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The proliferative activity of peripheral blood and bone marrow hematopoietic cells expressing protein Ki-67 in CML patients with different Sokal score and response to tyrosine kinase inhibitors therapy

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Abstract
The aim – to establish the prognostic significance of expression of Ki-67 protein by hematopoietic cells in peripheral blood and bone marrow of patients with CML in the formation of response to tyrosine kinase inhibitors therapy based on its collating with the Socal score. *Methods*. The expression of hematopoietic cells in peripheral blood and bone marrow of the proliferation marker Ki-67 of 79 CML patients was measured by flow cytometry in direct immunofluorescence test using monoclonal antibodies from a set of PE Mouse Anti-Human Ki-67 Set (BD Pharmingen, USA). Sokal score was determined in the debut of the disease. The recommendations from The European LeukemiaNet, update 2010, for the management of CML have used. *Results*. The expression of intranuclear protein in patients with intermediate Sokal score was more than three times higher compared to the low-risk group in peripheral blood and more than twice higher in bone marrow ($p < 0.05$). In the high-risk group the number of cells that expressed Ki-67 was 4 times higher in the region of granulocytes and three times in the region of monocytes in peripheral blood compared to the low Socal risk group ($p < 0.05$). In the bone marrow the expression of Ki-67 in patients with high Sokal risk group was twice higher than with low-risk Sokal ($p < 0.05$). *Conclusions*. The relationship between the Socal score, the expression of Ki-67 and the response to imatinib therapy in CML patients treated with imatinib have been shown. An optimal response is achieved in patients with low Socal group and low intranuclear expression of Ki-67 protein, the worst results of therapy in patients was determined with high Sokal risk group and increased expression of Ki-67.

Keywords: chronic myeloid leukemia, Ki-67, Socal score, optimal, suboptimal response, treatment failure.

1. Introduction

Chronic myeloid leukemia (CML) is characterized by staging of disease, which necessitated the selection phase of the disease: chronic phase (CP), phase of acceleration (AP) and blast phase (BP). The division into phases based on the use of various prognostic factors of survival. Throughout the world, the most commonly use the criteria for determining phase of Cancer Center MD Anderson (USA), which were developed by HM Kantarjian *et al*. [1]. CML phase is determined at the beginning of the disease, the appearance of clinical progression and / or in the case of a change of therapy. To determine the prognosis of the disease, it is important to determine not only the phase CML, but also risk of disease progression, which is formed on calculated indices only in the debut of the disease, that includes data from the initial examination of patients. To identify the criteria that could be used as predictive in determining the expected duration of the disease and assessing response to therapy, the role of various clinical and hematological symptoms were analyzed. Most studies have shown the relationship with the size of the spleen, percentage of blasts and basophils in the blood and bone marrow at diagnosis. The correlation with the level of hemoglobin, platelet count, the liver size and patients' age was found. It was suggested a lot of predictive models based on many of these symptoms. However, most informative and now widely applied in the world is Socal score [2]. With the help of computer programs and mathematical model of Cox authors analyzed and determined the relative importance of different symptoms to select only the features of independent significance for the duration of the disease and to exclude those which play an indirect role. As a result of this analysis, studying the disease of 1635 CML patients, the predictive model based on four characteristics: the patient's age at diagnosis, number of platelets, blasts in the blood and spleen size, was proposed.

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Despite the fact that the risk criteria were determined for patients receiving therapy with cytostatic and IFN-α, their importance has shown for patients treated with tyrosine kinase inhibitors (TKI) - worse results of therapy were observed for patients with high risk group [3-5]. It is known that, despite the pronounced efficacy and low toxicity of TKI therapy the part of patients in CP, as well as in most of AP and BP primary insensitive to this group of drugs or lose their sensitivity during the treatment. The problem of resistance to TKI therapy has not been fully resolved today. One possible reason for the poor response to treatment is considered a high proliferative activity of hematopoietic cells of CML patients, which is manifested the increase of expression of intranuclear protein Ki-67 [6, 7]. The study to determine the relationship between the expression of this protein and Socal risk group is considered to be interesting. However, such research has not been conducted yet. These data will be useful in the formation of new criteria for prognosis of the CML and response to TKI therapy. The aim of our study was to establish the prognostic significance of expression of Ki-67 protein by hematopoietic cells in peripheral blood and bone marrow of patients with CML in the formation of response to tyrosine kinase inhibitors therapy based on its collating with the Socal score.

2. Materials and Methods
The study included 79 patients with CML CP in age from 22 to 65 years receiving treatment with imatinib. To assess the expression of protein Ki-67 by hematopoietic cells in peripheral blood (PB) and bone marrow (BM) of patients the method of direct cytometry was used. For this purpose, monoclonal antibodies PE Mouse Anti-Human Ki-67 Set (BD Pharmingen, USA) were applied. Cytometry study was performed using a flow cytometer laser FACS can (Becton Dickinson, USA) with an argon laser which has a wavelength of 488 nm. Data collection was performed by flow cytometry using the LYSYS-II Ver. 1.1 (Becton Dickinson) software. The program Win MDI 2.8 (Joseph Trotter, Scripps Institute, La Jolla, CA) was used to analyze the results. For analysis the parameters of forward and side light scatter were selected and related to the regions of granulocytes and monocytes (Fig. 1). The number of positive cells was determined by fluorescence histogram distribution parameter. Threshold set by the fluorescence of cells labeled with isotope, which is a specified set. The result expressed as percentage of positive cells. The probability of differences between groups was determined by Student's t test.
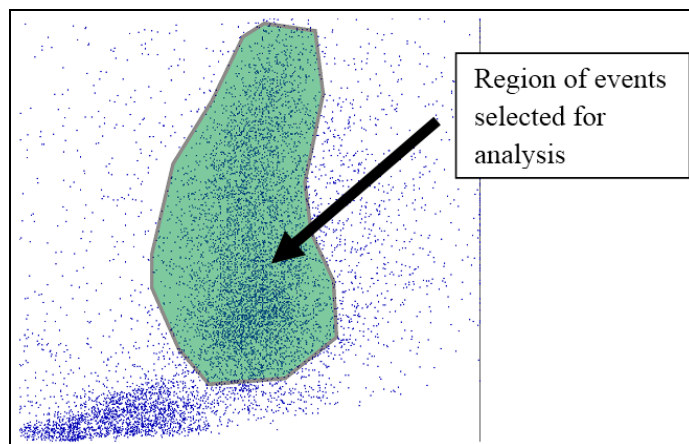

Fig 1: The choice of events to analyze the parameters of the front and side light scattering

We applied Socal score in CML patients for risk stratification at the time of presentation by using four clinical variables: age; size of spleen; percentage of blast cells and platelet count. The hazard ratio (Socal score) was calculated by entering data in the following equation.

$$\text{Exp}\{0.0116 \times (\text{age in years} - 43.4) + 0.0345 \times (\text{spleen size} - 7.51) + 0.188 \times [(\text{platelet count} \div 700)^2 - 0.563] + 0.0887 \times (\text{blast cells \% - 2.10})\}; \exp = 2{,}718.$$

The classification divides patients into three groups: low risk group (Socal score < 0.8), intermediate risk (Socal score 0.8-1.2) and high risk group in which Socal score was >1.2.
According to the criteria for assessing the effectiveness of TKI of The European Leukemia Net (update 2010) the patients were divided into three groups: with optimal, suboptimal response and failure of the imatinib therapy. The optimal response means a complete cytogenetic response after 12 months of therapy with imatinib (0% Ph-positive cells). Suboptimal response was defined when the bone marrow research showed from 1% to 35% of Ph chromosome cells after 12 months of imatinib therapy. The failure of treatment indicated the presence of a large number of cells with Ph chromosome, which is greater than 35% in the bone marrow.

3. Results and Discussion
We studied the expression of Ki-67 in patients with different Socal score. The research results are presented in Table 1.

Table 1: The expression of proliferation marker Ki-67 in CML patients with different prognostic Socal score treated with imatinib

Region	Expression of Ki-67 (% + m%) with different Socal score		
	Low (n=25)	Intermediate (n=26)	High (n=28)
Granulocytes PB	3.29 ± 0.60	10.78 ± 1.80*	13.64 ± 1.86*
Monocytes PB	3.46 ± 0.63	10.96 ± 1.93*	11.87 ± 1.30*
Granulocytes BM	7.32 ± 2.37	17.82 ± 3.64*	15.63 ± 1.78*
Monocytes BM	8.08 ± 2.08	21.99 ± 3.65*	16.00 ± 1.62*

* - Statistically significant difference with low Socal score

The expression of Ki-67 protein in patients with intermediate Sokal risk was more than three times higher in contrast with the low risk group in PB and more than twice time higher in BM ($p < 0.05$). In the high Sokal risk group the number of cells that expressed Ki-67 was 4 times higher in the region of granulocytes and three times higher in the region of monocytes in PB compared with low risk group ($p < 0.05$). In the bone marrow the expression of Ki-67 in patients with high Sokal score was twice higher compared to low Sokal risk group ($p < 0.05$).

Analysis of the response to imatinib therapy according to Sokal score was carried out (Table 2). Thus, among the 28 patients from high Sokal risk group at 12 month of imatinib treatment only two patients achieved the optimal response, six patients had suboptimal response, and twenty patients had treatment failure. Among the 26 patients from intermediate Sokal score, six achieved the optimal response at 12 month of treatment monitoring, a suboptimal response was obtained in five patients, and fifteen patients had treatment failure. Patients with low risk practically all achieved the optimal response after one year of imatinib therapy, and only two had an inadequate response to treatment (suboptimal response and treatment failure).

Table 2: Response to imatinib therapy based on prognostic Sokal score

Response rate	Low risk group (n = 25)		Intermediate risk group (n = 26)		High risk group (n = 28)	
	n	%±m%	N	%±m%	n	%±m%
Optimal response	23	92±5,4	6	23,1±8,3***	2	7,2±4,9
Suboptimal response	1	4±3,9	5	19,2±7,7*	6	21,4±7,8*
Failure	1	4±3,9	15	57,7±9,7*	20	71,4±8,5*

* - Statistically significant difference with low Socal score
* - Statistically significant difference with high Socal score

The results indicate that patients with low expression of Ki-67 and low-risk Sokal group achieve optimal response to treatment in most cases. Patients with a high Sokal score have increased expression of Ki-67 and ineffective treatment with imatinib. In order to predict the potential effectiveness of therapy, we compared the expression of Ki-67, a prognostic Sokal group and the response to therapy in some patients. Patients with low risk and low expression of Ki-67 achieved an optimal response as well as at 12th and 18th months of treatment. There have been several cases where patients had an initially low Socal score, a small number of Ki-67+ cells in the PB (about 3%) and suboptimal response to treatment after 12 months of treatment. However, at 18th months the response became optimal. In the case of intermediate Socal risk the number of hematopoietic cells in the PB with co-expression of Ki-67 was about 9 %, and the response to imatinib therapy after 12 months of treatment was optimal or suboptimal. But after 18 months of treatment the response was ineffective. Patients with high risk had increased expression of Ki-67 and after 12 months of imatinib therapy a failure of treatment or suboptimal response was observed. During the next cytogenetic analysis in all patients in that group a treatment failure was determined.

4. Conclusions
Studies indicate that there is a relationship between the value of the Socal score, the expression of Ki-67 protein and the response to imatinib therapy in CML patients. The results of TKI therapy were the best for patients with low Socal score and low expression of intranuclear protein Ki-67, the worst in patients with high Sokal risk and increased expression of Ki-67. Proceeding from the fact that the index Sokal considered an effective factor in prognosis of CML, the further research will develop a prognostic test system for evaluating response to TKI therapy based on the assessment of expression of proliferation marker Ki-67.

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