after the exposure. This is because; following relatively large radiation exposure, an abortive rise in the numbers of peripheral blood lymphocytes is observed which reflects the highly dynamic changes in the maturation of lymphocyte reserves from the bone marrow. Thus, the maximum frequency of appearance of LTN should be expected one month after irradiation. This was confirmed by data on patients with Hodgkin's disease (patient C) after radiotherapy. It was after 1-2 months, rather than during the first week, that the frequency of LTN attains its maximum.

In Chernobyl liquidators, binuclear lymphocytes with chromosomal bridges (Fig. 8) were also observed with an average frequency of 0.057%, whilst in male control donors similar cells were recorded with a frequency of 0.005%.

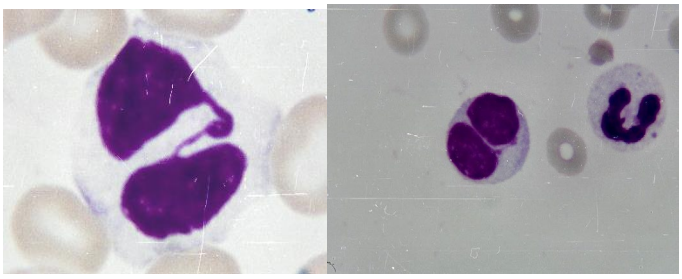


Fig. 8. Photomicrographs of lymphocytes with internuclei bridge. Romanowsky stained peripheral blood smears. Magnification 1000x.

When performing the count of tailed nuclei, the incidence of lymphocytes with internuclear chromatin bridges should be also taken into account. Bridges, however, are a much rare phenomenon than "tails". Therefore, we focus on the incidence of "tailed" nuclei in lymphocytes. Lymphocytes are circulating cells, so their division is always followed by cytokinesis. In this article we discuss biomarkers in vivo mainly as exemplified by "tailed" nuclei rather than bridges in lymphocytes. The incidence of LTN is significantly higher than that of bridges. Apparently, this is due to the circulation of lymphocytes in the body and the specifics of their maturation followed by compulsory cytokinesis. Rarely proliferating epithelial cells in persons exposed to radiation are usually found to have bridges rather than "tailed" nuclei. For example, the bridges can persist for decades in thyrocytes of subjects exposed as children to radioactive iodine in Chernobyl. Thus, the exposure of the thyroid gland to radioiodine is shown by such biomarker as strongly stretched internuclear bridges in the cells of follicular epithelium of thyroid, although thyrocytes are also observed to have "tailed" nuclei [10].

We would like to emphasize that the simplest biomarker (LTN) we suggest for radiation exposure detection in vivo is not an alternative to classical radiation biomarkers in vitro [1,3,4], as well as to other modern approaches [5,11-13]. Detection of chromosomal aberrations (dicentrics) and the cytochalasine B micronucleus and "Cytome" methods [2] are reliable bio-indication and dosimetry methods. However, in an emergency situation, when hundreds and even thousands of people may be subject to radiation exposure, these testing methods are unlikely to be available for all the exposed subjects, which require the cultivation of lymphocytes in vitro. In this article, we describe a biomarker that has the same origin as dicentrics (double stroke chromosomal aberrations) but occurs and persists in vivo. This biomarker can be detected in fingertip blood smears, and the simplicity and low cost of the method make it practical in emergency bioindication of the impact of radiation factors.

The degree of incidence of LTN in vivo is a relevant bioindicator, and the analysis can be conducted on the same fingertip blood smears in parallel with a standard lymphocytes and neutrophils count.

Conclusion

Lymphocytes with "tailed" nuclei (LTN) which are easily observed in routine smears of peripheral blood are a biological response to radiation. In emergency situations, we recommend to use LTN as the simplest biomarker in conjunction with hematological tests of the blood smears of the exposed persons.

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