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QUANTITATIVE AND MORPHOLOGICAL ASSESSMENT OF CHANGES IN HEMATOLOGICAL LABORATORY INDICATORS IN ACUTE MYELOBLASTIC LEUKEMIA

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Abstract
Aim of the study: To analyze the range of laboratory changes in patients with acute myeloblastic leukemia.
Material and methods: The study was conducted with the participation of 103 patients with AML and 104 healthy donors without a history of cancer. The selection of patients was carried out when applying to the RSSPMCH from 2015 to 2023. The subjects' hematological blood parameters were analyzed on an automatic hematological analyzer "Sysmex" using test systems from the company "HUMAN, Germany".
Results: Quantitative analysis of hematological parameters in groups of patients showed the presence of characteristic changes for acute leukemia, manifested by a significant decrease in hemoglobin content and the number of erythrocytes, as well as platelets, and an increase in the number of immature blast cells. This was accompanied by an increase in the median number of leukocytes, a decrease in the number of mature neutrophils with an acceleration of the ESR level. At the same time, quantitative and morphological analysis of the hematological parameters of the myelogram showed the presence of the main diagnostic indicator of acute leukemia - an increased number of blast cells of more than 30%. Carrying out cytochemical studies of blast cells made it possible to determine the variant of the disease, i.e. the presence of a positive reaction to myeloperoxidase and the detection of glycogen in the form of diffuse small granules are characteristic cytochemical signs of AML. At the same time, it is important to emphasize that more intense disturbances in bone marrow hematopoiesis and in peripheral blood were observed in the group of patients with a resistant course.

Keywords: acute myeloid leukemia, resistance, hematological parameters, laboratory abnormalities, myelogram, blasts.

Introduction

Acute myeloblastic leukemia (AML) is an important oncohematologic problem among myeloid neoplasia - a clonal heterogeneous pathology of the hematopoietic system associated with mutational changes in the stem cell leading to blockade of differentiation processes and aberrant proliferation of myeloid cells [5,9]. The mechanisms leading to AML are not fully

disclosed [11], however, it is known that specific genetic mutations at the early stage of leukemogenesis during clonal expansion, disrupting normal hematopoiesis, eventually form AML [1,8].

With a high prevalence of 3-8 cases per 100,000 people per year, AML affects people of all age categories [2,4]. Modern induction chemotherapy leads to complete remission in 40-90% of cases [7]. However, the discrepancy between favorable primary response rates and significantly lower long-term survival rates in ALL is due to the fact that a high proportion of patients eventually relapse and associated therapy resistance represents one of the central problems in the treatment of ALL [3,6,10].

In connection with the above-mentioned, the analysis of laboratory hematologic changes in AML is important for early prediction of the possible development of resistant forms of AML at the stages of examination.

Aim of the study. To analyze the spectrum of laboratory changes in patients with acute myeloblastic leukemia.

Material and Methods

The study included 103 patients with AML and 104 healthy donors without a history of cancer. The selection of patients was carried out when applying to the Republican Specialized Scientific and Practical Medical Center of Hematology (RSSPMCH) in the period 2015-2023. The subjects were aged from 18 to 74 years, with the age median being 41.4±1.7 years.

The diagnosis of AML was verified based on national standards of clinical and laboratory diagnostics. Patients with AML (n=103), in the main group, were divided depending on the response to polychemotherapy (PCT) into two groups:

a) non-resistant (n=67) and

b) resistant to PCT (n=36).

Laboratory examination included:

- clinical (general) blood test (CBC), cellular elements of the CBC were determined on the hematological automatic analyzer “SYSMEX. GLOBAL IMPEX" (Japan), using reagents from the company "HUMAN", (Germany) and of the cellular composition of bone marrow aspirate to determine cytochemical changes in blast cells when staging specific cytochemical reactions was carried out using manual microscopy using microscopes "LEICA ICC50 E" (Germany) with a digital color camera of 5-megapixel resolution (2592 x 1944). Erythrocyte sedimentation rate (ESR) was determined using a Panchenkov apparatus (Russia);

Informed consent for participation in the study was obtained from all subjects.

Statistical calculations of the obtained results were performed using Microsoft Office Excel-2019 statistical software package with calculation of the mean square deviation and arithmetic mean error by the method of moments (M±m), Student's difference reliability criterion (t) and degree of confidence (p).

Results and Discussion

It is well known that one of the primary key methods of diagnosing acute myeloblastic leukemia is the analysis of laboratory hematologic parameters, i.e., clinical blood count and

myelogram. Quantitative analysis of cellular elements in clinical blood analysis showed a decrease in hemoglobin content in the main group of patients with AML (Table 1).

Table 1. **Quantitative analysis of hematologic parameters of clinical (general) blood test (CBC) in the main group with AML before treatment, (M±m)**

Indicators	Surveyed groups			
	Control, n=104	Main (AML), n=103	Non-resistant AML, n=67	Resistant AML, n=36
Нв, g/L	139,5±2,8	74.5±1.4**	80,3±1,4*	61,6±1,6***
RBC ($x10^{12}$/L)	5,4±0,7	2.2±0.06**	2,5±0,07**	1,9±0,06***
PLT ($x10^9$/L)	298,4±4,2	64.2±3.7***	87,8±2,6***	20,3±2,7***
MCV (fL)	91,1±2,5	80.2±0.6	80,0±0,8	80,5±0,9
MCH (pg)	29,3±0,6	27.4±0.5	28,6±0,7	25,3±0,5
MCHC (g/%)	34,8±1,1	32.5±0.3	33,0±0,4	31,5±0,6
RDW (%)	13,2±0,2	12.4±0.3	11,4±0,2	14,3±0,8
WBC ($x10^9$/L)	6,2±1,1	55.0±4.1***	27,3±1,1***	106,5±4,1***
BLAST (%)	-	28.2±1.8***	23,3±2,3***	37,4±2,2***
NEU (%)	67,3±2,6	42.0±1.4*	46,9±1,8	32,7±1,1*
LYM (%)	34,2±1,8	26.7±0.7	25,4±0,7	29,2±1,3
MON (%)	5,1±0,2	4.0±0.3	3,7±0,4	4,5±0,6
EOS (%)	2,3±0,1	0.7±0.1	0,6±0,1***	1,03±0,2***
ESR, mm/h	5,4±1,8	27.4±1.9***	14,8±0,7***	51,0±1,7***

Note: *- statistical reliability of differences when comparing with healthy controls: * -P<0.05; ** -P<0.01; *** -P<0.001

In the main group of AML compared to healthy controls, there was a statistically significant decrease in hemoglobin 1.9-fold (74.5±1.4 g/L vs 139.5±2.8 g/L, p<0.01) and erythrocytes 2.4-fold (2.2±0.06 $x10^{12}$/L vs 5.4±0.7 x1012/L, p<0.01). Another important sign of AML was a significant 4.6-fold decrease in platelet count among patients compared with healthy controls (64.2±3.7 $x10^9$/L versus 298.4±4.2 $x10^9$/L; p<0.001). Along with this, in the mean values of erythrocyte indices despite their decrease in the group with OML their median did not deviate from the borders of normal level. Thus, in comparison with the control among the patients the median MCV decreased from 91,1±2,5 fl to 80,2±0,6 fl (p>0.05), MSN from 29,3±0,6 pg to 27,4±0,5 pg (p>0.05), MSNS from 34,8±1,1 g/% vs. 32,5±0,3 g/% (p>0.05). In contrast, the median RDW increased from 13.2±0.2% to 15.4±0.8% (P>0.05).

At the same time, the median leukocyte count in the group with AML was statistically significantly higher by 8.9 times (55.0±4.1$x10^9$/l vs. 6.2±1.1$x10^9$/l; P<0.001). The increase in the number of leukocytes was observed due to the main diagnostic indicator of the disease - blast cells, i.e. the substrate of this neoplasia, the proportion of which amounted to 28.2±1.8% (p<0.001).

At the same time, the median of leukocytes in the group of patients with AML, when compared with healthy ones, was statistically significantly increased by 8.9 times (55.0±4.1x109/l versus 6.2±1.1x109/l; P<0.001). An increase in the number of leukocytes was

observed due to the main diagnostic indicator of the disease - blast cells, i.e. substrate of this neoplasia to 28.2±1.8% ($p<0.001$). In addition, there was a decrease in the proportion of mature leukocyte cell elements - neutrophils by 1.6 times (67.3 ± 2.6% versus 42.0 ± 1.4%; $p<0.05$), with an insignificant decrease in the median of lymphocytes (26.7 ±0.7% versus 34.2±1.8%; $p>0.05$) and monocytes (4.0±0.3% versus 5.1±0.2%; $p>0.05$) and with a significant decrease in the average content eosinophils by 3.3 times (0.7±0.1% versus 2.3±0.1%; $p>0.05$).

In the group with AML, a statistically significant increase was observed in the ESR indicator by 5.1 times (27.4±1.9 mm/h versus 5.4±1.8 mm/h; $P<0.001$).

Comparison of quantitative changes in hematological parameters in groups of patients with non-resistant and resistant AML revealed some features (Table 1).

In particular, if in the group of patients with non-resistant AML, compared with the control, the hemoglobin content decreased by 1.7 times (80.3±1.4 g/L versus 139.5±2.8 g/L; $p<0.05$), and red blood cells by 2.2 times (2.5±0.07 $x10^{12}$/L versus 5.4±0.7 $x10^{12}$/L; $p<0.01$), then in the group of patients with a resistant course these indicators decreased more intensively by 2 .3 (61.6±1.6 g/l versus 139.5±2.8 g/L; $p<0.001$) and 2.8 times (1.9±0.06$x10^{12}$/L versus 5.4±0 .7x1012/L; $p<0.001$), respectively.

Similar changes were observed in platelets, the median of which decreased statistically significantly in the non-resistant group by 3.4 times (87.8±2.6 $x10^9$/L versus 298.4±4.2 $x10^9$/L; $p<0.001$), and among resistant patients by 14.7 times (20.3±2.7 $x10^9$/L versus 298.4±4.2 $x10^9$/L; $p<0.001$).

The median erythrocyte indices in the non-resistant and resistant groups decreased compared to control values, and again did not deviate from the normal level.

Thus, in the non-resistant group, the median MCV decreased from 91.1±2.5 fL to 80.0±0.8 fL ($P>0.05$), MCH from 29.3±0.6 pg to 28.6±0, 7 pg ($p>0.05$), MCHC from 34.8±1.1 g/% to 33.0±0.4 g/% ($P>0.05$), and median RDW increased from 13.2±0.2% up to 11.4±0.2% ($p>0.05$). In the resistant group, the median MCV decreased from 91.1±2.5 fL to 80.5±0.9 fL ($p>0.05$), MCH from 29.3±0.6 pg to 25.3±0.5 pg ($p>0.05$), MCHC from 34.8±1.1 g/% to 31.5±0.6 g/% ($p>0.05$), with an increase in median RDW from 13.2±0.2% to 14 .3±0.8% ($p>0.05$).

Meanwhile, the average number of leukocytes in the group with non-resistant AML compared to the control group statistically significantly increased by 4.4 times (27.3±1.1$x10^9$/L versus 6.2±1.1$x10^9$/L; $p<0.001$) over due to an increase in the number of blast cells to 23.3±2.3% ($p<0.001$). Similar indicators in the resistant group in relation to the control increased most significantly to 106.5±4.1$x10^9$/L ($p<0.001$) and 37.4±2.2% ($p<0.001$), respectively.

In the non-resistant and resistant groups, mature elements of the leukocyte formula, such as neutrophils, decreased by 1.4 (46.9±1.8% versus 67.3±2.6%; $p>0.05$) and 2.1 times (32.7±1.1% versus 67.3±2.6%; $p<0.05$). At the same time, in these groups of patients, the median of lymphocytes and monocytes increased to 25.4±0.7% ($p>0.05$) and 3.7±0.4% ($p>0.05$), as well as to 29.2±1 .3% ($p>0.05$) and 4.5±0.6% ($p>0.05$), respectively. The median of eosinophils in this group of patients compared to the control statistically significantly decreased by 3.8 times (0.6±0.1% versus 2.3±0.1% versus; $p>0.05$) and 2.2 times (1.03±0.2% versus 2.3±0.1%; $p>0.05$), respectively.

Moreover, in the non-resistant and resistant AML groups, a statistically significant increase was observed in the median ESR by 2.7 times (14.8±0.7 mm/h versus 5.4±1.8 mm/h; p<0.001) and 9.4 times (51.0±1.7 mm/h versus 5.4±1.8 mm/h; p<0.001), respectively.

The decisive verification step in the diagnosis of AML is the analysis of bone marrow aspirate (myelogram), obtained by puncture from the upper third of the sternum of patients, which allows identifying abnormalities observed in the red bone marrow.

In patients with AML, the picture of red bone marrow was represented by medium cellularity with infiltration of the bone marrow by blast cells of medium and large sizes, with nuclei of round, bilobular and lobed shape. At the same time, blasts were revealed with signs of anaplasia in the form of fragmentations and depressions with a delicate chromatin structure, clear nucleoli, wide basophilic cytoplasm with pronounced vacuolization phenomena (Table 2).

Table 2. **Analysis of myelogram parameters in AML before treatment, (M±m)**

Indicators	Reference	Groups of patients		
		Main group c AML, n=103	Non-resistant AML, n=67	Resistant AML, n=36
Blasts, %	0,1-1,1	46,4±1,7***	36,9±0,9***	64,3±2,5***
Neutrophil elements, %	52,7-68,9	34,6±1,4*	43,5±0,7	18,2±1,8**
Eosinophilic elements,%	0,5-3,5	1,2±0,2	1,3±0,2	1,1±0,03
Basophilic elements, %	0-0,5	0,2±0,05	0,3±0,06	0,2±0,06
Monocytes, %	0,7-3,1	5,2±0,3	7,1±0,2*	1,9±0,4
Lymphocytes, %	4,3-13,7	11,8±0,4	10,2±0,2	14,6±1,0
Plasma cells, %	0,1-1,8	0,4±0,06	0,4±0,08	0,3±0,1
Total number of erythroid elements, %	14,5-26,5	11,7±0,9	12,6±1,3	10,0±0,5
Leukocytes - RBC	2,1-4,5:1	8,5:1***	7,9:1***	10,1:1***
MHK	5-12	2,9±0,2***	3,9±0,7*	1,1±0,2***

Note: *- statistical reliability of differences when comparing with healthy controls: * -P<0.05; ** -P<0.01; *** -P<0.001

Consequently, the main diagnostic sign of acute leukemia was an increase in the number of blast cells in all groups of patients: in the main group up to 46.4±1.7% (P<0.001), in the non-resistant group up to 36.9±0.9% (P <0.001) and in the resistant group up to 64.3±2.5% (P<0.001).

The cytochemical picture was characterized by the presence in blast cells of a positive reaction to myeloperoxidase and glycogen in the form of diffuse small granules, which confirmed the presence of the myeloid variant of acute leukemia in patients. At the same time, signs of inhibition of normal granulocytic, erythrocyte and megakaryocytic hematopoietic lineages were revealed.

In particular, in the main group of AML, with non-resistant and resistant forms, the sum of neutrophil elements decreased to 34.6±1.4% (P<0.05); 43.5±0.7% (P>0.05) and 18.2±1.8% (P<0.01), respectively. Meanwhile, the median values of eosinophilic and basophilic

elements, as well as plasma cells in all groups were within normal values. While the number of monocytes in the main and resistant groups increased to 5.2±0.3% (P>0.05) and 7.1±0.2% (P<0.05), with no deviations from the norm in the non-resistant group (1.9±0.4%; P>0.05).
At the same time, lymphocytes according to the groups amounted to 11.8±0.4% (P>0.05); 10.2±0.2% (P>0.05) and 14.6±1.0% (P>0.05), respectively, for the groups of patients.
A decrease in the activity of erythroid hematopoiesis was manifested by a decrease in the number of erythroid elements, the sum of which, compared with the minimum normal value, decreased in each group of patients to 11.7±0.9% (P>0.05), 12.6±1.3% (P>0.05) and 10.0±0.5% (P>0.05).
An increase in the sum of leukocyte elements and a decrease in the sum of erythrocyte elements, respectively, was accompanied by an increase in the leukocyte-erythroid ratio (L:E) in each group of patients to 8.5:1 (P<0.001); 7.9:1 (P<0.001) and 10.1:1 (P<0.001), respectively. Along with these features, there was also a decrease in the number of MGCs viewed in the preparation according to the groups of patients to 2.9±0.2 (P<0.001); 3.9±0.7 (P<0.01) and 1.1±0.2 (P<0.001).

Conclusion

Thus, a quantitative analysis of hematological parameters in groups of patients showed the presence of characteristic changes for acute leukemia, manifested by a significant decrease in hemoglobin content and the number of red blood cells, as well as platelets. In addition, a key sign of the disease was an increase in the number of immature blast cells. This was accompanied by an increase in the median number of leukocytes, a decrease in the number of mature neutrophils with an acceleration of the ESR level. At the same time, the severity of changes in these indicators was more intense in the group of patients with resistant AML, which was confirmed by more significant correlations in this group between a decrease in hemoglobin and erythrocytes with an increase in the number of leukocytes, blasts and ESR. At the same time, quantitative and morphological analysis of the hematological parameters of the myelogram showed the presence of the main diagnostic indicator of acute leukemia - an increased number of blast cells of more than 30%. Carrying out cytochemical studies of blast cells made it possible to determine the variant of the disease, i.e. the presence of a positive reaction to myeloperoxidase and the detection of glycogen in the form of diffuse small granules are characteristic cytochemical signs of AML. At the same time, it is important to emphasize that more intense disturbances in bone marrow hematopoiesis were observed in the group of patients with a resistant course.

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