Імунні цитопенії у хворих на хронічну лімфоцитарну лейкемію (особливості перебігу, прогностичні маркери)

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Вступ. Імунні цитопенії (ІЦ) є одними з поширених ускладнень при хронічній лімфоцитарній лейкемії (ХЛЛ). Описано особливості різних імунних цитопеній у пацієнтів з ХЛЛ і значення окремих прогностичних маркерів у їхньому перебігу.

Методи дослідження. Під нашим спостереженням перебувало 62 хворих на хронічну лімфоцитарну лейкемію, ускладнену імунними цитопеніями, а саме: у 30 хворих спостерігалась автоімунна гемолітична анемія (АІГА), у 18 – імунна тромбоцитопенія (ІТП), у 10 – синдром Фішер-Івенса, у 3 – парціальна червоноклітинна аплазія (ПЧА) та в 1-ї хворої - імунна нейтропенія (ІН).

Крім загального обстеження та лабораторних досліджень, проводили додаткові обстеження: імунофенотипування лімфоцитів периферичної крові, методом проточної цитометрії (CD5; CD19; CD20; CD23; CD38; ZAP70), тест Кумбса, молекулярно-цитогенетичне дослідження лімфоцитів периферичної крові методом FISH з використанням зондів TP 53 та ATM, визначення рівня $ß_2$МГ.

Результати дослідження. З'ясовано, що загальне виживання хворих на ХЛЛ, ускладнену ІЦ, залежить від форми ІЦ. Медіана загального виживання у хворих з синдромом Фішер-Івенса була найкоротшою (75 місяців), дещо ліпше виживання спостерігалось у хворих з АІГА (медіана 80 місяців), найкраще виживання виявилось у хворих ІТП (медіана не досягнута). ІЦ частіше виникала на ранніх стадіях хвороби. Різниці у загальному виживанні хворих з раннім чи пізнім ускладненням ХЛЛ ІЦ не виявлено.

Із несприятливих маркерів перебігу ХЛЛ з ІЦ варто відзначити наявність del 11q22.3. Несприятливим прогностичним маркером був позитивний тест Кумбса, високі рівні експресії ZAP 70, а також високий рівень $ß_2$МГ.

Ключові слова: хронічна лімфоцитарна лейкемія, імунні цитопенії, аутоімунна гемолітична анемія, імунна тромбоцитопенія, синдром Фішер-Івенса, парціальна червоноклітинна анемія.

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Immune cytopenias in patients with chronic lymphocytic leukemia (peculiarities, prognostic markers)

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Introduction. Immune cytopenia (IC) is one of the major complications in chronic lymphocytic leukemia (CLL). The paper describes the peculiarities of different immune cytopenia in CLL patients and the importance of individual prognostic markers in the course of the disease.

Methods. We observed 62 patients with CLL complicated by immune cytopenia. Among these patients 30 had autoimmune hemolytic anemia (AIHA), 18 experienced immune thrombocytopenia (ITP), 10 had Fisher-Evans syndrome (FES), 3 were diagnosed with partial red cell aplasia (PRCA), and immune neutropenia (IN) was revealed in 1 patient.

In addition to general examination and laboratory studies, the following examinations were performed: immunophenotyping of peripheral blood lymphocytes, flow cytometry (CD5; CD19; CD20; CD23; CD38; ZAP70), Coombs test, a molecular cytogenetic study of peripheral blood lymphocytes using the FISH method with TP53 and ATM probes, the level of $ß_2$-microglobulin.

Results. It was established that the overall survival of CLL patients with IC depends on the form of the latter. The median overall survival in patients with Fisher-Evans syndrome was the shortest (75 months), slightly better survival was observed in patients with AIHA (median 80 months), the best survival was found in patients with ITP (median not reached).

Among unfavorable markers of CLL with IC, there is the presence of del 11q22.3. Unfavorable prognostic markers were also the following: a positive Coombs test, high levels of ZAP 70 expression, and high levels of $ß_2$-microglobulin.

Keywords: Chronic lymphocytic leukemia, immune cytopenia, autoimmune hemolytic anemia, immune thrombocytopenia, Fisher-Evans syndrome, partial red cell aplasia.

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Immune cytopenia (IC) are one of the major complications in chronic lymphocytic leukemia (CLL) [1]. According to literature data, the frequency of IC in CLL patients varies from 4.3% to 9.7%. The most common complication of CLL is autoimmune hemolytic anemia (AIHA), which is observed in 5-10% of patients [2, 3]. Immune thrombocytopenia (ITP) is less common and is seen in 1-5% of the patients [2, 4]. Zent and Kay reported that Fisher-Evans syndrome (FES) is diagnosed in 55 – 66% of patients with IC [2]. Pure red cell aplasia (PRCA) and immune neutropenia (IN) are among rare immune complications [5].

Patients with anemia and thrombocytopenia are diagnosed with III or IV Rai stages regardless of the genesis of cytopenia. However, IC should be distinguished from cytopenia, caused by bone marrow insufficiency, because these conditions require different therapeutic management.

We observed 62 patients with CLL complicated by immune cytopenia, 37 of them were males aged 40–78 (median 63.5) and 25 females aged 42–76 (median 65.6). The study was reviewed and approved by the Ethics Commission (Committee on Ethics and Deontology at the SI "Institute of Blood Pathology and Transfusion Medicine of the NAMS of Ukraine") on August 30, 2021 (protocol number 08/05). The Informed Consent form was received from all study participants.

The diagnosis was established by performing a clinic-laboratory examination in accordance with international criteria [6, 7, 8]. Immunophenotyping of lymphoid cells from peripheral blood or bone marrow was performed using the flow cytometry technique. The level of β_2-microglobulin was measured using the ECLIA method (analyzer and test-systems COBUS 600, Roche Diagnostics, Switzerland). Molecular cytogenetic analysis of lymphocytes in peripheral blood was done with FISH using TP53 and ATM probes. Diagnosis of CLL in all patients was confirmed with immunophenotyping of peripheral blood by the presence of CD 5+, CD 19+, CD 20+, CD 23+ population.

For AIHA diagnosis, the following criteria were used: anemia with a hemoglobin level below 100 g/l, reticulocytosis, indirect bilirubinemia, positive direct Coombs test, elevated LDH level, retained erythroid lineage in the bone marrow. ITP was diagnosed according to such criteria as a rapid decrease in platelets level below 100 x 10^9/L or 2-fold decrease from baseline level, presence of megakaryocytes in bone marrow, no hypersplenism and more than 4–8 weeks after the last course of chemotherapy. PRCA was revealed when the following criteria were noticed: normochromic anemia with a hemoglobin level below 100 g/l, reticulocytopenia, erythroid lineage in bone marrow < 1%, negative direct Coombs test, normal level of hemoglobin and more than 4–8 weeks after the last course of chemotherapy. Diagnosis of IN was established based on the following parameters: continuous unexplained granulocytopenia, decreased or absence of granulocyte progenitors in the bone marrow. For FES diagnosis, we used the following criteria: anemia with a hemoglobin level below 100 g/l, reticulocytosis, indirect bilirubinemia, positive direct Coombs test, elevated LDH level, rapid decrease in platelets level below 100 x 10^9/L, retained erythroid lineage and presence of megakaryocytes in the bone marrow.

Obtained results were evaluated using Statistical Analysis System and visualization program "Statistica for Windows 6.0" (Statsoft, USA). For describing parametric indicators, descriptive statistics was used to determine medians and quartiles. The overall survival of patients was assessed using Kaplan-Mayer curves, which were compared using Cox F-test, the median survival was also determined.

Results

The most frequent form of immune cytopenia in the group of observed patients was AIHA, diagnosed in 30 of them (III Rai stage). ITP was recorded less often, 18 patients suffered from this complication (IV Rai stage), 10 patients had FES (IV Rai stage), 1 patient had IN (II Rai stage), and PRCA developed in 3 patients (III Rai stage).

Table 1 shows the distribution of CLL patients by sex and the form of IC. As can be seen from the table, the most common form of IC was AIHA, which was observed with equal frequency among females and males. ITP and Fisher-Evans syndrome were more common in men.

IC may complicate CLL at any time of disease course, starting with the diagnosis. Time from

Table 1

The distribution of CLL patients by sex and the form of IC

Immune cytopenia	Males	Females	All
Autoimmune hemolytic anemia	17	13	30
Immune thrombocytopenia	12	6	18
Fisher-Evans syndrome	7	3	10
Immune neutropenia	0	1	1
Partial red cell aplasia	1	2	3

CLL diagnosis to the development of IC is presented in Table 2.

AIHA developed predominantly in males at the early stage of the disease. Specifically, hemolysis occurred in 6 patients at the onset of disease, in 7 males within a year from diagnosis and only in 4 patients, this complication was diagnosed on the 24th year from diagnosis or later. Among females, only 2 CLL patients debuted with hemolysis. In most cases, hemolysis developed on the 36th month from the onset of disease or later.

ITP was the first sign of disease in 10 patients (8 males and 2 females). Hemorrhagic syndrome, thrombocytopenia were the reason for examination of patients, which allowed to diagnose CLL

In 6 patients, Fisher-Evans syndrome occurred in the early stage of disease, and in 4 patients, on the 36th month from the disease onset or later. PRCA was diagnosed in 2 females and one male on the 1st, 34th and 43rd months from CLL diagnosis. IN occurred on the 156th month from disease onset in one female patient, who was diagnosed with CLL at the age of 40.

Table 2

Time from diagnosis of chronic lymphocytic leukemia to the development of immune cytopenia

Time to development of IC	Males			Females		
	AIHA	ITP	FES	AIHA	ITP	FES
Onset	6	8		2	2	2
Up to 12 months	7	1	4			
Up to 24 months	1	1		1		
Up to 36 months	1	2	1	2	1	1
Up to 48 months			1	1	1	
Up to 60 months	1		1	4		
> 60 months	1			3	2	

Figure 1 shows the overall survival curves for CLL patients depending on the time of IC onset. As can be seen in the figure, the overall survival of patients with CLL with early or late complications of IC did not differ significantly.

Overall survival curves for CLL patients depending on the type of IC are presented in fig. 2. As can be seen from the figure, overall survival was worse in patients with CLL com-

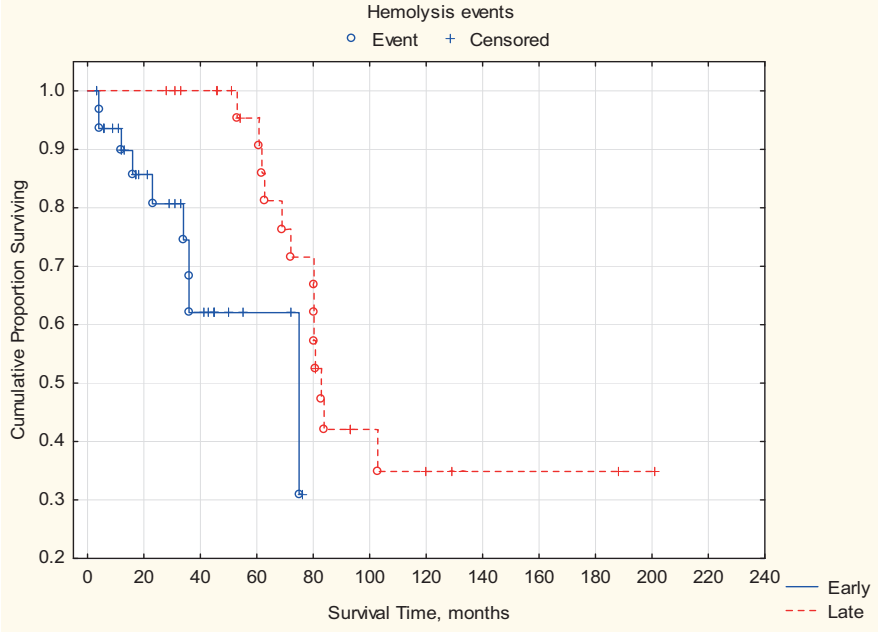

Figure 1. Overall survival curves for CLL patients depending on the time of onset of IC

Table 3

Parameters of peripheral blood in patients with CLL, complicated with immune cytopenias

Type of complication	Hemoglobin, (ґ/л) Median, lower-upper quartile	RBC, ($\times 10^{12}$/L) Median, lower-upper quartile	WBC ($\times 10^{9}$/L) Median, lower-upper quartile	Lympho-cytes, (%) Median, lower-upper quartile	Platelets, ($\times 10^{9}$/л) Median, lower-upper quartile	Reticulocytes, (‰) Median, lower-upper quartile	Bilirubin, umol/L Median, lower-upper quartile
AIHA	71 [55-88.5]	2.35 [1.8-3.05]	102 [31.5-189]	83 [74.5-96]	195 [146-236]	73,5 [52-113]	44,5 [31-80]
ITP	119 [108-135]	4.0 [3.8-4.0]	46.5 [12-74]	74 [66-81]	20 [17-49]	-	-
Fisher-Evans syndrome	52 [49-86]	1.7 [1.4-2.7]	155 [181-225]	95.5 [93-96]	59 [14.5-82]	29 [14-88]	37 [16-46]

plicated by Fisher-Evans syndrome (median OS – 75 months). Similar data were observed in case of AIGA complication (median OS – 80 months). Better survival was found in patients with ITP (median OS – not reached).

Two patients developed AIHA after the administration of the COP (CVP) course. One of them suffered from repeated hemolysis after RFC therapy. Two more patients had AIHA and one was diagnosed with Fisher-Evans syndrome while taking chlorambucil. IC in other patients was not associated with administered treatment.

Table 3 contains peripheral blood parameters in patients with different types of immune cytopenia.

All patients experienced elevated white blood cell count levels, lymphocytosis.

Patients with ITP had a lower WBC count than in two other groups. RBC and hemoglobin were within normal ranges. PLT level was markedly decreased.

In case of AIHA, low hemoglobin and the RBC level were revealed as well as reticulocytosis, increased indirect bilirubin concentration and normal PLT count.

Patients with Fisher-Evans syndrome had low parameters of red blood and platelet count.

Twenty-one patients with AIHA had warm antibodies (positive direct Coombs test), titer from 1:8 to 1:2048. One female patient had a high titer of cold antibodies (1:8000).

Positive Coombs test had also 4 patients with

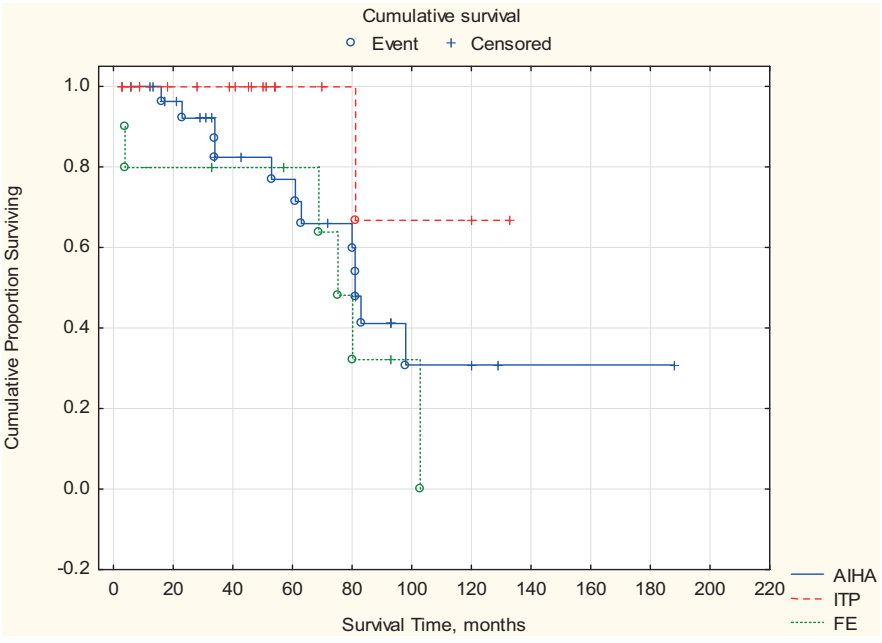

Figure 2. Overall survival curves for CLL patients depending on the type of IC

ITP (titer 1:4 – 1:64) and 3 patients with Fisher-Evans syndrome (titer 1:512).

The overall survival curve for CLL patients with IC complications depending on Coombs test results is presented in fig.3. As it can be seen, the median overall survival in patients with positive Coombs test was 64 months and was not reached in patients with a negative Coombs test. Thereby, overall survival in patients with positive Coombs test was significantly poorer than in subjects with negative Coombs test.

FISH was performed in 23 patients with probes to ATM genes (chromosome 11q22.3) and TP53 (chromosome 17p13.1). The deletion of chromosome 11q22.3 was revealed in 5 of 10 patients with AIHA in 11% – 100% of analyzed cells. The remaining 5 patients had a deletion of neither chromosome 11q22.3 nor 17p13.1.

In patients with ITP, the deletion of chromosome 11q22.3 was revealed in 3 of 5 examined patients in 16%-18% of analyzed cells. Two of them also had a concomitant deletion of chromosome 17p13.1 (in 15% and 19% of analyzed cells). In the group of 6 patients with Fisher-Evans syndrome, the deletion of chromosome 11q22.3 was found in 2 of them in 14% and 44% of analyzed cells. The deletion of chromosome 17p13.1 was not revealed in these patients. No aberrations were found in one patient with PRCA and one patient with IN.

Summing up, the deletion of chromosome 11q22.3 was revealed in 10 of 23 examined CLL patients with IC (43.3%), i.e., significantly more frequently than in CLL patients without immune complications.

Figure 4 shows that the median overall survival in patients with the deletion of chromosome 11q22.3 was 60 months, and was not reached in patients without this abnormality. Overall survival of CLL patients with IC and the deletion of chromosome 11q22.3 was poorer than in patients without this aberration, but the difference was not statistically significant.

Other prognostic factors for CLL in patients with immune complications were investigated as well. Levels of β_2 microglobulin, CD 38 expression, ZAP 70 were detected.

Fig. 5 shows overall survival curves for CLL patients with IC depending on the level of β_2 microglobulin.

The median overall survival in patients with the level of β_2 microglobulin above 2.0 mg/L was 80 months and did not reach a lower level of this protein.

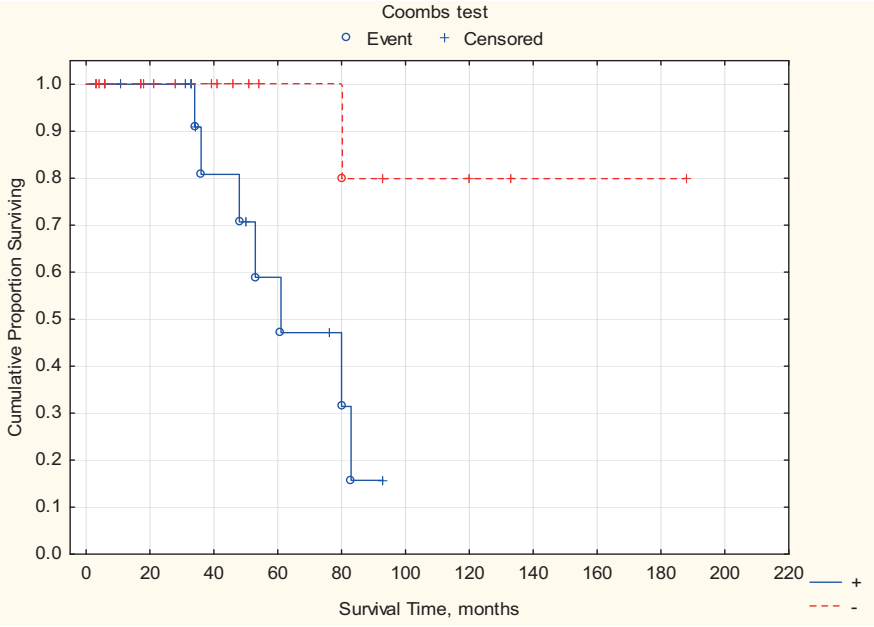

Figure 3. Overall survival curves in CLL patients with IC, depending on Coombs test results

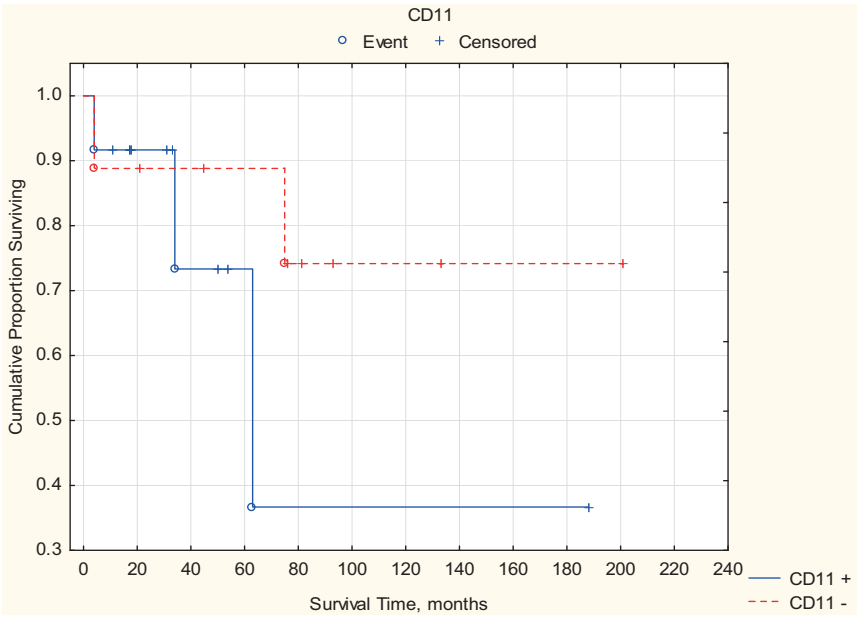

Figure 4. Overall survival curves for CLL patients with immune cytopenias depending on the presence of deletion 11q22.3

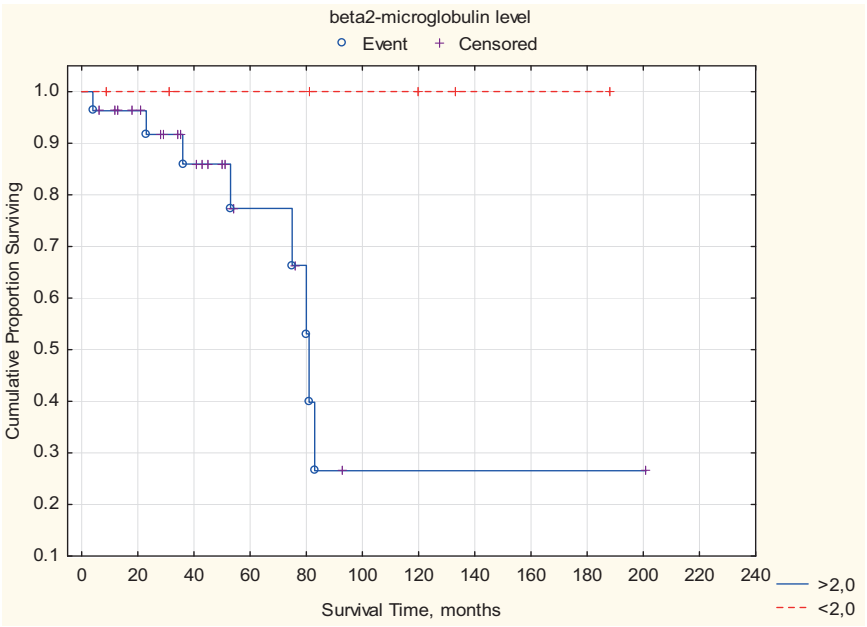

Figure 5. Overall survival curves for CLL patients with IC depending on the level of β_2 microglobulin

Overall survival in patients with the level of β_2 microglobulin above 2.0 mg/L was markedly shorter in comparison with the same in subjects with a lower level.

Fig. 6 presents overall survival curves in CLL patients with IC depending on the level of CD 38. No significant difference was revealed in the overall survival of CLL patients with IC and CD 38 expression above and below 20%.

Fig. 7 presents overall survival curves in CLL patients with IC depending on ZAP70 expression. Median overall survival in CLL patients with IC and ZAP70 expression above 20% was 60 months, and in patients with ZAP70 expression below 20%, it was not reached.

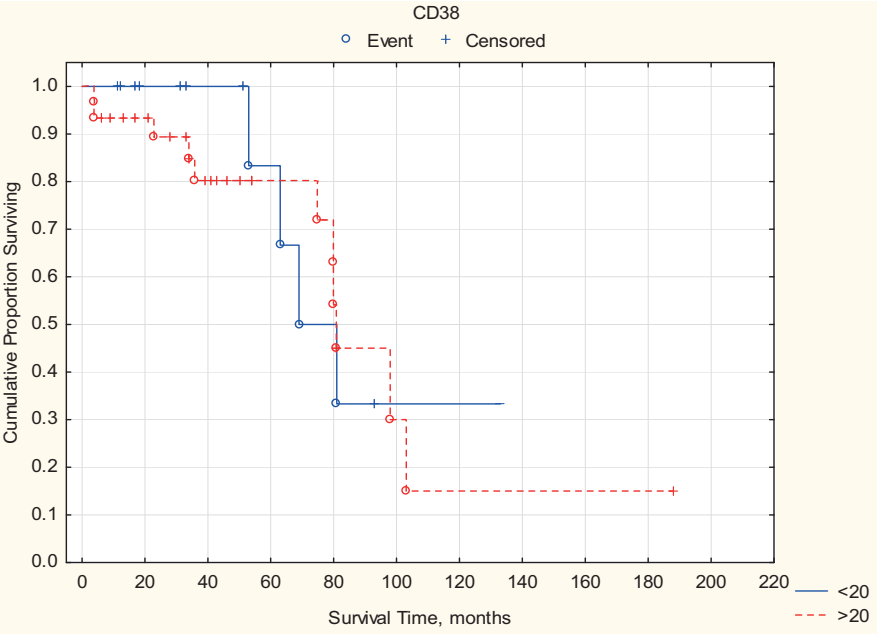

Figure 6. Overall survival curves for CLL patients with IC depending on the level of CD 38

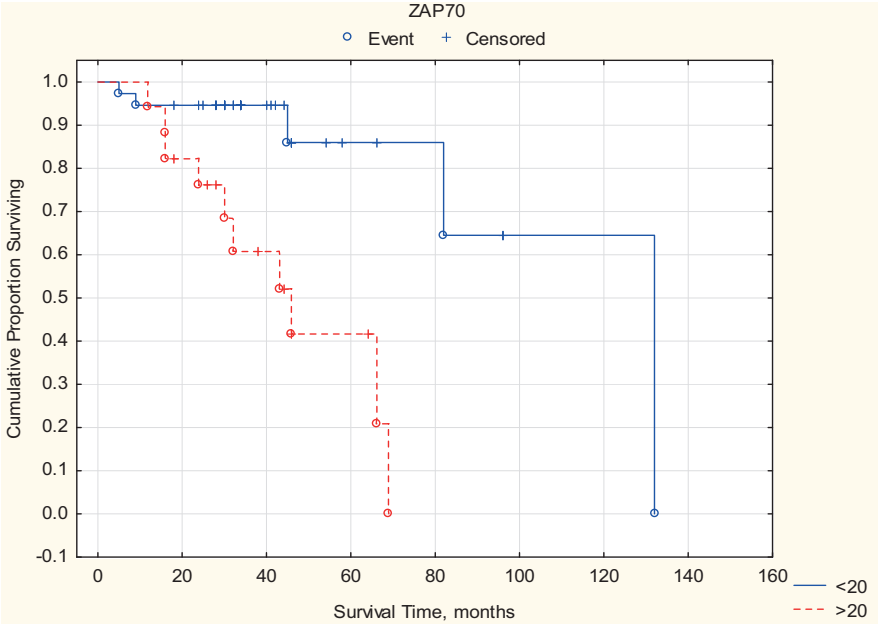

Figure 7. Overall survival curves for CLL patients with IC depending on the level of ZAP70 expression

Discussion

Among examined patients, the most common immune complication of CLL was AIHA (30 patients), ITP was observed less often (18 patients), which corresponds to the literature data. We found the occurrence of Fisher-Evans syndrome only in 10 out of 62 patients, which does not coincide with the data of C. Zent and N. Kay, who diagnosed Fisher-Evans syndrome in 50-60% of all patients with IC [2]. A rare complication was PRCA (3 patients) and IN (1 patient). Literature data regarding the effect of IC on the life expectancy of CLL patients are ambiguous. Thus, a large observational study including 960 CLL patients revealed no difference in the overall survival when 70 patients with IC were compared with the rest of the patients without IC. Patients without AIHA,

reportedly, have better survival rates in comparison with AIHA patients [9, 10].

According to other researchers, immune cytopenia, in contrast to cytopenia due to bone marrow insufficiency, have a better response to treatment and do not significantly affect patients' longevity [5, 11]. Our results indicate that the overall survival of patients depends on the form of IC. More specifically, the median overall survival of patients with Fisher-Evans syndrome was the shortest (75 months). Slightly better overall survival rates were observed in patients with AIHA (80 months). And the best overall survival rates were observed in patients with ITP (median was not reached).

According to the literature, the time of IC occurrence has major importance. It is noted that patients with the early onset of IC have a shorter overall survival rate in comparison with patients without IC. [5, 10]. However, patients with advanced CLL stages with IC have better survival rates than patients with cytopenia due to insufficiency of bone marrow hematopoiesis [12, 13]. Thus, according to literature data, the median survival rate of CLL patients complicated with ITP is 5.7 years, while in patients with thrombocytopenia due to insufficiency of bone marrow hematopoiesis, this parameter reaches only 2.8 years [14].

Among examined patients, IC occurred more often in the early stages of CLL, i.e., 32 CLL patients experienced this complication during the first year of the diagnosis. When comparing the overall survival curves of patients with early and late onset of IC, no significant difference was found.

Alkylating drugs are widely known to induce AIHA in CLL patients [10]. Moreno et al. observed IC in 5% of CLL patients treated with chlorambucil [13]. According to other researchers, IC occurred in 7.7% of patients receiving fludarabine as monotherapy, in 2.8% of patients treated with FC regimen and in 1% of patients after FCR therapy [3, 15]. Cases of AIHA after treatment with bendamustine and ibrutinib have also been described [16, 17, 18, 19].

Among the examined patients, AIHA was developed in 2 patients treated with chlorambucil. Another patient was treated with chlorambucil for Fisher-Evans syndrome. Acute hemolysis developed in 2 CLL patients immediately after a cycle of CVP. In one of them, hemolysis was eliminated by a course of R-CHOP therapy, remission started. With the disease progression (without hemolysis), the patient was prescribed a course of RC, after which acute hemolysis re-developed, which was not treated. The patient died 34 months after being diagnosed with CLL. In other patients, the cause of IC was not identified.

Despite a significant number of studies, so far, no prognostic markers have been established according to which the development of IC in a particular patient can be predicted. Some publications report on the prognostic significance of the Coombs test. A positive Coombs test was found in 5.7 to 27.6% of patients with CLL and was considered a poor prognostic marker, but a positive Coombs test is not an accurate predictor of AIGA, and its negative result does not preclude future development of IC [10, 20, 21]. Other researchers do not report the prognostic value of the Coombs test [22]. In a large study including 378 patients with CLL, a positive Coombs test was found in 14% of patients, but AIGA developed in only 5% of them [21]. In our studies, the Coombs test was performed at the time of IC occurrence. Coombs test was positive in 23 patients with AIGA (titer from 1: 8 to 1: 2048), i.e. hemolysis was caused by warm antibodies. One patient had a high titer of cold antibodies (1:8000). Coombs test was also positive in 4 patients with ITP and 3 patients with Fisher-Evans syndrome.

When comparing survival curves of patients with positive and negative Coombs tests, it was revealed that the overall survival length of patients with negative Coombs test is significantly longer, which indicates the prognostic value of the Coombs test.

Studies of the prognostic value of other indicators were conducted. Older age, male gender correlated with the development of ITP [14]. It was reported that IC is more common in patients with unmutated IGHV status [23, 24]. It is considered that a high level of β2-microglobulin [24, 25], increased CD 38 [22, 25], high ZAP70 [24, 26, 27, 28] may be antecedents of IC. We studied the prognostic significance of individual markers during the IC period. The

level of β2-microglobulin, CD 38 expression, ZAP70, genetic aberrations were determined.

The comparison of the overall survival of patients with CD38 + lymphocytes <20% and patients with CD 38> 20% did not show a significant difference. A significant distinction was observed in patients with different expressions of ZAP70. Thus, the median overall survival of patients with ZAP70> 20% was 60 months. In the group of patients with ZAP70 expression, <20% of the median is not reached, which underlines the prognostic value of ZAP70.

When comparing the curves of overall survival of patients depending on the level of β2-microglobulin, the median overall survival of patients with high β2-microglobulin was found to be 80 months, in patients with β2-microglobulin <2 mg/L, the median was not reached, i.e., survival patients were feeling much better at the level of β2-microglobulin > 2 mg/L.

It is known that genetic aberrations, in particular the deletion of 11q22.3 and 17p13.1, are an important prognostic marker of CLL. Regarding the immune complications of CLL, the increased frequency of ITP was observed in patients with the deletion of 11q22.3 [23]. According to our results, the deletion of 11q22.3 was found in 10 of 23 examined patients (43.4%). In addition, two of them had a simultaneous 17p13.1 deletion, which according to the literature, is observed in 1% of patients and is a poor prognostic marker. Despite the small number of studies, it can be concluded that the frequency of 11q22.3 deletion in CLL patients with IC is significantly higher than in patients without such complications. P. Greipp et al. (2013) described prognostic markers in 2184 patients with CLL, in particular, 17p13.1 was found in 7% of patients, 11q22.3 deletion – in 11% of patients, the association 17p13.1 deletion and 11q22.3 deletion in only 1% of patients [29]. The average life expectancy of this group of patients was the shortest (1.9 years), while with 17p13.1 deletion and 11q22.3 deletion, this parameter reached 3.1 and 4.8 years respectively.

When comparing survival curves of patients with and without 11q22.3 deletion, it was found that the overall survival of patients with mentioned abnormality was shorter (median 60 months) compared with OS patients without 11q22.3 deletion (median not reached).

Thus, it was found that the severity of the course of CLL complicated by IC depends on the form of cytopenia. Determination of prognostic markers (presence of 11q22.3 deletion, Coombs test, ZAP-70 expression, $ß_2$-microglobulin level) allows predicting the course of the disease.

A possible limitation of the study is that only one-third of patients were examined using molecular cytogenetic methods since they are not reimbursed to patients. In conclusions: 1. The overall survival of patients with CLL complicated by IC depends on the form of cytopenia. The shortest overall survival was observed in patients with Fisher-Evans syndrome, the longest was revealed in patients with ITP. 2. There was no difference in overall survival of patients with CLL with early or late onset of immune cytopenia. 3. Unfavourable markers of CLL with IC are a positive Coombs test, high expression of ZAP-70 and a high level of $ß_2$- microglobulin. 4. In patients with CLL complicated with IC, deletion of chromosome 11q22.3 and simultaneous deletion of chromosome 11q22.3 and chromosome 17p13.1 are more common than in patients with CLL without IC. The presence of 11q22.3 deletion is an unfavorable prognostic marker.

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