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CLINICAL-HEMATOLOGICAL AND MOLECULAR-GENETIC FEATURES OF CHRONIC MYELOID LEUKEMIA

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Abstract:

Molecular genetic studies have shown the need for an analysis for the presence of a quantitative determination of the chimeric oncogene BCR-ABL according to standardized methods to determine the effectiveness of the therapy used in patients with CML, and the need to determine the minimal residual disease (MRD) on the IS scale at 3, 6 and 9 months of treatment. The results of our studies indicate the effectiveness of imatinib therapy for CML, which is consistent with the results of international studies. The presence of a large molecular response in patients with CML to treatment with imatinib determines its high efficacy. Mutation analysis should be performed in patients who have never reached LMR, or when the BCR-ABL transcript level is more than 10-fold. If a patient loses LMR or shows an increase in the BCR-ABL transcript level, molecular testing should be performed every 1 to 3 months. Patients with partial and absent molecular response are indicated for therapy with the latest generation of TKI drugs or bone marrow transplantation.

Keywords: CML, LMR, PMR, CCR, PCR, imatinib, minimal residual disease.

INTRODUCTION.

Among all tumor diseases of the hematopoietic system, scientists are especially interested in studying chronic myeloid leukemia (CML), which accounts for 20% in the structure of leukemia. According to European studies of CML in Europe and North America, it ranks third in terms of prevalence after acute leukemia and chronic lymphocytic leukemia [1]. At the same time, the annual incidence of CML is 1–1.5 per 100 thousand population in all countries, and has remained practically stable over the past 50 years. Men get sick more often than women (55-60%). Literature sources indicate that CML can develop in both children and adults, however, the disease develops more often in the category of people aged 30-50 years [2,3].

Today it is known that the pathogenesis of CML is based on the formation of the BCR-ABL gene, which produces a protein with a significantly more pronounced tyrosine kinase activity than its normal prototype [4,5]. Activation of various regions of the ABL and BCR genes, resulting from their fusion, determines a sequential chain of processes in cells, which ultimately lead to an increase in cell proliferation, impaired differentiation of progenitor cells. Cells carrying the BCR-ABL gene are less sensitive than normal to factors causing apoptosis, which leads to an increase in the mass of tumor cells [6,7].

Despite the presence of studies in the course of which a number of aspects of the disease have been disclosed, nevertheless, today many questions regarding the characteristics of the development of CML remain open. In this connection, the study of the clinical features of the course of CML in Uzbekistan is relevant. Chronic myeloid leukemia (CML) is a myeloproliferative disease that occurs as a result of an acquired cytogenetic anomaly - reciprocal translocation t (9; 22) (q34; q11), the so-called "Philadelphia" or Ph-chromosome [8,9]. As a result of the indicated translocation on chromosome 22, the chimeric gene BCR-ABL1, encoding BCR-ABL1 tyrosine kinase, is formed [10]. Protein BCR-ABL1 has a constant high tyrosine kinase activity, which leads to the activation of signaling pathways that increase the proliferative activity of cells, inhibition of apoptosis; reduces the dependence of cells on external regulatory mechanisms and lowers cell adhesion [11]. Abnormal production of BCR-ABL1 tyrosine kinase plays a major role in leukemic cell modification and is considered the main cause of CML development [12]. The study of the molecular basis of the pathogenesis of CML paved the

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way for the development of targeted therapy for this disease, which consists in the use of specific inhibitors of BCR-ABL tyrosine kinase (TKI). Successful treatment of patients with CML is impossible without appropriate monitoring of the effectiveness of the therapy. This position has become especially obvious since the introduction into clinical practice of the first drug of the group of tyrosine kinase inhibitors - imatinib mesylate. The creation of imatinib was a revolutionary breakthrough in the field of targeted anticancer therapy [13]. A standard cytogenetic study (SCyS) of bone marrow cells allows for reliable diagnosis and monitoring of CML treatment. SCyS is the only method that allows you to analyze the entire chromosome set of a cell. With the help of SCyS, additional chromosomal aberrations can be detected, which in some cases indicate an unfavorable prognosis of the disease [14]. It should be understood that the resolution of this method is relatively low and amounts to 1-5% of the cells. The Philadelphia chromosome using standard cytogenetics is detected in almost 90% of newly diagnosed untreated patients with CML. In accordance with the recommendations of the European Leukemia Net [15], control SCyS against the background of CML TKI therapy is performed once every 6 months until a complete total genetic response is achieved, and then once a year.

Polymerase chain reaction (PCR) is used both to diagnose CML and to monitor minimal residual disease (MRD) during therapy. With the help of PCR or FISH analysis, the diagnosis is confirmed in those 5% of cases in which the SDH Ph-chromosome does not reveal. For testing, you can use samples of both blood and bone marrow. The sensitivity of this method is high and allows the detection of one single cell with specific DNA or RNA between 104-106 cells. In more than a quarter of Ph-negative patients, the chimeric BCR-ABL gene is detected by PCR. Molecular monitoring of BCR-ABL transcript levels by real-time quantitative PCR is increasingly being used to assess the response to treatment in patients with CML. This method becomes especially important in the era of CML TKI therapy, when the residual level of leukemic cells is usually below the sensitivity level of cytogenetic studies [16]. Molecular monitoring in patients receiving imatinib is performed by determining the expression level of the BCR-ABL transcript prior to initiation of therapy and then every 3 months. This approach allows you to assess the dynamics of therapy and predict the possibility of a relapse. The significance of molecular analysis is also determined by the fact that the level of molecular response is a predictor of disease-free survival. An increase in the expression level of the BCR-ABL transcript during imatinib therapy may indirectly indicate the presence of mutations in the Bcr-Abl kinase gene, which are the leading cause of imatinib resistance. As a result, it is advisable to use molecular monitoring as a screening strategy for mutational analysis [17].

PURPOSE OF THE STUDY.

Study of the features of the clinical-hematological and molecular-genetic characteristics of chronic myeloid leukemia.

MATERIALS AND METHODS.

The material of the study was RNA samples isolated from the peripheral blood of 150 patients with a clinically and cytogenetically established diagnosis of CML, examined at the Republican Specialized Scientific and Practical Medical Center of Hematology of the Republic of Uzbekistan. In order to determine the nature of the occurrence of CML, depending on the sex difference during the study, the subjects were divided into two subgroups. Depending on the effectiveness of imatinib treatment in accordance with the ELN criteria (2020), the examined patients were divided into 4 groups, Table 1. The first group consisted of 30 patients with a newly diagnosed CML prior to the initiation of imatinib therapy. The second group consisted of 50 patients with an optimal response to imatinib therapy. The third group consisted of 40 patients with a suboptimal response to imatinib therapy in accordance with the ELN guidelines. The fourth group - 30 patients refractory to imatinib therapy. All patients received treatment with imatinib at a dose of 400, 600 mg / day. The maximum duration of therapy was 18 months, the minimum - 3 months.

Cytogenetic studies. The work used the generally accepted direct method and the method of bone marrow culture for 24 hours. For SCyS, preparations were stained by the GTG method (differential G stain). The results of karyotyping were described in accordance with the requirements of the International Cytogenetic Nomenclature ISCN 2009, as well as the criteria for no response, were determined in accordance with the ELN guidelines.

RT-PCR for quantitative determination of the BCR-ABL gene (p210). The method for detecting mRNA of the bcr-abl gene in clinical material is based on: a) extraction of total RNA from peripheral blood cells; b) carrying out the reverse transcription reaction; c) subsequent amplification with detection in "real time" with two mixtures of oligonucleotides: amplification of the mRNA region of the chimeric gene M-bcr-abl (p210), corresponding to the region where the bcr and abl genes (b2a2 and b3a2) and the mRNA fragment of the abl gene splicing region are linked (recommended by the Europe Against Cancer Working Group, EAC), as an endogenous internal control and normalizer gene. Amplification products are detected

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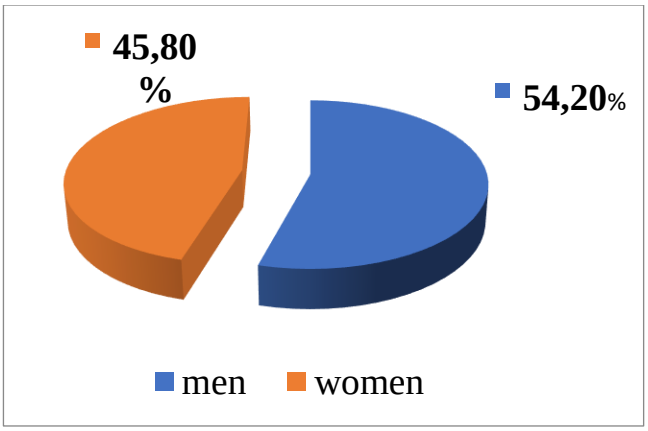
in real time. The result of bcr-abl cDNA amplification is recorded by the Yellow / JOE / HEX fluorescence channel, the abl amplification result is recorded by the Yellow / JOE / HEX channel. To assess the dynamics of changes in the BCR-ABL gene expression, the International Scale (IS) recommended by the ELN expert group was used. In this case, the expression of the control gene (in our case, the ABL gene) is taken as 100%. The relative expression of the BCR-ABL gene according to IS was defined as the ratio of the average copy number of the BCR-ABL gene to the average copy number of the ABL gene, multiplied by 100%. According to the ELN recommendations, the detection of the BCR-ABL gene in more than 1% of cells indicates a pronounced expression of BCR-ABL, which can be assessed using ScyS. Expression of the BCR-ABL gene in 0.1–1% of cells is characteristic for a moderate level of expression, which is no longer possible to assess using SCyS. Determination of the expression of the BCR-ABL gene at a level of less than 0.1% indicates that the patient has achieved a large molecular response.

Tab. 1. The number of performed cytogenetic and molecular genetic studies at the stage of diagnosis and in the dynamics of observation of patients with CML

Groups (n=150)	Duration of imatinib therapy (months)			
	0	6	12	18
Standard cytogenetic study				
1-group (n=30)	30 (100%)	-	-	-
2-group (n=50)	50 (100%)	5 (10%)	10 (20%)	-
3-group (n=40)	40 (100%)	10 (25%)	10 (25%)	30 (75%)
4-group (n=30)	30 (100%)	25 (83%)	28 (93%)	30 (100%)
Real-time PCR studies of BCR-ABL gene expression				
1-group (n=30)	25 (83%)	-	-	-
2-group (n=50)	43 (86%)	23 (46%)	50 (100%)	25 (50%)
3-group (n=40)	38 (95%)	23 (57%)	35 (87%)	40 (100%)
4-group (n=30)	25 (83%)	12 (40%)	30 (100%)	30 (100%)

RESULTS AND DISCUSSION.

The results of the study showed, that CML, by gender distribution, is somewhat more common in men than in women. Of the total number of patients with CML (150 people), 81 were men, which is 54.2%, and 69 patients were women, which amounted to 45.8% (Fig. 1). The analysis shows a higher incidence of CML among men, which, according to nosology, exceeded that in women in 1.05. At the same time, the median of the average age of the subjects at the time of diagnosis was from 40 to 59 years. In particular, the average age of the examined patients with CML was 50.4 ± 1.4 years. These data are consistent with the literature data on the incidence of CML such indicators in other countries of the world [1,6].



Rice. 1. Distribution of CML patients depending on gender.

CLINICAL AND HEMATOLOGICAL FEATURES.

Since many patients do not notice any changes in the initial stage of CML, they turn to the second stage of the disease. Almost all patients (100%) complained of increasing weakness and decreased performance, increased fatigue and sweating caused by leukocytosis and increased cell decay. 39 (26%) patients reported dizziness, and 25 (16.4%) - tinnitus.

As a rule, in patients with CML, a combination of asthenovegetative syndrome with splenomegaly was observed. 122 (81.5%) patients had severity, of which 80 (53.4%), along with this, felt some pain in the left hypochondrium, which was directly related to the enlargement of the spleen due to its myeloid metaplasia. In 29 (19.2%) patients, dyspnea was detected when walking, and 17 (11%) complained of palpitations, which were more likely due to the appearance of splenomegaly (table 2.).

Table 2. Clinical manifestations of CML in patients

№	Complaints	Number of patients	
		n	%
1	Weakness and decreased performance	150	100
2	Increased fatigue	150	100
3	Excessive sweating	150	100
4	Dizziness	39	26

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5	Noise in ears	25	16,4
6	Dyspnea	29	19,2
7	Palpitations	17	11
8	Severity in the left hypochondrium	122	81,5
9	Pain in the left hypochondrium	80	53,4

There were no differences in the complaints presented among men and women.

Clinical changes were accompanied by hematological changes, both in the general analysis of peripheral blood and in the myelogram.

The general blood test was characterized by the presence of mild anemia in 70 (46.6%) and moderate anemia in 45 (30.1%) patients. Along with this, all patients (100%) had leukocytosis, but its degree was different: moderate leukocytosis in the range of 20-30 thousand X 109 / L was registered in 56 (37.6%), sharp leukocytosis in the range of 50-200 thousand. X109 / L was observed in 94 (62.3%) patients. The degree of leukocytosis correlated with the clinical manifestations of CML. So, in patients with a sharp leukocytosis, there was a greater increase in the size of the spleen and a feeling of pain in its projection.

In the leukocyte formula, an increase in intermediate cellular elements, consisting of myelocytes and promyelocytes, was observed, as well as a basophilic-eosinophilic association (Table 3.).

Table 3. Parameters of peripheral blood in patients with CML (M ± m).

Indicators and units	Patients with moderate leukocytosis, n = 56	Patients with severe leukocytosis, n = 94	p
Hb	92,3±1,8	78,2±0,59*	<0,0001
Er x10^{12}/л	4,1±0,03	3,2 ±0,03*	<0,0001
WLB x10 ^{9}/л	26,6±1,1	152,5±1,18*	<0,0001
PLT x10 ^{9}/л	450,0±3,5	650,0±1,8*	<0,0001
EO %	3,0±0,09	5,0±0,09*	<0,0001
Baso, %	7,05±0,04	10,1±0,06*	<0,0001

Note: * - p <0.05 compared with the value of the indicator among patients with moderate leukocytosis

Table 4. shows the results of the study of the main indicators of the myelogram. From the above information, it can be seen that all patients with CML had bone marrow hypercellularity, which is expressed in the rejuvenation of the granulocytic lineage due to an increase in the number of intermediate cells, which differs depending on the degree of leukocytosis: with moderate leukocytosis, promyelocytes were 11.6 ± 0.03; myelocytes 15.8 ± 0.12; metamyelocytes 20.3 ± 1.2; basophils 6.3 ± 0.04; eosinophils 7.2 ± 0.06; whereas in patients with acute leukocytosis, these indicators were as follows: promyelocytes 19.5 ± 0.1; myelocytes 20.4 ± 0.18; metamyelocytes 32.5 ± 1.5; basophils 9.4 ± 0.04; eosinophils 10.5 ± 0.08 (Table 4.).

Table 4. Indicators of myelogram in patients with CML (M ± m).

Indicators and units	Patients with moderate leukocytosis, n = 56	Patients with severe leukocytosis, n = 94	p
Blast cells%	0,1±0,03	2,0±0,09*	<0,0001
Promyelocytes	11,6 ±0,03	19,5 ±0,1*	<0,0001
Myelocytes	15,8±0,12	20,4±0,18*	<0,0001
Metamyelocytes	20,3±1,2	32,5±1,5*	<0,0001
All neutrophil cells,%	86,5±3,1	96,3±3,8	>0.05
Basophils,%	6,3±0,04	9,4±0,04*	<0,0001
Eosinophils of all generations,%	7,2±0,06	10,5±0,08*	<0,0001

Note: * - p <0.05 compared with the value of the indicator among patients with moderate leukocytosis

Cytogenetic and molecular genetic research. A complete cytogenetic response (CCR) was achieved in 10 out of 30 (33%) patients with a suboptimal response to imatinib therapy for 18 months, and in 5 out of 10 (50%) patients with an optimal response to imatinib therapy for 12 months. A partial cytogenetic response (PCR) was achieved in 5 out of 10 (50%) patients, with an optimal response to imatinib therapy for 12 months, in 2 out of 10 (20%) patients, with a suboptimal response to imatinib therapy for 12 months, in 2 of 5 (40%) patients, with an optimal response to imatinib therapy for 6 months, in 3 out of 10 (30%) patients, with a suboptimal response to imatinib therapy for 6 months (Table 5).

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Volume-4, November 2021
ISSN: 2749-3644

Table 5. Frequency of complete and partial cytogenetic response to imatinib in CML patients

Groups (n=150)	Duration of imatinib therapy (months)		
	6	12	18
2-group (n=50)	40% PCR	50% PCR 50% CCR	-
3-group (n=40)	30% PCR	20% PCR	33% CCR

A large molecular response (LMR) was achieved in 20 of 25 (80%) patients, with an optimal response to imatinib therapy for 18 months, in 12 of 40 (30%) patients, with a suboptimal response to imatinib therapy for 18 months, in 40 of 50 (80%) patients with an optimal response to imatinib therapy for 12 months, in 20 out of 35 (57%) patients, with a suboptimal response to imatinib therapy for 12 months, in 18 out of 23 (78%) patients, with optimal response to imatinib therapy for 6 months, in 10 out of 23 (43%) patients, with a suboptimal response to imatinib therapy for 6 months, in 12 out of 43 (28%) patients, with an optimal response to imatinib therapy for less than 3 months, and in 8 of 38 (21%) patients with a suboptimal response to imatinib therapy for less than 3 months.

A partial molecular response (PMR) was achieved in 5 of 25 (20%) patients, with an optimal response to imatinib therapy for 18 months, and in 28 of 40 (70%) patients, with a suboptimal response to imatinib therapy for 18 months, in 10 of 50 (20%) patients with an optimal response to imatinib therapy for 12 months, in 15 of 35 (43%) patients, with a suboptimal response to imatinib therapy for 12 months, in 5 of 23 (22%) patients, with an optimal response to imatinib therapy for 6 months, in 13 out of 23 (43%) patients, with a suboptimal response to imatinib therapy for 6 months, in 31 out of 43 (72%) patients, with an optimal response to imatinib therapy for a duration of therapy less than 3 months, and in 30 out of 38 (79%) patients, with an optimal response to imatinib therapy with a duration of therapy less than 3 months (Table 6).

The results of the study indicate that the increase in the progression of CML disease with imatinib therapy is reduced.

Table 6. Frequency of large and partial molecular responses to imatinib in CML patients

Groups (n=150)	Duration of imatinib therapy (months)			
	0-3	6	12	18
2-group (n=50)	28% LMR 72% PMR	78% LMR 22% PMR	80% LMR 20% PMR	80% LMR 20% PMR
3-group (n=40)	21% LMR 79% PMR	43% LMR 57% PMR	57% LMR 43% PMR	30% LMR 70% PMR

CONCLUSIONS.

Thus, in the examined CML patients, the disease was more often registered among men. Their average age at the time of treatment was 50.4 ± 1.4 years. Almost all patients were treated only at the height of CML, while the clinical picture showed the presence of mixed symptoms of the disease. On admission, the majority of patients showed general weakness, decreased performance, fatigue, sweating, heaviness in the left hypochondrium, which correlated with anemia and leukocytosis.

Molecular genetic studies have shown the need for an analysis for the presence of a quantitative determination of the chimeric oncogene BCR-ABL according to standardized methods to determine the effectiveness of the therapy used in patients with CML, and the need to determine the minimal residual disease (MRD) on the IS scale at 3, 6 and 9 months of treatment. The results of our studies indicate the effectiveness of imatinib therapy for CML, which is consistent with the results of international studies. The presence of a large molecular response in patients with CML to treatment with imatinib determines its high efficacy. Mutation analysis should be performed in patients who have never reached LMR, or when the BCR-ABL transcript level is more than 10-fold. If a patient loses LMR or shows an increase in the BCR-ABL transcript level, molecular testing should be performed every 1 to 3 months. Patients with partial and absent molecular response are indicated for therapy with the latest generation of TKI drugs or bone marrow transplantation.

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