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ANALYSIS OF THE EXPRESSION OF APOPTOSIS MARKERS ON PERIPHERAL BLOOD LYMPHOCYTES AND BLAST CELLS IN ACUTE LEUKEMIA IN CHILDREN

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ABSTRACT

Relevance: *A topical issue of oncohematology is the search for effective approaches to therapy and methods of predicting the course of acute leukemia. A promising direction in this field is the study of changes in the expression of molecular markers on the cell surface in the dynamics of chemotherapy.*

The study aimed to *determine the expression level of proteins annexin V, Bcl-2, CD95, and p53 on peripheral blood lymphocytes and leukemic bone marrow cells in children diagnosed with acute leukemia.*

Methods: *the study design was cross-sectional. Research method: immunophenotyping. Peripheral blood and bone marrow of 106 patients with acute leukemia diagnosed for the first time at 1 month to 16 years (study group) and peripheral blood of 23 conditionally healthy children aged 2 to 17 years (control group) served as the study materials. The obtained data were subjected to statistical processing.*

Results: *The study of Bcl-2 expression in B-ALL did not reveal reliable differences; in T-ALL, Bcl-2 expression was significantly higher on peripheral blood lymphocytes than on blasts. In OML, Bcl-2 expression was significantly higher in the blast cell population than in lymphocytes. When CD95 expression was analyzed in ALL, expression was significantly higher on peripheral blood lymphocytes than on the membrane of leukemic cells. The results of the analysis of the expression of annexin V showed that lymphocytes expressed a marker of early apoptosis significantly more than bone marrow blasts. This phenomenon is a dangerous sign indicating a decrease in antitumor immunity. Comparative analysis of p53 protein expression on the surface of lymphocytes and blast cells showed no significant differences in leukemia variants.*

Conclusion: *The study indicated the prognostic significance of Bcl-2 and CD95 in acute leukemia. Annexin V and p53 did not show reliable sensitivity and specificity, which allows not to include these markers in the leukemia immunophenotyping panel.*

Keywords: *acute leucosis, blast cells, markers of apoptosis, Bcl-2, CD95.*

Introduction: Significant progress has been made in diagnosing and treating acute leukemia (AL) over the past decade. The clinicians' main problem remains the presence of resistant forms of the disease and the frequency of relapses. Accordingly, the search for effective methods of treatment, diagnostics, and prognosis of the AL course is one of the main topics of domestic and foreign oncohematology. In this area, the study of the biological characteristics of leukemia cells, particularly changes in the expression of molecular markers on the surface of cells during chemotherapy dynamics, seems to be a promising area [1, 2].

It is known that an important role is played by an imbalance between proliferation and the ability of cells to die naturally (apoptosis) in the development of most malignant tumors. The main function of apoptosis is to eliminate transformed cells, including virus-infected, tumor-infected, or irreversibly damaged cells. Induction of apoptosis is the main mechanism of action of most chemotherapy drugs used in the intensive treatment of AL [2-11]. Currently, two main interrelated mechanisms of apoptosis are actively investigated: mitochondrial and receptor ones. They function with a balanced interaction of pro- and anti-apoptotic factors, such as proteins of the Bcl-2 family, p53, and CD95 proteins [2].

Thus, H.F. Ebian et al. found that increased regulation of Bcl-2 was paradoxically associated with increased apoptosis and low rates of early mortality in AML patients [12]. Low Bcl-2 expression levels indicated inhibition of antiapoptosis and chemosensitivity in malignant cells [2].

The CD95 protein is known to be directly involved in the initiation and regulation of apoptosis. The results of studies showing a significant increase in CD95 expression on the surface of cells obtained from patients with acute leukemia, breast cancer, and glioblastoma after chemotherapy or radiation treatment are presented in [13-16]. A high level of CD95 expression predicted a favorable response to chemotherapy in acute lymphocytic leukemia (ALL) [2]. At the same time, M. Tiribelli et al. have shown an association between increased Bcl-2 expression and chemotherapy resistance and low survival in AML [17].

A key element in ensuring the genomic stability of a cell is the transcription factor p53, known as the "im-

mortality protein" or "guardian of the genome." It is involved in the regulation of l death receptor genes (DR5, Fas); l genes responsible for stopping cell division (P21, GADD45, etc.); l genes that trigger apoptosis (I-VM, KILLER DR5, PIG, etc.); l causes repression of genes that inhibit apoptosis (BCL-2, RELLA). Dysfunction of p53 is found among many malignant diseases, including AL. In adult ALL, p53 gene mutations are found in 13% of cases, but in children, this figure is much lower at 2% of cases. It may lead to a more favorable prognosis and a high frequency of remission in children [2]. Studying the p53 expression in patients with various AML variants, A. Ahmádzadeh et al. found a poor prognosis in patients with high levels of this protein expression [18]. Therefore, studying the complex of apoptotic (annexin V, CD95, p53) and anti-apoptotic antigens (Bcl-2) will make it possible to identify differences in the signaling pathways of apoptosis in different variants of acute leukemia. It may be helpful to look for abnormalities in programmed cell death mechanisms to determine methods to predict leukemia cells' sensitivity and/or resistance. Analysis of the role of annexin V, CD95, p53, and Bcl-2 markers controlling apoptosis will make it possible to develop recommendations for improvement of the panel of surface antigens in cell immunophenotyping at various stages of therapy.

The study aimed to determine the expression level of proteins annexin V, Bcl-2, CD95, and p53 on peripheral blood lymphocytes and leukemic bone marrow cells in children diagnosed with acute leukemia.

Materials and methods:

The study design is cross-sectional.

The study materials were the peripheral blood and bone marrow from 106 patients (the study group) diagnosed with acute leukemia and the peripheral blood of 23 conditionally healthy children (the control group). The patients' age varied from 1 month up to 16 years in the study group and from 2 to 17 years in the control group.

All patients with AL received appropriate therapy at the Scientific Center for Pediatrics and Pediatric Surgery (Almaty, Kazakhstan). The stay of patients diagnosed with ALL in the inpatient regime was 8 months, for patients with AML - 4-5 months.

Samples from patients with marked leukopenia and in the absence of signed informed consent or informed waiver from the study prior to initiation of therapy were excluded from the study.

Research method: immunophenotyping of bone marrow and peripheral blood. The acute leukemia diagnostic panel included 51 immunophenotypic markers. The samples were analyzed on a flow cytometer FacsCantoII (Planet Dickéncon, USA) in the DIVA program. The data were collected under several parameters in the DIVA software of the flow-through cytometer FacsCantoII: forward light scattering (FSC), side light scattering (SSC), and 8 fluorescence channels. The linearity and subvariant of acute leukemia were determined at the first stage using a multicolor panel of monoclonal antibodies. The same samples on bone marrow blast cells and peripheral blood lymphocytes of patients with AL were tested for annexin V, Bcl2, CD95, and p53 protein expression before treatment.

Statistical methods: The SPSS statistical program was used for statistical data processing. The Mann-Whitney U-test was used to analyze the differences between the two independent variables. The significance level was set to $p<0.05$ to determine reliability.

Results: The linearity and subvariant of acute leukemia in children were determined with the use of a panel of monoclonal antibodies; the expression of apoptotic (annexin-V, CD95, p53) and anti-apoptotic antigen (Bcl-2) on bone marrow blast cells and peripheral blood lymphocytes was found for the same sample.

The structure of AL immunological variants in the studied patients is presented in Figure 1.

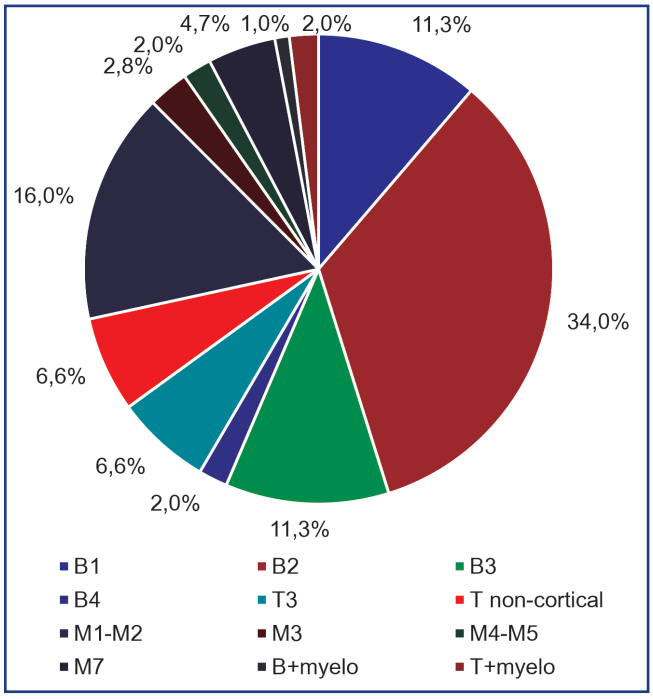


Figure 1 – Structure of AL immunologic variants (106 patients)

Thus, most ALL cases were B-ALL cases (46.1%), among which the B2 variant prevailed (34.0%). The next most common cases were AML, which accounted for 38.7% of all ALs. T-ALL accounted for 13.2% of cases, while T-cortical (T3) and non-cortical (non-T3) variants were equally common – 6.6% of the total number of ALLs. The mixed-cell phenotype of AL (biphenotypic OL) was the rarest, accounting for 2% of all leukemias. Figure 2 shows a CD45/SSC scattergram showing the gating of a blast population (R2) that has a paler (CD45) luminescence for the total leukocyte antigen CD45 compared to the lymphocyte population (R1).

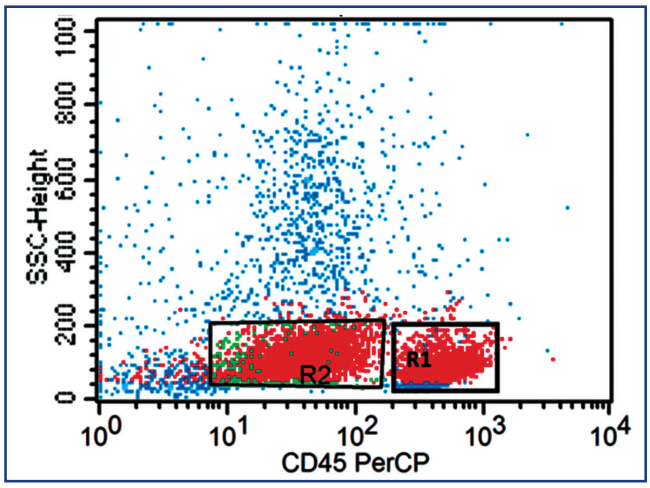


Figure 2 – CD45/SSC scattergram

Comparison of the expression of apoptotic antigens on peripheral blood lymphocytes in patients with AL and conditionally healthy children. Table 1 presents the analysis results for the expression level of the proteins - annexin V, Bcl-2, CD95, and p53 on peripheral blood lymphocytes in patients with ALL and conditionally healthy children.

A comparison of the early apoptosis marker, annexin V, expression on peripheral blood lymphocytes found the following. The mean expression of this marker in the control group did not significantly differ from the corresponding indicator in patients with almost all leukemia subvariants except for the mature B4 variant and the M7 variant of AL. At that, annexin expression was notably lower than in the control group.

Table 1 – Comparison of the expression levels of apoptosis markers on peripheral blood lymphocytes in patients with ALL (n=106) and the control group (n=23), M±m

AL variant Number of patients (N)	Markers of apoptosis							
	annexin V		P53		Bcl-2		CD95	
	Early apoptosis,%	Medium value	%	Medium value	%	Medium value	%	Medium value
B2 (n=36)	60.8±3.4	42.5±6.1	2.9±1.3	7.1±1.9	17.8±5.8	6.0±3.3	33.3±4.3	11.6±2.5
B1 (n=12)	68.5±7.3	50.4±5.6	2.8±1.5	7.6±1.7	12.9±1.5	5.6±1.4	23.1±1.9	12.0±3.3
B3 (n=12)	57.2±3.2	48.3±6.8	4.7±2.2	8.8±2.9	8.9±2.9	6.0±0.5	25.9±3.2	11.1±3.6
B4 (n=2)	50.4±5.5	39.2±2.1	11.4±2.9	11.1±2.7	15.4±4.6	4.5±2.3	46.5±2.1	9.4±3.9
T3 (n=7)	70.9±1.6	40.5±3.9	1.7±1.7	9.6±1.7	10.1±3.6	4.3±0.8	43.1±3.9	12.1±3.8
T4 (n=7)	70.7±4.8	47.5±3.9	3.8±4.5	8.4±1.5	17.7±4.0	3.6±1.4	37.6±2.9	7.4±4.1
M1-M2 (n=17)	54.1±2.9	51.9±4.4	3.5±4.3	7.8±2.1	26.0±3.2	6.1±2.3	30.2±2.8	8.9±2.8
M3 (n=3)	57.6±3.4	32.1±3.4	3.6±3.3	7.6±1.6	6.0±5.1	4.2±1.6	27.2±1.3	7.9±1.1
M4-M5 (n=2)	53.7±11.7	28.7±7.8	5.9±1.9	6.4±1.2	2.8±2.8	6.6±2.9	8.3±12.7	12.2±8.8
M7 (n=5)	49.2±4.5	64.8±7.1	4.1±4.2	7.1±1.7	9.6±1.9	5.5±2.6	16.0±2.5	10.0±1.5
T+myelo (n=2)	55.6±11.5	42.6±5.5	2.8±6.0	7.4±2.7	8.8±10.9	7.1±3.9	21.1±3.4	11.5±2.0
B+myelo (n=1)	65.0	3025.2	2.9	15.8	11.6	11.7	39.1	12.1
Control group (n=23)	66.1±3.7	21.9±2.5	3.6±2.1	6.1±0.9	10.9±2.3	4.4±1.2	20.3±3.2	7.3±1.1

Note: Arithmetic Mean (M), Standard Deviation (±m)

Analysis of annexin expression within the leukemia variant showed a significant difference between the group with B1 ALL (68.57.3, $p<0.05$) and B4 ALL (50.4±5.5%) among B-lymphoblastic ALs. No significant difference was found within the T-ALL groups. There was also no significant difference in annexin expression on peripheral blood lymphocytes among the myeloblastic and biphenotypic leukemia variants.

More significant changes were observed in the study of the annexin fluorescence intensity, which turned out to be significantly lower in the control group than in the corresponding indicator in all other subgroups. It should be noted that the fluorescence intensity of annexin V in the biphenotypic variant (B+myelo) of leukemia increased manifold, even though its expression did not differ from that of the control group. Fluorescence intensity is probably a more sensitive indicator for determining the course and outcome of the disease.

The apoptotic marker p53 expression ($t>2.2$; $p<0.05$) was significantly higher only in the subvariant with the mature B-form of ALL (B4), both compared to the control group and other leukemia subvariants. However, no convincing changes in fluorescence intensity (mean for p53 antigen) were determined in the analysis between the control group and leukemia variants, as well as within the groups, in contrast to annexin. According to these findings, p53 is not specific in its expression or fluorescence intensity in oncohematological diseases. However, the limited number of patients in each group reduced the reliability of the presented findings. The number of leukemia subvariant studies shall be increased to achieve statistically significant groups.

Comparing the anti-apoptotic marker Bcl-2 expressions on lymphocytes in conditionally healthy children and patients with leukemia, we found that the Bcl-2 expression on peripheral blood lymphocytes in healthy children was significantly higher than in patients with any ALL subvariant. At the same time, no significant differences in the Bcl-2 protein expression were found in the control group and patients with AML and biphe-

notypic variant of AL, and there were no differences in the fluorescence intensity. There is an assumption that there will be an increase in the expression level of the Bcl-2 anti-apoptotic marker on the patients' lymphocytes due to the activation of the cellular link [19]. However, this hypothesis was confirmed only in patients with ALL. This could be due to the statistically small number of studies conducted, which indicates the need for further study.

In the CD95 apoptosis marker parameter analysis, the mean value of the control group was significantly lower than the corresponding value in ALL and biphenotypic leukemia patients. In contrast, high CD95 expression was determined only in M1-M2 variants of AML in the groups of AML patients. No significant difference in fluorescence intensity was determined between the control and study groups. We assumed that increased CD95 expression on peripheral blood lymphocytes in leukemia patients indicates inhibition of immune system activity by suppressor factors produced by tumor cells to avoid immunological surveillance. This assumption was confirmed only for ALL. In comparing the expression and intensity of CD95 fluorescence between leukemia subvariants, the intensity reached the maximum in the B4 and T3-cortical variants and the minimum in the M4-M5 variants. However, interpreting these findings is impossible due to the small sample size (n=1).

Expression analysis of apoptotic markers on peripheral blood lymphocytes and bone marrow blast cells in patients with lymphoblastic AL. Analysis of the apoptotic marker annexin V expression showed a significant increase in protein expression on peripheral blood lymphocytes of patients compared to bone marrow blasts. Among B-ALL, a significant increase was characteristic of the B1 and B2 subvariants, T-ALL for the T3-cortical subvariant, AML for the M1-M2 and M4-M5 subvariants, as well as in biphenotypic variants of AL (B+myelo and T+myelo) ($p<0.05$, $t>2.2$). The fluorescence intensity was significantly higher in T3, M7, and biphenotypic leukemia. In our opinion, the pronounced expression of the early apoptosis marker on lymphocytes is an unfavorable prognostic factor, indicating the focus of immunocompetent cells on programmed death and a decrease in antitumor immunological surveillance.

Table 2 – Expression of apoptosis markers in bone marrow blast cells in patients with acute lymphoblastic leukemia, M±m

AL variant, quantity Patients (N)	Apoptosis markers							
	annexin V		P53		Bcl-2		CD95	
	Early apoptosis,%	Mean	%	Mean	%	Mean	%	Mean
B2 (n=36)	44.4±7.8	27.8¥5.4	4.8¥7.2	8.7¥2.3	17.2¥16.4	6.9¥8.7	8.1¥2.7	6.1¥7.2
B1 (n=12)	29.0¥13.0	33.5¥26.5	4.3¥4.5	7.6¥1.0	10.8¥13.2	5.7¥1.6	2.8¥2.2	4.8¥1.4
B3 (n=12)	55.6¥8.7	37.7¥10.1	3.9¥5.2	8.6¥1.2	26.5¥19.0	6.6¥2.1	5.3¥3.1	5.2¥1.0
B4 (n=2)	37.8¥18.2	15.6¥2.5	29.2¥20.7	16.8¥7.8	9.6¥1.3	3.4¥1.8	3.0¥0.4	4.8¥0.1
T3(n=7)	36.4¥7.2	16.7¥4.8	3.0¥1.5	6.2¥2.6	2.1¥1.3	3.8¥2.5	3.0¥1.5	5.0¥1.8
T-non-cort (n=7)	62.7¥2.8	39.3¥5.5	4.2¥3.1	6.4¥3.3	8.3¥11.8	5.1¥4.3	4.2¥3.1	5.3¥2.5
M1-M2 (n=17)	37.1¥14.7	44.5¥24.5	5.0¥6.0	13.0¥4.2	11.0¥17.4	8.0¥6.0	16.4¥13.2	13.3¥4.9
M3 (n=3)	56.5¥11.2	36.9¥7.5	1.9¥2.4	14.6¥1.6	57.5¥2.0	12.9¥0.7	10.5¥6.8	16.8¥1.7
M4-M5 (n=2)	35.0¥11.7	37.4¥7.8	3.5¥1.9	11.3¥1.2	2.9¥2.8	7.3¥2.9	30.6¥12.7	17.5¥8.8
M7 (n=5)	53¥14.5	43¥6.6	5.2¥2.0	13.4¥1.1	1.8¥1.1	5.5¥1.1	3.8¥2.3	8.6¥2.9
T+myelo (n=2)	23.9¥11.5	27.5¥5.5	6.8¥6.0	11.0¥2.7	15.9¥10.9	7.8¥3.9	3.5¥3.4	8.1¥2.0
Biphenotypic B+myelo (n=1)	40.1	33.1	84.7	31.1	0.21	17.9	24.2	8.7

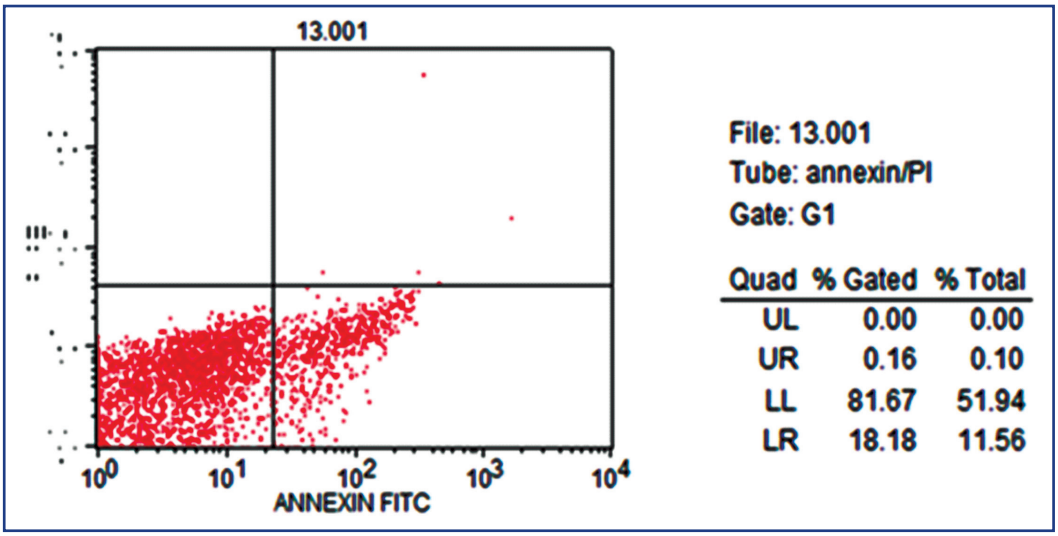
Note: Mean is the arithmetic mean (M). The data is presented as M±m, where m is the standard deviation

While comparing the annexin V expression on bone marrow blasts in different ALL subvariants, the minimum expression was found in the B1 variant and the biphenotypic form (T+myelo), indicating the pronounced viability of the blasts. The maximum expression was found in the B2, B3, T4, M3, and M7 variants. An increase in fluorescence intensity was also characteristic of these leukemia subvariants. It has been shown that B3 and M3 linear leukemias have a favorable prognosis, and T-non-cortical ones are more favorable compared to the cortical form. Probably, the presence of a larger number of apoptotic proteins on the surface of blasts will contribute to their accelerated death. It is not the expression of annexin itself that should be considered but the indicator that has shown great sensitivity, i.e., the fluorescence intensity. An increase in the patient samples and future studies could affect the search results. However, we can say that even based on the data obtained, it is necessary to analyze this marker on the blasts and lymphocytes of patients in order to predict the disease course.

Among T-ALL, the early apoptosis marker expression on blast cells was significantly reduced in the group with the T3-cortical variant (36.4±7.2%) compared to the group of non-cortical T-ALL (62.7±2.8%) ($p<0.05$, $t=2.9$), while the annexin expression was maximum in peripheral blood lymphocytes in these types of leukemia. The mean value is also lower in patients with T3-cort ALL (16.7± 4.8 c.u.) compared to the corresponding indicator of non-cortical T-ALL (39.3±5.5 c.u.).

Among AML, the annexin-V expression on peripheral blood lymphocytes was significantly higher than on blast cells in the M1-M2 and M4 subvariants of AML. Acute promyelocytic leukemia (AML M3) and acute megakaryocytic leukemia (AML M7) are known to have an aggressive course and an unfavorable outcome compared to other types of AML. Thus, it was implied that the annexin-V marker expression on tumor cells in these AML subvariants would be lower than in the groups with B-ALL characterized with a more favorable course, but no significant differences were found.

In our opinion, the significantly low expression of annexin on blasts in the biphenotypic (T+myelo) variant compared to the B+myelo variant is interesting.

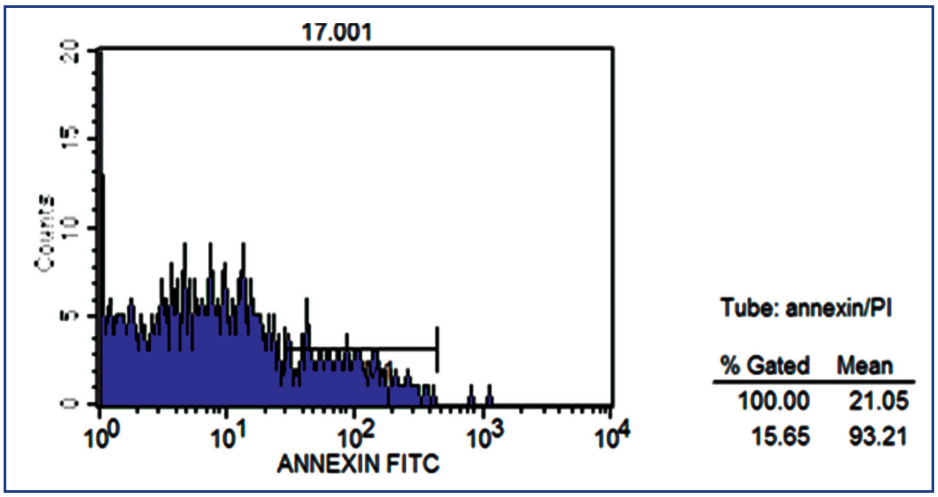


Legend: UL (upper left) – upper left quadrant, UR (upper right) – upper right quadrant, LL (lower left) – lower left quadrant, LR (lower right) – lower right quadrant, Gated – area of cells, Quad – quadrant, Total – total percentage

Figure 3 – Expression of fluorescein-labeled annexin V (FITC) on blast cells

The lower right quadrant of the graph (LR) shows blast cells that are annexin-V positive, i.e., at the stage of early apoptosis (18.18%). The left lower quadrant (LL) contains cells that are annexin-V negative, hence alive (81.57%), the left upper quadrant (UL) contains dead cells (0.00%), and the right upper quadrant (UR) contains cells in the late apoptosis stage (0.16%) (Figure 3).

The mean value of FITC-positive cells containing annexin-V was 21.05 units (Figure 4).



Legend: Gate is the area of cells; Mean is the average intensity of fluorescence

Figure 4 – One-parameter histogram of annexin V fluorescence intensity (mean)

In the groups with myeloid ALs, the annexin V expression on peripheral blood lymphocytes was significantly higher than on blast cells in leukemia subvariants M1-M2 and M4-M5 ($p<0.05$, $t=11.2$). At the same time, no significant differences were found in the groups with M3 and M7 subvariants. High expression of annexin-V on lymphocytes may signal a more unfavorable course of the disease due to the accelerated withdrawal of lymphocytes from participation in antitumor immunity. In the future, we intend to analyze the correlation between the disease's clinical course and the expression of apoptosis markers. No signif-

icant differences have been determined in annexin mean fluorescence intensity.

Thus, the analysis of an early marker of apoptosis in patients with ALL and AML shows that this antigen is expressed higher on peripheral blood lymphocytes than on tumor cells. A similar picture was observed concerning the fluorescence intensity.

Expression analysis of p53 protein. The analysis of the p53 protein expression on peripheral blood lymphocytes and blast cells in various AL subvariants showed a significant increase in this marker expression only in peripheral blood lymphocytes of patients with the B4 subvariant compared to lymphocytes of other groups. The same pattern was observed when comparing the p53 expression on blast cells. ($p<0,05$; $t>2.2$) (Figure 5, Table 1).

No significant difference in p53 protein expression was found among T-ALL (3.0±1.5% and 4.2±3.1%, respectively) ($p<0.05$; $t>2.2$). No significant difference was also found in the groups with AML (Table 1). Assessment of fluorescence intensity did not show significant differences in the studied groups. Therefore, it can be assumed that the p53 marker is not functionally significant for this study (Figure 5).

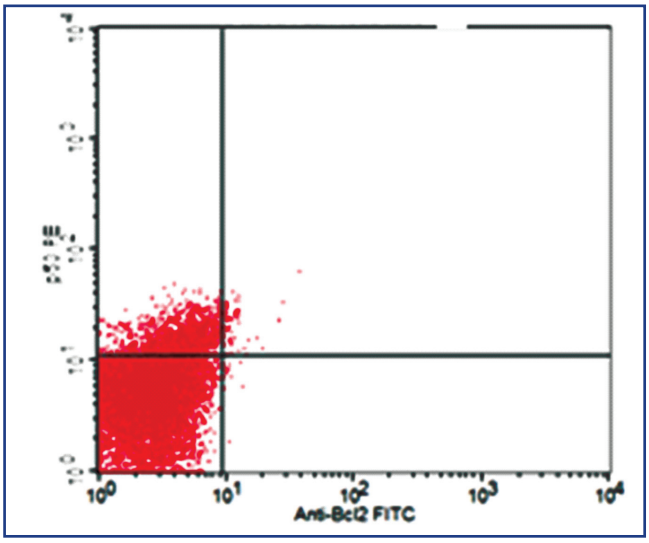


Figure 5 – Dot plot of the Bcl-2 marker and p53 protein expression

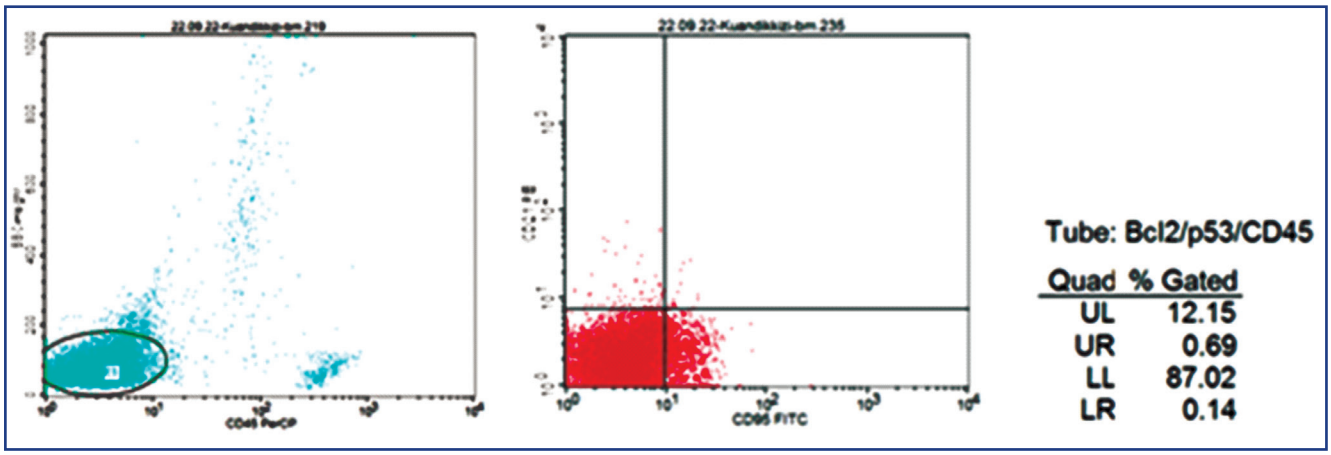
Expression analysis of anti-apoptotic marker Bcl-2. In our study, this marker's expression on peripheral blood lymphocytes was significantly lower in all patient groups before treatment than in healthy patients, except for the M1-M2 variants, where the Bcl-2 expression did not differ from the control group.

A comparative analysis of this marker expression on lymphocytes within groups showed that the highest expression was observed in the M1-M2 subtypes. We consider it a prognostically favorable sign, as it indicates the preservation of a certain functional activity of lymphocytes in AML. The expression of Bcl-2 in the blast cell population in different variants of B-ALL did not differ significantly but was significantly higher than in patients with T-ALL.

The bcl-2 expression was at minimum in the M3 variant on peripheral blood lymphocytes of patients in the group with AML and at maximum in the M1-M2 variant. The Bcl-2 expression on blast cells was the maximum in the M3 variant. The wide range of Bcl-2 expressions in the AML group compared to ALL could be due to the origin of blasts from different hematopoietic sprouts (myelocytic and lymphocytic ones). At the same time, it evidences a greater prognostic value of Bcl-2 in AML. A comparison of the expression results of this parameter between all the studied groups demonstrated that the minimum expression of the anti-apoptotic marker on blast cells was observed in ALL T3 and AML M4-M5. In contrast, the maximum was observed in AML M3 (Figure 6).

Expression analysis of the Fas ligand CD 95. Analysis of CD95 expression on peripheral blood lymphocytes within the groups did not show significant differences. At the same time, CD95 expression on blasts in all types of leukemia was notably lower than on the peripheral blood lymphocytes of these patients.

The percentage of antigen in the B2 group (8.1±2.7%) was increased compared with the B1, B3, and B4 subvariants (Table 1). There was no significant difference in the expression of the CD95 surface marker on the blasts within the groups of patients with T-ALL. Among AML, the expression of the CD95 ligand was significantly higher in the M4-M5 variants than in others. In the M7 variant, the expression of CD95 on the blasts was minimal, significantly lower than other AML variants. The highest expression of the CD95 marker was found in the M4-M5 OL group ($t=4.0$, $p<0.001$) (Figure 6) in summary table 1 for variants (B, T, myelo). The mean fluorescence intensity was also higher in the AML group (16.4±13.2 c.u.).



Legend: Gate is the region of cells, Q is the quadrant

Figure 6 – Gating strategy: 1) isolation of the blast population gate, 2) detection of expression of Bcl-2 and p53 antigens

Discussion: Apoptosis is known to be the primary mechanism by which most chemotherapeutic agents induce tumor cell death. It is more likely that the balance of expression (annexin V, CD95, p53) and anti-apoptotic protein (Bcl-2) can control the response of leukemia cells to chemotherapy and subsequently affect the patient's prognosis. Therefore, the task of this study was to determine the markers that will have prognostic significance for the studied leukemia variants.

This study area is relatively new, so only a few publications are available that determine the functional significance of apoptosis markers in predicting the course and response to therapy in patients with oncological and oncohematological diseases. In the presented work, we obtained data that partially corresponded to the already available results.

We also found a high expression of an early apoptosis marker, annexin V, on the lymphocytes of healthy patients. It does not correspond to the literature data, where the expression of annexin V in the healthy population is much lower. This result indicates the need for larger studies on a larger population sample. At the same time, the intensity of annexin fluorescence on lymphocytes in the healthy population is significantly lower than that of patients with leukemia. In the future, fluorescence intensity will probably be considered a more specific indicator for annexin V. However, we have not found any publications highlighting changes in its fluorescence intensity. Besides, a comparative analysis of annexin V expression on lymphocytes (the combined index of all groups of lymphoblastic leukemias is 63.8%) and blasts (44.3%) showed that this marker was significantly more expressed on lymphocytes than in the blast population, and it is an alarming sign in relation to the suppression of antitumor immunity. The expression of annexin V on lymphocytes was 58%I in myeloid leukemias, while it was 45.4% on blast cells but these differences were insignificant. No significant differences were also found when the expression of annexin within leukemia subvariants was considered. Therefore, this marker did not show pronounced specificity and cannot be recommended as a prognostic for monitoring and predicting the course of the pathological process.

Comparative analysis of p53 protein expression on the surface of lymphocytes and blast cells revealed no significant differences in leukemia variants. Available publications present the data from immunohistochemical studies of solid tumors (ovarian cancer, lung cancer, etc.), where this marker shows a certain diagnostic significance [20]. However, no significant differences in p53 expression were found in our study. Perhaps this marker is more specific for tumor cells of solid tumors. However, it is possible that there are no significant changes due to the small size of groups with rare leukemia subvariants, and therefore, there is a need for further study of this marker.

The study of the Bcl-2 protein expression on lymphocytes and blast cells in B-cell variants did not show significant differences, whereas this marker was expressed in significantly greater numbers in the T-cell variant on peripheral blood lymphocytes than in blasts. In AML, Bcl-2 expression was significantly higher in the blast cell population compared to lymphocytes. It may be one of the mechanisms of "tumor avoidance of immunological surveillance." As an anti-apoptotic marker, Bcl-2 contributes to prolonging the viability of the blast population, causing the phenomenon of "immortalization." At the same time, A. Cahyadi et al. [21] found no correlation between Bcl-2 expression and both response to induction chemotherapy and relapse rates in ALL, indicating that Bcl-2 expression levels have rather low prognostic significance. At the same time, it was quite surprising that all cell samples that showed a good response to the initial prednisolone therapy showed a significantly higher expression of Bcl-2 than those that did not respond well. Thus, high levels of Bcl-2 expression in ALL may indicate that in vivo tumor cell survival is dependent on cytokines. Glucocorticoids are known to have a potent anti-inflammatory ef-

fect due to the suppression of cytokine gene expression [22], and the treatment with prednisolone in vivo may result in an overall decrease in cytokine production. Thus, it is interesting to suggest that the prednisolone-induced reduction in cytokine expression may be responsible for the favorable response of blast cells (decreased apoptosis intensity) to prednisolone in ALL cell samples with high levels of Bcl-2 expression. The results of a study of Bcl-2 expression in ALL suggest that Bcl-2 expression levels may be higher in patients with a favorable response to treatment [23]. In the absence of published results of comparative analysis of the expression of apoptotic annexin-V, p53, CD95, and anti-apoptotic Bcl-2 markers on leukemia cells and lymphocytes, it is necessary to compare the level of expression with the clinical course of the disease in each case in order to establish the diagnostic significance of each marker, and it will determine the direction of further research.

According to the studies, the most diagnostically significant changes were shown in a comparative analysis of CD95 expression on lymphocytes and the blast population. CD95 expression on peripheral blood lymphocytes was significantly higher than on the blast population for any form of ALL, any subvariant of T-AL and B-AL. The results are consistent with the findings of A.Yu. Varishnikov et al., who showed that CD95 expression in blast cells is a favorable prognostic sign associated with an increase in relapse-free and overall survival. In contrast, the absence of CD95 antigen in blasts is an unfavorable sign for developing the disease. Thus, monitoring CD95 expression and CD95 function during in vivo chemotherapy may help to further determine the prognostic value of CD95 for drug-induced apoptosis in all patients. No significant differences were found in AML. It indicates the functional preservation of lymphocytes, but such a factor as a small number of patients could affect the results obtained.

Conclusion The results of the present studies indicate the prognostic significance of Bcl-2 and CD95 in acute leukemia. Further studies of these molecules are required and included in the standard panel of immunophenotyping in leukemia to identify favorable and/or unfavorable prognostic value in ALL in children. Annexin V and p53 showed no significant sensitivity and specificity. It allows not to include these markers in the immunophenotyping panel of leukemia.

References:

1. Hodunova E.E. Issledovanie markerov programmirovannoj kletochnoj gibeli (Bcl-2, Bax, r53 i CD95): dis. – Moskva: FGBU NMIC gematologii Minzdrava Rossii, 2013. – 117 s. [Khodunova E.E. Study of markers of programmed cell death (Bcl-2, Bax, p53 and CD95): diss. - Moscow: Federal State Budgetary Institution "National Medical Research Center of Hematology" of the Ministry of Health of the Russian Federation, 2013. – 117 p. (in Russ.)]. https://www.dissercat.com/content/issledovanie-markerov-programmirovannoi-kletochnoi-gibeli-bcl-2-bax-p53-i-cd95-v-period-indu

2. Parovichnikova E.N., Hodunova E.E., Gal'ceva I.V., Kulikov S.M., Troickaya V.V., Kuz'mina L.A., Shcheblyakov D.V., Savchenko V.G. Markery apoptoza v CD34-pozitivnyh kletkah pri ostryh lejkozah // Klinicheskaya onkogematologiya. Fundamental'nye issledovaniya i klinicheskaya praktika. - 2013. – Vol 4(6). - P. 373-378 x Parovichnikova E.N., Khodunova E.E., Galtseva I.V., Kulikov S.M., Troitskaya V.V., Kuzmina L.A., Shcheblyakov D.V., Savchenko V.G. Apoptosis markers in CD34-positive cells in acute leukemia // Clinical oncohematology. Fundamental research and clinical practice. – 2013. – Vol. 4 (6). – P. 373-378 (in Russ.)]. https://old.bloodjournal.ru/markery-apoptoza-v-cd34-pozitivnyh-kletkah-pri-ostryh-lejkozah/

3. Newton K., Strasser A., Kayagaki N., Dixit V.M. Cell death // Cell. - 2024. – Vol. 187(2). - P. 235-256. https://doi.org/10.1016/j.cell.2023.11.044

4. Obeng E. Apoptosis (programmed cell death) and its signals // Braz. J. Biol. - 2021. – Vol. 81(4). - P. 1133-1143. https://doi.org/10.1590/1519-6984.228437

5. Nagata S., Tanaka M. Programmed cell death and the immune system // Nat. Rev. Immunol. - 2017. – Vol. 17(5). - P. 333-340. https://doi.org/10.1038/nri.2016.153

6. McBride A., Houtmann S., Wilde L. The Role of Inhibition of Apoptosis in Acute Leukemias and Myelodysplastic Syndrome // Front Oncol. - 2019. – Vol. 9. - P. 192. https://doi.org/10.3389/fonc.2019.00192

7. Ling V.Y., Straube J., Godfrey W., Haldar R., Janardhanan Y., Cooper L., Bruedigam C., Cooper E., Tavakoli S.P., Jacquelin S., Tey S.K., Baell J., Huang F., Jin J., Zhao Y., Bullinger L., Bywater M.J., Lane S.W. Targeting cell cycle and apoptosis to overcome chemotherapy resistance in acute myeloid leukemia // Leukemia. - 2023. – Vol. 37(1). - P. 143-153. https://doi.org/10.1038/s41375-022-01755-2

8. Qian S., Wei Z., Yang W., Huang J., Yang Y., Wang J. The role of BCL-2 family proteins in regulating apoptosis and cancer therapy // Front. Oncol. - 2022. – Vol. 12. - P. 985363. https://doi.org/10.3389/fonc.2022.985363

9. Hussar P. Apoptosis Regulators Bcl-2 and Caspase-3 // Encyclopedia. - 2022. – Vol. 2(4). - P. 1624-1636. https://doi.org/10.3390/encyclopedia2040111

10. Wang H., Guo M., Wei H. Targeting P53 pathways: mechanisms, structures and advances in therapy // Sig. Transduct. Target Ther. - 2023. – Vol. 8. - P. 92. https://doi.org/10.1038/s41392-023-01347-1

11. Neophytou C.M., Trougakos I.P., Erin N., Papageorgis P. Apoptosis Deregulation and the Development of Cancer Multi-Drug Resistance // Cancers. - 2021. – Vol. 13(17). - P. 4363. https://doi.org/10.3390/cancers13174363

12. Ebian H.F., El-korashi L.A., Embaby A. Spontaneous apoptosis and BCL2 gene expression as predictors of early death and short overall survival in acute leukemia patients: a prospective, case cohort study // Egypt J. Med. Hum. Genet. - 2021. – Vol. 22. - P. 91. https://doi.org/10.1186/s43042-021-00210-8

13. Levoin N., Jean M., Legembre P. CD95 Structure, Aggregation, and Cell Signaling // Front Cell Dev Biol. - 2020. – Vol. 8. - P. 314. https://doi.org/10.3389/fcell.2020.00314

14. Hanna J.R.A. Expression of CD95 in Acute Lymphocytic Leukemia (ALL) in Egyptian Children before and after Treatment // J. Blood Disorders Trans. - 2015. – Vol. 6. - P. 250. https://doi.org/10.4172/2155-9864.1000250

15. Qadir A.S., Ceppi P., Brockway S., Law C., Mu L., Khodarev N.N., Kim J., Zhao J.C., Putzbach W., Murmann A.E., Chen Z., Chen W., Liu X., Salomon A.R., Liu H., Weichselbaum R.R., Yu J., Peter M.E. CD95/Fas Increases Stemness in Cancer Cells by Inducing a STAT1-Dependent Type I Interferon Response // Cell Rep. - 2017. – Vol. 18(10). - P. 2373-2386. https://doi.org/10.1016/j.celrep.2017.02.037

16. Akın D.F., Ozkan Didem T., Nejatd K.E.A. Screening of single nucleotide polymorphism in CD95 (APO-1/FAS) promoter region (G-1377A) in children with acute leukemia // The Egyptian Journal of Haematology. - 2018. – Vol. 43(2). - P. 45-48. https://doi.org/10.4236/oalib.1104385

17. Tiribelli M., Michelutti A, Cavallin M, Di Giusto S, Simeone E, Fanin R, Damiani D. BCL-2 Expression in AML Patients over 65 Years: Impact on Outcomes across Different Therapeutic Strategies // J. Clin. Med. - 2021. – Vol. 10(21). - P. 5096. https://doi.org/10.3390/jcm10215096

18. Ahmadzadeh A., Hossein Mohammadi M., Mezginezhad F., Aghaee N.H., Parkhideh S., Khosravi M., Khazaei Z., Adineh H.A., Allahbakhshian F.M. The expression of the TP53 gene in various classes of acute myeloid leukemia // WCRJ. - 2018. – Vol. 5(4). – P. e1178. https://doi.org/10.32113/wcrj_201812_1178

19. Saddam M., Paul S.K., Habib M.A., Fahim M.A., Mimi A., Islam S., Paul B., Helal M.M.U. Emerging biomarkers and potential therapeutics of the BCL-2 protein family: the apoptotic and anti-apoptotic context // Egypt J. Med. Hum. Genet. - 2024. – Vol. 25. – Art. no. 12. https://doi.org/10.1186/s43042-024-00485-7

20. Pundir D.S., Pundir C.S. Detection of tumor suppressor protein p53 with special emphasis on biosensors // Analyt. Biochem. - 2020. – Vol. 588. – Art. no. 113473. https://doi.org/10.1016/j.ab.2019.113473

21. Cahyadi A., Ugrasena I.D.G., Andarsini M.R., Larasati M.C.S., Aryati A., Arumsari D.K. Relationship between Bax and Bcl-2 Protein Expression and Outcome of Induction Phase Chemotherapy in Pediatric Acute Lymphoblastic Leukemia // Asian Pac. J. Cancer Prev. - 2022. – Vol. 23(5). - P. 1679-1685. https://doi.org/10.31557/APJCP.2022.23.5.1679

22. Huang P., Sun R., Xu C. Glucocorticoid activates STAT3 and NF-κB synergistically with inflammatory cytokines to enhance the anti-inflammatory factor TSG6 expression in mesenchymal stem/stromal cells // Cell Death Dis. - 2024. – Vol. 15. - P. 70. https://doi.org/10.1016/j.redox.2016.03.005

23. Erkmen T., Serdar B.S., Ates H., Korkmaz M., Koçtürk S. Borax Pentahydrate and Disodium Pentaborate Decahydrate Are Candidates as Anti-leukemic Drug Components by Inducing Apoptosis and Changing Bax/Bcl-2 Ratio in HL-60 Cell Line // Biol. Trace Elem. Res. - 2022. – Vol. 4(200). - P. 1608-1616. https://doi.org/10.1007/s12011-021-02802-2

АҢДАТПА

БАЛАЛАРДАҒЫ ЖЕДЕЛ ЛЕЙКОЗДАРДАҒЫ ПЕРИФЕРИЯЛЫҚ ҚАН ЛИМФОЦИТТЕРІНДЕ ЖӘНЕ БЛАСТ ЖАСУШАЛАРЫНДА АПОПТОЗ МАРКЕРЛЕРІНІҢ ЭКСПРЕССИЯСЫН ТАЛДАУ

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Өзектілігі: *оЖедел лейкоз емінің тиімді тәсілдерін және емнің барысын болжау әдістерін іздеу онкогематологияның өзекті мәселесі. Бұл саладағы перспективалық бағыт химиотерапия барысында жасуша бетіндегі молекулалық маркерлер экспрессиясының өзгеруін зерттеу болып табылады.*

Зерттеудің мақсаты *– алғаш рет анықталған жедел лейкозы бар науқастарда перифериялық қан лимфоциттерінде және сүйек кемігінің бласт жасушаларында аннексин V, Bcl-2, CD95, p53 ақуыздарының экспрессиясын зерттеу.*

Әдістері: *Зерттеу дизайны – көлденең. Зерттеу әдісі: иммунофенотиптеу. Зерттеу материалдары 1 айдан 16 жасқа дейінгі "жедел лейкоз" диагнозы қойылған 106 пациенттің перифериялық қаны мен сүйек кемігі (зерттеу тобы) және 2 жастан 17 жасқа дейінгі шартты түрде сау 23 баланың перифериялық қаны (бақылау тобы) болды. Алынған мәліметтер статистикалық өңдеуден өтті.*

Зерттеу нәтижелері: *В-жасушалы жедел лейкоздарда BCL-2 экспрессиясын зерттеу сенімді айырмашылықтарды анықтаған жоқ, перифериялық қан лимфоциттеріндегі Т-жасушалы лейкоздарда бұл маркер бласттарға қарағанда сенімді түрде көбірек көрсетілді. Жедел миелобластты лейкозда BCL-2 экспрессиясы лимфоциттермен салыстырғанда бласттар популяциясында айтарлықтай жоғары болды. CD95 экспрессиясы лимфобластты лейкоздарда бласт жасушаларының мембранасына қарағанда перифериялық қан лимфоциттерінде айтарлықтай жоғары болды. Аннексин V экспрессиясын талдау ерте апоптоз маркерының экспрессиясы лимфоциттерде жоғары екенін анықтады. Бұл ісікке қарсы иммунитеттің төмен екенін көрсететін белгі. Лимфоциттер мен бласт жасушаларының бетіндегі p53 ақуызының экспрессиясын салыстырмалы талдау лейкоздың әртүрлі варианттарында сенімді айырмашылықтарды анықтаған жоқ.*

Қорытынды: *Жүргізілген зерттеудің нәтижесі жедел лейкоз кезінде BCL-2 және CD95 болжамдық маңыздылығының болуын көрсетті. Annexin V және p53 сенімді сезімталдық пен ерекшелікті анықтаған жоқ, бұл лейкоздың иммунофенотиптік панеліне осы маркерлерді қоспауға мүмкіндік береді.*

Түйінді сөздер: *жедел лейкоз, бласт жасушалар, апоптоз маркерлері, Bcl-2, CD95.*

АННОТАЦИЯ

АНАЛИЗ ЭКСПРЕССИИ МАРКЕРОВ АПОПТОЗА НА ЛИМФОЦИТАХ ПЕРИФЕРИЧЕСКОЙ КРОВИ И БЛАСТНЫХ КЛЕТКАХ ПРИ ОСТРЫХ ЛЕЙКОЗАХ У ДЕТЕЙ

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Актуальность: *Актуальным вопросом онкогематологии является поиск эффективных подходов терапии и методов прогнозирования течения острых лейкозов. Перспективным направлением в этой области является изучение изменений экспрессии молекулярных маркеров на поверхности клеток в динамике химиотерапии.*

Цель исследования *– определить уровень экспрессии белков annexin V, Bcl-2, CD95, p53 на лимфоцитах периферической крови и лейкемических клетках костного мозга у детей с диагнозом «острый лейкоз».*

Методы: *Дизайн исследования – поперечный. Метод исследования: иммунофенотипирование. Материалами исследования послужили периферическая кровь и костный мозг 106 пациентов с впервые установленным диагнозом «острый лейкоз» в возрасте от 1 мес. до 16 лет (исследуемая группа) и периферическая кровь 23 условно здоровых детей в возрасте от 2 до 17 лет (контрольная группа). Полученные данные были подвергнуты статистической обработке.*

Результаты: *Исследование экспрессии Bcl-2 при В-клеточных вариантах достоверных различий не выявило, при Т-клеточном варианте на лимфоцитах периферической крови данный маркер экспрессировался в достоверно большем количестве, нежели на*

бластах. При остром миелоидном лейкозе экспрессия Bcl-2 была достоверно выше на бластной популяции клеток, по сравнению с лимфоцитами. При анализе экспрессии CD95 при острых лимфобластных вариантах экспрессия была достоверно выше на лимфоцитах периферической крови, чем на мембране лейкемических клеток. Результаты анализа экспрессии annexin V показали, что лимфоциты экспрессировали маркер раннего апоптоза достоверно больше, чем бласты костного мозга. Данное явление является опасным признаком, указывающим на снижение противоопухолевого иммунитета. Сравнительный анализ экспрессии белка p53 на поверхности лимфоцитов и бластных клеток достоверных различий при различных вариантах лейкозов не выявил.

***Заключение:** Результаты проведенных исследований указывают на наличие прогностической значимости Bcl-2 и CD95 при остром лейкозе. Annexin V и p53 не выявили достоверной чувствительности и специфичности, что позволяет не включать данные маркеры в панель иммунофенотипирования лейкозов.*

***Ключевые слова:** острый лейкоз, бластные клетки, маркеры апоптоза, Bcl-2, CD95.*

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