The use of optical microscope equipped with multispectral detector to distinguish different types of acute lymphoblastic leukemia

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Abstract. The article describes the use of a computer optical microscopy with multispectral camera to characterize the texture of blasts bone marrow of patients with different variants of acute lymphoblastic leukemia: B- and T- types. Specific characteristics of the chromatin of the nuclei of blasts for different types of acute lymphoblastic leukemia were obtained.

1. Introduction

Diagnosis of acute lymphoblastic leukemia (ALL) and their variants is based on morphological, cytochemical and immunophenotypic characterization of leukemic cells pool. In light microscopy an important role plays accurate assessment of some parameters of blasts: cell shape and size, shape of the nuclei, features of the chromatin structure, taking into consideration thin loop texture, lumpy texture, dense structure. Polymorphism of blasts associated with a variety of phenotypic, genetic and molecular biological characteristics. So far, it failed to show a significant correlation between the structure of blasts and immunophenotypical status. In conventional light microscopy heterogeneity of the blast population is ascertained in cases of T- and B- types of acute lymphoblastic leukemia [1-3]. Computer optical microscopy using multispectral camera has more features for the study of the structure of nuclear chromatin fibers and allows to objectify data in the form of a numeric indexes. Comparative study of blasts at different variants of acute lymphoblastic leukemia with the help of modern high technologies will expand our knowledge of the biology of leukemic cells and to identify possible patterns in the structure of the nuclear blasts differences in different types of ALL [4-10].

The aim of this work is to study features of nuclear chromatin structure of bone marrow blasts T- and B- types of acute lymphoblastic leukemia cases the using computer optical microscopy with multispectral camera and formalization of the resulting figures for comparative analysis.

2. Materials and methods

Computer microscopy was performed on stained bone marrow smears of 26 ALL patients, 11 of which have been set T-ALL, 15 of which have been set B-ALL. Morphocytochemical and

immunophenotypic studies were conducted in the laboratory of immunology hematopoietic of N.N. Blokhin Russian Cancer Scientific Center (Head of Laboratory, Professor Dr. med Tupitsyn N.N.).

Images of cells were obtained in the RGB color model based on measurements of the function of brightness in three spectral ranges corresponding to red (R), green (G) and blue (B) regions of spectrum of visible electromagnetic radiation (automated microscope Olympus BX43 with a multispectral camera Imperx IPX-4M1ST-GCFB). Images were saved in BMP format, color-coded RGB24 (over 16 million colors).

4866 images of bone marrow lymphoblasts were received, 2164 of these were from patients with T-ALL, 2702 cells with B-ALL.

Investigation of the nuclei chromatin structure was performed with data transformation into the components of color models RGB, XYZ, HSL, Lab, Luv, LHC, HLS, HSV, YUV, YIQ, YCbCr, CMY[8]. Texture features were based on spatial adjacency matrices and matrices of the lengths of the series. These features were considered separately. Averages on group characteristics of blasts nuclei were calculated for T-ALL and B-ALL. The support vector machine and Bayes classifier were used with Euclidean, Manhattan and Chebyshev distance functions in two-dimensional feature space to separate the data with different acute lymphoblastic leukemia variants.

3. Results

Characteristics of the structure of blasts were calculated in a 360 features specific to each of the 26 cases. Among them a couple of features was identified that characterized all the events with minimal error. First feature P_{IPS} is the value of the texture characteristic "fraction of image in the series", it was calculated according to the matrix of the series in the HSV color space for color tone H. Second feature P_{OMS} is the value of the texture characteristic "reverse moment of series", it was calculated from the matrix of series length in LAB color space for the component B. The variability characteristics of the cells was estimated within each type. The statistical characteristics P_{IPS} and P_{OMS} for T- and B-cells (Table 1): $(\bar{x}, S, D_{\bar{x}}, \text{Min}, \text{Max}, t)$ were calculated. Here $\bar{x}$ – arithmetic mean of characteristic value, $D_{\bar{x}}$ – estimation of the mean arithmetic value variance of the characteristic, Min – minimum value of the characteristic, Max - maximum value of characteristic for the sample corresponding variant T-ALL cells and B-ALL cells, t – student's t-criterion to test the hypothesis of equality of sample averages.

Table 1. Statistical characteristics of features P_{IPS} and P_{OMS} for T- and B- blast cells

	number of cells	P_{IPS}						P_{OMS}					
		$\bar{X}$	S	$S_{\bar{x}}$	Min	Max	t	$\bar{x} *10$	S	$S_{\bar{x}}$	Min	Max	t
T-cells	2164	3,909	0,007	2,96	5,52	2164	3	6,165	0,008	0,48	0,73	6,165	15
B-cells	2702	3,880	0,006	2,90	6,52	2702		6,327	0,007	0,45	0,79	6,327	

Student's t-criterion was calculated according to the formula $t = \dfrac{\bar{x}_T - \bar{x}_B}{\sqrt{D_{\bar{x}_T} + D_{\bar{x}_B}}}$ to test the hypothesis about equality of means for samples of T-blasts and B-blasts from the experimental data. Here the indexes T and B define cell types. For a significance level of 0.05 t-test values greater than 1.96 indicate statistical significance of differences in mean values for the T- and B- blast cells.

Table 2. Generalized characteristics M_{IPS} and M_{OMS} for group T- and B-cells patients with acute lymphoblastic leukemia.

The patient (type of blasts)	Quantity of cells	M_{IPS}	M_{OMS}	The patient (type of blasts)	Quantity of cells	M_{IPS}	M_{IPS}
P1(B)	222	4,16	0,643	P 14(B)	202	3,89	0,626
P 2(B)	181	3,93	0,626	P 15(B)	205	3,71	0,633
P 3(B)	202	3,68	0,648	P 16(T)	206	4,14	0,623
P 4(B)	122	3,76	0,642	P 17(T)	209	3,91	0,600
P 5(B)	210	3,91	0,643	P 18(T)	204	4,04	0,624
P 6(B)	204	3,83	0,671	P 19(T)	200	3,77	0,579
P 7(B)	226	3,90	0,642	P 20(T)	169	3,74	0,595
P 8(B)	193	4,19	0,582	P 21(T)	208	3,76	0,601
P 9(B)	125	3,85	0,625	P 22(T)	161	3,87	0,604
P 10(B)	200	3,48	0,629	P 23(T)	193	4,32	0,635
P 11(B)	125	3,73	0,628	P 24(T)	200	3,82	0,652
P 12(B)	204	3,86	0,640	P 25(T)	206	3,64	0,626
P 13(B)	81	4,16	0,646	P 26(T)	208	3,83	0,601

Thus on the average values of these features can be judged on ALL version (T or B-type). At the same time ranges of attribute values for the T blasts completely cover the range of values of B-blasts, that does not allow determine the type of individual cells for the values of the considered features.

In the next phase of the study generalized characteristics M_{IPS} and M_{OMS} were examined, the average values of characteristics P_{IPS} и P_{OMS} were defined for a group of cells of the same patient. The values for the generalized characteristics are presented in table 2.

A graphical representation of the results of table 2 is shown in Figure 1.

As you can see, most of the features of the B-blast cells are located in the area above the line segment with the coordinates M0(3,4;0,598) and M1(4,4;0,649), while T-blast cells are concentrated in areas below the specified line segment. The exceptions were in two cases of T-ALL for P24 - P25 patients and one case of B-ALL for patient P8. Consequently, in 23 (87%) of the 26 patients had a certain distribution of the data pattern. In three cases this association was absent. Therefore, the study of the texture of nuclei blasts allows in most cases to establish a definite link between research results and ALL variant. Thus, the data obtained with the use of optical microscopy with multispectral camera can be used for the determination of objectifying study of acute lymphoblastic leukemia variants.

The present observation is that the characteristic of blast chromatin texture is essential biological criterion that gives grounds to assume the relationship between the orientation of cell differentiation and the nuclei chromatin structure. Further refinement of research methods and the extension of the sample of patients will allow to characterize in depth this problem.
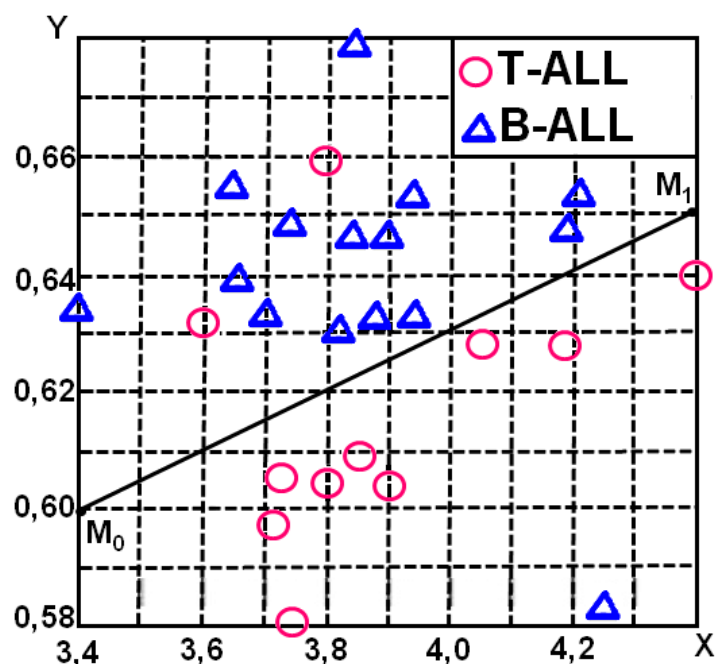
Figure 1. The distribution of the two classes (T-ALL and B-ALL) in two-dimensional feature space. Features are represented according to the coordinate axes: first feature plotted along the Y-axis $Y = M_{OMS}$, : second feature plotted along the X-axis $X = M_{IPS}$.

4. Conclusion
The study showed the possibility of using a computer optical microscopy with multispectral camera to establish T - or B - variants of acute lymphoblastic leukemia.

Planned step for further research is analysis of possibilities of application of computer optical microscopy with multispectral camera to define subtypes within T-ALL and B-ALL.

Acknowledgments

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