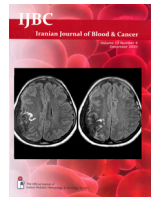
IJBC 2021; 13(1): 11-16

Iranian Journal of Blood & Cancer

Journal Home Page: www.ijbc.ir



ORIGINAL ARTICLE

Prognostic Impact of Interleukin-10 in Patients with Chronic Lymphocytic Leukemia

Waiel Mohmed Al-Kahiry[1*], Maha Abubakr Feissal Rabie[2], Homam Mohamed Sharshira[3], Amal Mostafa Ghanem[3], Maha Mohamed El-gammal[3], Irene Lewis Mikhael[3]

[1]Department of Hematology, Faculty of Medicine and Health Sciences, University of Aden, Yemen
[2]Department of Medical Laboratory Technology, Pharos University in Alexandria, Egypt
[3]Department of Hematology, Medical Research Institute, University of Alexandria, Egypt

ARTICLE INFO

Article History:
Received: 25.10.2020
Accepted: 13.01.2021

Keywords:
Chronic lymphocytic leukemia
Egypt
IL-10 level
ZAP-70
Binet stage
Rai stage
Prognosis
Correlation

**Corresponding author:*
Waiel Mohmed Al-Kahiry,
Department of Hematology, Faculty of Medicine and Health Sciences, University of Aden, Postal code: 21500, Yemen
Tel: +96-77-39252585
Email: kahiry13@gmail.com

ABSTRACT

Background: Several markers are investigated for prognostic purpose in CLL patients among which interleukin-10 (IL-10) is under more investigation. We aimed to study IL-10 level in a group of newly diagnosed patients with CLL in a single center in Alexandria, Egypt.
Methods: In this study, 80 newly diagnosed patients with CLL referring to Hematology Department of the Medical Research Institute of Alexandria University, Egypt, and a control group including 40 healthy volunteers were included. ZAP-70 was determined by flowcytometry and serum IL-10 concentration was measured using IL-10 sandwich ELISA method.
Results: Mean serum IL-10 levels were significantly higher among patients with CLL. IL-10 level was higher in those who were positive for ZAP-70, Binet stage C, Rai stage III-IV and patients with high scores for CLL prognostic index (≥8). It showed significant positive correlation with the percentage of ZAP-70 expression and significant negative correlation to hemoglobin and platelets count.
Conclusion: Serum IL-10 in patients with CLL at presentation could be used as a prognostic marker for disease progression. Measurement of IL-10 in low resources areas where flowcytometry is not available could be recommended as a substitute investigation.

Please cite this article as: Al-Kahiry WM, Rabie MAF, Sharshira HM, Ghanem AM, El-gammal MM, Mikhael IL. Prognostic Impact of Interleukin-10 in Patients with Chronic Lymphocytic Leukemia. IJBC 2021; 13(1): 11-16.

Introduction

Chronic lymphocytic leukemia (CLL) is a clonal disorder characterized by a progressive accumulation of incompetent lymphocytes. It is the most common form of leukemia found in adults in Western countries,[1] with highly variable clinical course and a male preponderance.[2]

Because most CLL patients are asymptomatic, the true incidence is unknown. CLL is the most common type of leukemia in adults accounting for 25% of all leukemias.[3] In the USA, it accounts for approximately 37.6% of ALL in the Central Arkansas Veterans Healthcare System (CAVHS) which was attributed to an increase in the use of peripheral blood immunophenotyping as the only diagnostic test for CLL over the time period of the study.[4]

In a literature review, Redaelli and colleagues studied the clinical and epidemiological burden of CLL.[5] They observed that the incidence of CLL is variable among countries around the world. The highest rates were found in Australia, North America, and Europe (especially Ireland, Italy, and Switzerland). Lower rates are found in Asia and South America.

Among Arab countries, CLL represented 10% of all registered leukemias in the Saudi Cancer Registry,[6] 8.6% of all leukemia cases in Kuwait,[7] and 16% of all types of leukemias in Jordan.[8] In Egypt, CLL was the most common subtype of leukemia according to the National Cancer Registry Program of Egypt, so that over 80% of leukemias occurring in adulthood are CLL.[9, 10]

Several markers are investigated for prognostic purpose in patients with CLL among which are the interleukin-10 (IL-10). IL-10 is an anti-inflammatory class-2 cytokine with pleiotropic effects in immunoregulation and inflammation.[11] It has a potent stimulating effect on B cells inducing proliferation and differentiation.[12]

There are various reports describing elevated levels of IL-10 expression in patients with particular cancers including malignant melanoma,[13] ovarian cancer,[14] lymphoma, and myeloma.[15, 16] There are scarce data available in the literature regarding IL-10 as a prognostic marker in patients with CLL. We aimed to study IL-10 level in a group of newly diagnosed patients with CLL in a single center in Alexandria, Egypt.

Materials and Methods

This was a cross-sectional study on 80 newly diagnosed patients with CLL referring to Hematology Department of the Medical Research Institute, Alexandria University, Egypt. There was a control group consisting of 40 age and sex matched healthy volunteers. Patients with CLL with other morbidities such as secondary malignancy, infections and autoimmune disorders were excluded. Diagnosis of CLL was based on the finding of an absolute lymphocyte count of 5.0 x 10^9/L or more for at least 3 months, mature lymphocyte morphology and scoring system of 4 or 5 on immunophenotyping for diagnosis of CLL.

Beside the complete blood count, flowcytometry was used to determine the percentage of ZAP-70 expression and ELISA sandwich method for determination of serum IL-10 (KOMA BIOTECH INC). Serum LDH and β_2-microglobulin were also measured. Clinical staging (Binet Rain staging system) and CLL prognostic index which is based on the presence of risk factors were applied for the patients (table 1).[17, 18]

Data processing was performed by the Statistical Package for Social Sciences (SPSS) for windows which revealed normally distributed (parametric) data. Accordingly, parametric tests were conducted (t-test for two means, one-way ANOVA for more than two means, and the Pearson correlation test) with 95% confidence interval. This study was ethically approved by the ethical committee of Medical Research Institute, Alexandria University and an informed written consent was obtained from every participant.

Results

The mean±SD age of the patients was 58.95±7.9 years (range: 46-76 years), with a male predominence (M:F ratio of 2.3:1). Serum IL-10 levels ranged from 11-98 pg/ml among patients with CLL with a significant higher mean (36.09 pg/ml) compared with the control group (6.32 pg/ml, table 2).

Serum IL-10 levels showed significantly higher mean values among patients with CLL with positive ZAP-70, advanced stage disease (Binet stage C and advanced Rai stages) and those with a high-risk prognostic index (table 3).

The ROC curve was drawn for IL-10 level as a sensitive indicator of ZAP-70 positivity in patients with CLL. It showed a statistically significant high area under the curve (AUC=0.88, P=0.0001) in ZAP-70 positive patients.

Correlation tests were performed between IL-10 and other parameters. A significant positive correlation was observed between IL-10 levels and percentage of ZAP-70 expression (r=0.865, P=0.001, figure 1), total leukocyte counts (r=0.416, P=0.008, figure 2) and absolute lymphocyte counts (r=0.405, P=0.010, figure 3). There was a significant negative correlation with hemoglobin (r=-0.528, P=0.001, figure 4) and platelets counts (r=-0.559, P=0.001, figure 5). Other markers such as β_2-microglobulinand LDH did not show a significant correlation with IL-10 levels.

Table 1: CLL Prognostic index based on the presence of risk factors [17, 18]

Characteristics	Point contribution			
	0	1	2	3
Age (years)	-	<50	50–65	>65
β_2M (mg/L)	<ULN	1-2 x ULN	>2 x ULN	-
ALC (X10^9/L)	<20	20–50	>50	-
Sex	Female	Male	-	-
Rai stage	0–II	III–IV	-	-
Number of involved nodal groups	≤2	3	-	-

ULN: upper limit of normal, β_2M:Beta 2 microglobulin, ALC: absolute lymphocyte count, Score: 1-3 (low risk), 4-7 (intermediate risk), ≥8 (high risk)

Table 2: Sex, age and serum interleukin-10 levels in patients with CLL and control group

		B-CLL patients (n=80)	Control (n=40)	P value
Sex	Male	56 (70.0%)	28 (70.0%)	1.00
	Female	24 (30.0%)	12 (30.0%)	
Age (years)		58.95±7.9 (46-76)	54.8±8.7 (40-64)	0.069
Serum IL-10 level (pg/ml)		36.09±20.84 (11.0 - 98.0)	6.32±2.83 (1.8 - 10.4)	0.0001*

*Statistically significant

Table 3: Mean serum interleukin-10 levels according to ZAP-70, Binet and Rai staging in CLL patients

	Parameters	IL-10 (pg/ml) Mean±SD		P value
ZAP-70	Negative (n=58)	27.43±10.5	(11-50)	0.0001*
	Positive (n=22)	58.94±24.3	(16.7-98)	
Binet staging	A (n=16)	18.26±5.52	(11-28.9)	0.0001*
	B (n=54)	32.53±13.98	(16.7-72.6)	
	C (n=10)	57.5±19.8	(36.9-98.0)	
Rai staging	0-I(n=20)	22.70±6.4	(11-31.2)	0.0001*
	II (n=32)	26.77±11.9	(14.5-57.1)	
	III-IV (n=28)	56.3±20.7	(25.8-98.0)	
CLL-PI	Low risk (n=10)	22.36±7.51	(13.9-29.1)	0.0001*
	Intermediate (n=54)	30.53±14.18	(11.0-72.6)	
	High risk (n=16)	63.46±23.0	(28.9-98.0)	

CLL-PI: Chronic lymphocytic leukemia prognostic index, *Statistically significant

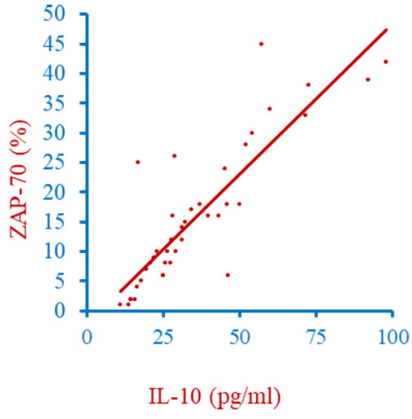


Figure 1: Positive correlation between IL-10 and ZAP-70 expression

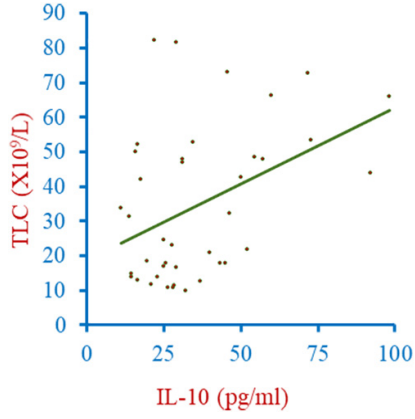


Figure 2: Positive correlation between IL-10 and total leucocytic count

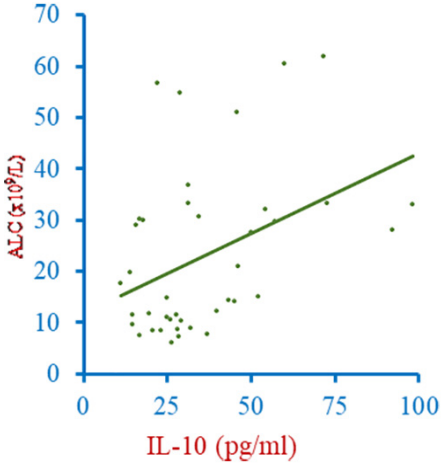


Figure 3: Positive correlation between IL-10 and absolute lymphocytic count

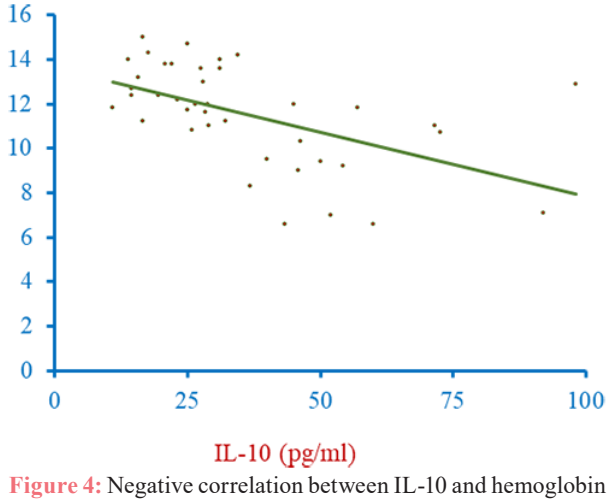


Figure 4: Negative correlation between IL-10 and hemoglobin concentration

Multiple regression analysis was conducted for all the studied variables in patients with CLL with respect to ZAP-70 as a dependent variable (criterion). Serum IL-10 was a significant predictor for prognosis in patients with CLL (P=0.001). Other variables did not show significant prognostic impact in the studied patients (table 4).

Discussion

IL-10 is a pleiotropic cytokine that is produced by type 2 helper cells. It is believed to be produced by CD5 positive B-lymphocytes through STAT3 and NFAT2 activation in patients with CLL.[19, 20]

It has been suggested that IL-10 inhibits proliferation of malignant cells in CLL.[21] In contrast, other authors have proposed that IL-10 might act as an autocrine growth factor for CLL cells since they observed spontaneous release of IL-10 in culture of CLL lymphocytes.[22]

In this study, mean and SD of IL-10 concentration in patients with CLL was 36.09±20.84pg/m with a range up to 100 pg/ml. Near to our finding, Kara et al. assessed the level of IL-10 in 22 healthy donors and 70 patients with B-CLL, and the correlation with disease stage and

Table 4: Multiple regression analysis for predictors of positive ZAP-70 in patients with CLL

Predictors	Standardized Beta Coefficients	*t*	P
Hemoglobin concentration (g/dl)	-0.157	-1.45	0.157
Total leukocyte count (×10^9/L)	0.243	0.760	0.453
Platelets count (×10^9/L)	0.098	0.952	0.349
Absolute lymphocytes count (×10^9/L)	0.026	0.082	0.935
β_2-microglobulin (mg/L)	-0.098	-0.965	0.342
LDH (U/l)	0.111	1.301	0.203
IL-10 (pg/ml)	0.773	6.150	0.001*

The test performed by using the enter method (F=15.58, P<0.0001). Adjusted R square=0.771

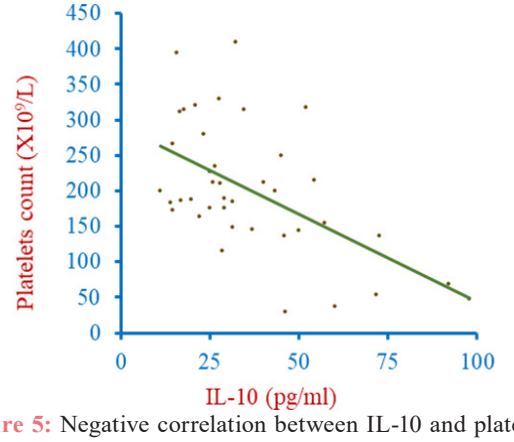
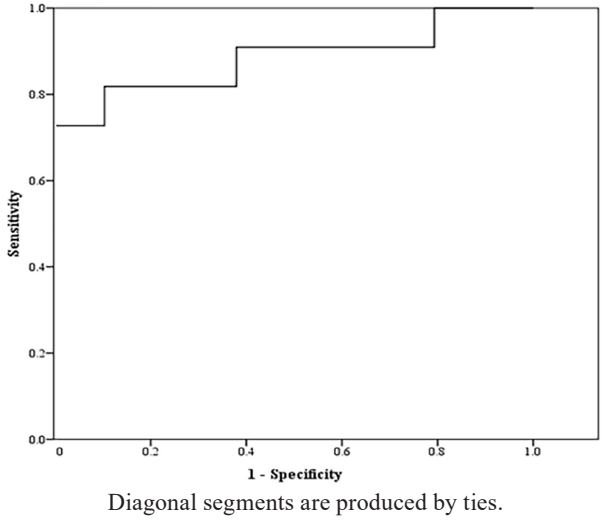


Figure 5: Negative correlation between IL-10 and platelets count



Diagonal segments are produced by ties.

Figure 6: ROC curve for detection of IL-10 as a sensitive predictor for ZAP-70 positivity

prognosis was performed. They reported a mean plasma IL-10 level of 26.152 pg/mL (0–100 pg/mL).[11]

In the current study, serum IL-10 was found to be higher among patients with advanced stage, similar to that reported by Egle and colleagues who observed that IL-10 levels were significantly higher in patients with Rai stages III and IV.[23] Kamper and co-workers reported that IL-10 was detected in all B-CLL patients, being higher (p:0.0153) in C than in A Binet stage patients, (10.76 [9.03–14.24] pg/mL vs. 7.15 [5.35–9.89] pg/mL, respectively.[24]

Serum IL-10 is a sensitive marker in predicting ZAP-70 positivity among CLL patients since the ROC curve for IL-10 showed higher significant AUC (Figure 6). So, the use of this simple marker by ELISA may be a useful surrogate marker for ZAP-70 positive expression which in turns is proved to be a substitute for immunoglobulin heavy chain variable region gene mutation.

In this study, the level of serum IL-10 in B-CLL patients showed significant association with disease progression. Advanced stages (Binet C and Rai III-IV) as well as patients with high risk prognostic indexes showed significantly higher mean IL-10 levels comparing to those with early stage disease or low and intermediate risk prognostic indexes.

The finding of higher IL-10 among B-CLL patients with advanced disease was observed, where IL-10 could enhance the survival of B-CLL cells in a dose-dependent fashion by inhibiting the process of apoptotic cell death. They observed also that B-CLL cells spontaneously release IL-10 in cultures, and serum IL-10 levels were elevated in five of the eleven B-CLL patients investigated.[25] An above mentioned study reported that plasma IL-10 was positively correlated with Rai stage (P=0.01) and significantly related to survival distributions for all parameters.[11] Egle et al. reported that serum IL-10 levels were significantly higher in patients with Rai stages III and IV.[23] In another study, IL-10 (viral and human) levels were elevated and correlated with adverse disease features and short survival.[26]

Serum IL-10 levels correlated with unfavorable phenotypic features of B-CLL such as advanced Binet and Rai stages, and high risk CLL prognostic indexes. It showed significant negative correlation with the hemoglobin and platelets counts. This adds to the unfavorable features of the disease. This is similar to that was reported in other studies.[11, 26]

Conclusion

Serum IL-10 concentration in patients with CLL could be used as a surrogate marker for disease progression. We recommend its measurement in low resource areas where flowcytometry is not available. It could also be used to substitute ZAP-70 as a marker for prognostic implications.

Compliance with ethical standards

Funding: this study was fully funded by the authors only.

- Author identifying information: are present on the title page that is separate from the manuscript.
- Conflict of interest: all authors declare that they have no conflict of interest.
- Ethical approval: all procedures performed in

this study involved CLL patients and the control group were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

- Informed consent: an informed written consent was obtained from all individual participants (patients and control) included in this study.

Conflict of Interest: None declared.

References

1. Nabhan C, Rosen ST. Chronic lymphocytic leukemia: a clinical review. JAMA. 2014;312(21):2265–76. doi: 10.1001/jama.2014.14553. PubMed PMID: 25461996.
2. Catovsky D, Wade R, Else M. The clinical significance of patients' sex in chronic lymphocytic leukemia. Haematologica. 2014;99(6):1088-94. doi: 10.3324/haematol.2013.101378.
3. Rozman C, Montserrat E. Current concepts: chronic lymphocytic leukemia. N Engl J Med. 1995;333:1052-7.
4. Zent CS, Kyasa MJ, Evans R, Schichman SA. Chronic lymphocytic leukemia incidence is substantially higher than estimated from tumor registry data. Cancer. 2001; 92: 1325-30. doi: 10.1002/1097-0142(20010901)92:5<1325::aid-cncr1454>3.0.co;2-e.
5. Redaelli A, Laskin BL, Stephens JM, Botteman MF, Pashos CL. The clinical and epidemiological burden of chronic lymphocytic leukaemia. Euro J Cancer Care 2004: 13; 279–87. doi: 10.1111/j.1365-2354.2004.00489.x.
6. Albakr RB, Khojah OT. Incidence trend of the leukemia reported cases in the Kingdom of Saudi Arabia, observational descriptive statistic from Saudi Cancer Registry. Inter J Biomed Res. 2014;5(8):522-4.
7. Al-Bahar S, Pandita R, Al-Muhannaha A, Al-Bahar E. The epidemiology of leukemia in Kuwait. Leuk Res. 1994;18(4):251-5. doi: 10.1016/0145-2126(94)90027-2.
8. Tarawneh M, Nimri O, Arkoob K, AL-Zaghal M. Cancer Incidence in Jordan, 2010. Jordan Cancer Registry. Ministry of Health: Amman; 2010.
9. Ibrahim AS, Mikhail NNH. Egypt National Cancer Registry: Aswan Profile-2008, Publication Number RR1. National Cancer Registry Program of Egypt: 2010.
10. Ibrahim AS, Mikhail NNH, Darwesh H, Heikel T. Egypt National Cancer Registry: Damietta Profile – 2009, Publication Number RR2. National Cancer Registry Program of Egypt: 2011.
11. Kara IO, Sahin B, Gunesacar R. Expression of soluble CD27 and interleukins-8 and-10 in B-cell chronic lymphocytic leukemia: correlation with disease stage and prognosis. Adv Ther. 2007; 24(1): 29-40. doi: 10.1007/BF02849990. PubMed PMID: 17526459.
12. Rousset F, Garcia E, Defrance T, Péronne C, Vezzio N, Hsu DH, et al. IL-10 is a potent growth factor for activated human B lymphocytes. Proc Natl Acad Sci USA. 1992;898:1890-3. doi: 10.1073/pnas.89.5.1890. PubMed PMID: 1371884.
13. Nemunaitis J, Fong T, Shabe P, Martineau D, Ando D. Comparison of serum interleukin-10 (IL-10) levels between normal volunteers and patients with advanced melanoma. Cancer Invest. 2001;19(3):239-47. doi: 10.1081/cnv-100102550.
14. Mustea A, Konsgen D, Braicu EI, Pirvulescu C, Sun P, Sofroni D, et al. Expression of IL-10 in patients with ovarian carcinoma. Anticancer Res. 2006;26(2c):1715-8.
15. Khatri VP, Caligiuri MA. A review of the association between interleukin-10 and human B-cell malignancies. Cancer Immunol Immunother. 1998;46(5):239-44. doi: 10.1007/s002620050483.
16. Otsuki T, Yamada O, Yata K, Sakaguchi H, Kurebayashi J, Yawata Y, et al. Expression and production of interleukin 10 in human myeloma cell lines. Br J Haematol. 2000;111(3):835-42. PubMed PMID: 11122145.
17. Wierda WG, O'Brien S, Wang X, Faderl S, Ferrajoli A, Do KA, et al. Prognostic nomogram and index for overall survival in previously untreated patients with chronic lymphocytic leukemia. Blood. 2007;109(11):4679–85. doi: 10.1182/blood-2005-12-051458.
18. Gentile M, Mauro FR, Rossi D, Vincelli I, Tripepi G, Recchia AG, et al. Italian external and multicentric validation of the MD Anderson Cancer Center nomogram and prognostic index for chronic lymphocytic leukaemia patients: analysis of 1502 cases. Br J Haematol. 2014;167(2):224–32. doi: 10.1111/bjh.13032.
19. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol. 2001;19:683-765.
20. Garaud S, Morva A, Lemoine S, Hillion S, Bordron A, Pers JO, et al. CD5 promotes IL-10 production in chronic lymphocytic leukemia B cells through STAT3 and NFAT2 activation. J Immunol. 2011;186(8):4835-44.
21. Sjoberg J, Aguilar-Santelises M, Sjogren AM, Pisa EK, Ljungdahl A, Bjorkholm M, et al. Interleukin-10 mRNA expression in B-cell chronic lymphocytic leukaemia inversely correlates with progression of disease. Br J Haematol. 1996;92(2):393-400. doi: 10.1046/j.1365-2141.1996.00358.x.
22. Jurlander J, Lai CF, Tan J, Chou CC, Geisler CH, Schriber J, et al. Characterization of interleukin-10 receptor expression on B-cell chronic lymphocytic leukemia cells. Blood. 1997;89(11):4146-52. PubMed PMID: 9166857.
23. Egle A, Marschitz I, Posch B, Herold M, Greil R. IL-10 serum levels in B-cell chronic lymphocytic leukaemia. Br J Haematol. 1996;94(1):211-2.
24. Kamper EF, Papaphilis AD, Angelopoulou MK, Kopeikina LT, Siakantaris MP, Pangalis GA, et al. Serum levels of tetranectin, intercellular adhesion molecule-1 and interleukin-10 in B-chronic lymphocytic leukemia. Clin Biochem. 1999;32(8):639-45.

25. Kitabayashi A, Hirokawa M, Miura AB. The role of interleukin-10 (IL-10) in chronic B-lymphocytic leukemia: IL-10 prevents leukemic cells from apoptotic cell death. Int J Hematol. 1995;62(2):99-106. doi: 10.1016/0925-5710(95)00395-9. PubMed PMID: 8590779.

26. Fayad L, Keating MJ, Reuben JM, O'Brien S, Lee BN, Lerner S, et al. Interleukin-6 and interleukin-10 levels in chronic lymphocytic leukemia: correlation with phenotypic characteristics and outcome. Blood. 2001;97(1):256-63. doi: 10.1182/blood.v97.1.256. PubMed PMID: 11133769.