Nature and Science

Websites:
http://www.sciencepub.net/nature
http://www.sciencepub.net

Emails:
naturesciencej@gmail.com
editor@sciencepub.net

MARSLAND PRESS
Multidisciplinary Academic Journal Publisher

Study of CD305, CD26 Expression in Chronic Lymphocytic Leukemia

Heba M. El-Erian[1], Mohamed K. Zahrah[1], Sahar Hazzaa[1], Atef M. Taha[2]

[1]Clinical Pathology Department, Faculty of Medicine, Tanta University, Tanta, Egypt
[2]Internal Medicine Department, Faculty of Medicine, Tanta University, Tanta, Egypt
heba.alaryan@yahoo.com

Abstract: Background: B-CLL is a heterogeneous disease with a highly variable clinical course and prognosis. Rai and Binet staging systems have been recognized as standard methods of assessing the survival and the treatment requirements in B-CLL patients. There is a need to seek out other prognostic factors in the early stage of the disease to identify stable or progressive forms of CLL that might facilitate risk-adapted treatment strategies. CD26 is a multifunctional type II cell surface glycoprotein that is widely expressed by many cells. LAIR1 (CD305) is a transmembrane glycoprotein acting as inhibitory receptor has been recently demonstrated in patients with CLL. **Aim of the Work:** Studying the role of CD305, CD26 expression in chronic lymphocytic leukemia and their correlation with CD38 and ZAP 70 expression. **Subjects and Methods:** This study was conducted on 50 newly diagnosed CLL cases from Internal Medicine Department, Tanta University Hospital and 20 healthy subjects as a control group. **Results:** Higher expression of ZAP 70, CD38, CD26, lower expression of CD305 is associated with more advanced staging. A negative correlation between CD305 expression and TLC, ALC, LDH, CD26, CD38 and ZAP 70 expression and a positive correlation with Hb level and platelet count. Where, positive correlation between CD26 expression and previous parameters and a negative correlation with Hb level, platelet count and CD305 expression. There was a significant longer overall survival and disease free survival in patient with high CD 305 expression and also in low CD 26 expression. **Conclusions:** CD26 and CD305 together with CD38 and ZAP 70 represent an important adverse prognostic markers, their expression should be routinely investigated for a better prognostic assessment of CLL patients and showed be taken in consideration in designing further therapeutic strategies based on patient specific risk factors.

[Heba M. El-Erian, Mohamed K. Zahrah, Sahar Hazzaa, Atef M. Tah. **Study of CD305, CD26 Expression in Chronic Lymphocytic Leukemia.** *Nat Sci* 2019;17(12):251-258]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 31. doi:10.7537/marsnsj171219.31.

Key words: CD305 Expression, CD26 Expression, Chronic Lymphocytic Leukemia.

1. Introduction:

Chronic lymphocytic leukemia (CLL) is the most common leukemia in the Western world, characterized by progressive accumulation of clonal B-lymphocytes coexpressing CD5 and CD19 with variable lymphadenopathy and splenomegaly and eventual bone marrow failure evidenced by anemia and thrombocytopenia **[1].**

The pathogenesis of B-CLL remains largely unknown, it may be due to defective apoptosis, genetic aberrations or cytokines **[2].**

It is more common in males mostly after the age of 60 years. Disease progress is quiet variable according to a number of factors. Life expectancy may be greater than 10 years in most patients with early stage; however patients with more advanced stage have a median survival of 18 months to 3 years. The Rai and the Binet systems are two commonly accepted staging methods in CLL **[3].**

The prognosis is related to particular biological parameters such as cytogenetic abnormalities, mutational status of IgVH, expression of ZAP70 and CD38, serum markers CD23, thymidine kinase and beta2-microglobulin (B2M), bone marrow examination and lymphocyte doubling time. Although those markers may help to predict the prognosis, the application of these tests is not suggested to be used in routine practice for the treatment. There are discrepancies between the usages of those molecular markers and their impact on the prognosis of the disease **[4].**

The most important prognostic factors in CLL have been reported as clinical stage, tumor load markers, both CD38 and ZAP70, and genetic parameters. Cellular proteins ZAP70 and CD38 have been found to be closely related to the prognosis of CLL **[5].**

CD26 is a multifunctional type II cell surface glycoprotein that is widely expressed by T lymphocytes, natural killer (NK), epithelial, endothelial and acinar cells of many tissues. CD26 expression is very low in B-cells; however, it is

upregulated following in vitro activation. CD26 has been suggested to play a role in the pathogenesis and progression of many types of tumors. Also, it may play a role in preventing apoptosis and metastasis as a result of its tumors ability to bind extracellular matrix proteins **[6].**

LAIR1 (Leukocyte-Associated Immunoglobulin-like Receptor-1), also known as CD305, is a transmembrane glycoprotein acting as inhibitory receptor, which is expressed by most immune cells. The known LAIR1 ligands are the extracellular matrix collagen and C1q, the first component of the complement. LAIR1 expression varies during B-cell differentiation and has been recently demonstrated in patients with CLL. In B-cells, the in-vivo role of LAIR1 consists in inhibiting B cell receptor (BCR)-mediated signaling and in controlling kinase pathways involved in cell proliferation **[7].**

2. Subjects and Methods:

The present study was performed on 50 newly diagnosed cases of B-CLL (32 males and 18 females) selected from Internal Medicine Department of Tanta University Hospital and 20 healthy persons as a control group. Informed consent was obtained from every patient for laboratory studies according to the guidelines of Committee of Medical Ethics of Tanta University Hospitals. This study was approved by the Committee of Human Research at Tanta University, Egypt. CLL diagnosis was based on NCI Working Group criteria and confirmed by a flowcytometry score >3. Patients were selected to be newly diagnosed cases before receiving any treatment.

Patients were subjected to the following laboratory tests:

Complete blood picture with examination of peripheral blood smears, immunophenotyping of all cases by flowcytometry using chronic lymphocytic panel, Lactate dehydrogenase enzyme (LDH), Erythrocyte sedimentation rate (ESR), Flowcytometry estimation of CD305, CD26, CD38 and ZAP 70 expression on peripheral blood samples for all cases.

Statistical Analysis

All of the statistical calculations were made using excel program and SPSS V.22. (Statistical package for social science) program. Qualitative data were presented as frequency and percentage. Statistical analysis of the present study was conducted using the mean value, standard deviation [SD], chi-square test, Standard student "t test", Analysis of variance [ANOVA] tests (f), Chi-square test, Linear Correlation Coefficient [r], ROC-curve, Kaplan-Meier test survival analysis. The best cutoff points for CD26, CD305 was done by (ROC) curves receiver operating characteristic. Regarding CD26 was 10% and CD305 was 30%. The cutoff point for ZAP 70 was ≥30% and for CD38 was ≥30%.

3. Results:

CLL was more prevalent in male gender and with advanced age. Lymphadenopathy was present in 86% of CLL patients, Splenomegaly was present in 60%of CLL patients, and Hepatomegaly was present in 38% of CLL patients.

Laboratory findings in the studied groups:

The disease was associated with increased TLC, ALC, lymphocyte percentage, LDH and ESR levels and decreased Hb and platelets (Table 1).

Table (1): Laboratory findings in the studied groups.

		Range	Mean ± S. D	t. test	p. value
Hb (g/dl)	Patient	5 – 13.5	10.31 ± 2.10	26.999	0.001*
	Control	11.5 – 15	12.85 ± 0.92		
PLT (x10^9/L)	Patient	50 – 348	146.12 ± 66.07	48.297	0.001*
	Control	185 – 390	267.80 ± 66.45		
TLC (x10^9/L)	Patient	15.8 – 320	76.74 ± 66.95	21.546	0.001*
	Control	4.1 – 10	6.94 ± 1.74		
Lymphocytes %	Patient	60 – 95	82.20 ± 9.47	387.259	0.001*
	Control	33 – 50	38.75 ± 4.25		
ALC (x10^9/L)	Patient	11.85 – 240	57.55 ± 50.21	21.546	0.001*
	Control	3.08 – 7.50	5.20 ± 1.30		
LDH (IU/L)	Patient	240 – 986	635.80 ± 223.96	42.584	0.001*
	Control	230 – 410	304.45 ± 49.64		
ESR (mm/hr)	Patient	10 – 58	32.04 ± 16.02	45.695	0.001*
	Control	4 – 10	7.65 ± 1.87		

CD 305 and CD 26 expression in the studied groups:

CD305 expression was significantly decreased in CLL patients as compared to control group and the expression of CD26 was significantly increased in CLL patients as compared to control group (Table 2).

Table (2): CD 305 (LAIR-1), CD 26 expression in the studied groups

		Range	Mean ± S. D	t. test	p. value
CD 305%	Patient	6.4 – 99	49.67 ± 38.50	12.845	0.001*
	Control	30.5 – 99	82.23 ± 19.98		
CD 26%	Patient	4.5 – 94.6	54.01 ± 36.81	33.687	0.001*
	Control	2.8 – 10.3	5.99 ± 2.14		

Relation between clinical data in CLL patients with the studied markers (CD305, CD26, CD38, ZAP 70 expression):

In this study, there was a significant positive association between ZAP 70 expression and degree of lymphadenopathy and significant negative association between CD305expression and degree of lymphadenopathy**,** where there was no significant association between CD26, CD38 expression and degree of lymphadenopathy. Also, there was a significant positive association between CD26, CD38, ZAP 70 expression and degree of splenomegaly and significant negative association between CD305 expression and degree of splenomegaly. While, there was a significant positive association between CD26, CD38, ZAP 70 expression and degree of hepatomegaly and significant negative association between CD305 expression and degree of hepatomegaly (Table 3). There was significant positive association between CD26, CD38, ZAP 70 expression and degree of Rai staging where there was significant negative association between CD305 expression and degree of Rai staging. High expression of ZAP 70, CD38, CD26, lower expression of CD305 are associated with more advanced staging (Table 4).

Table (3): Relation between clinical data and CD305, CD26, CD38, ZAP 70 expression.

			Range	Mean ± S. D	t. test	p. value
Lymphadenopathy	**ZAP 70**	Present	0.6 – 99.6	56.98 ± 38.56	4.598	0.037*
		Absent	1.8 – 99	23.71 ± 34.32		
	CD 38	Present	4.1 – 97.2	48.82 ± 28.32	2.385	0.129
		Absent	10 – 62.5	31.47 ± 21.45		
	CD 305	Present	6.4 – 99	44.34 ± 37.23	6.552	0.014*
		Absent	13 – 98.1	82.40 ± 30.80		
	CD 26	Present	4.5 – 94.6	54.96 ± 36.05	0.204	0.653
		Absent	4.8 – 94.6	48.13 ± 43.85		
Splenomegaly	**ZAP 70**	Present	9.9 – 99.6	75.07 ± 30.87	49.828	0.001*
		Absent	0.6 – 75	18.20 ± 22.64		
	CD 38	Present	4.1 – 97.2	59.47 ± 24.73	24.217	0.001*
		Absent	8.2 – 79.8	26.77 ± 20.13		
	CD 305	Present	6.4 – 99	29.46 ± 29.26	34.981	0.001*
		Absent	11.2 – 98.5	79.97 ± 30.06		
	CD 26	Present	5.6 – 94.6	67.32 ± 30.69	12.025	0.001*
		Absent	4.5 – 94.6	34.03 ± 36.84		
Hepatomegaly	**ZAP 70**	Present	11.7 – 99.6	72.44 ± 28.34	9.338	0.004*
		Absent	0.6 – 99	39.99 ± 40.55		
	CD 38	Present	5.3 – 97.2	57.73 ± 22.96	5.513	0.023*
		Absent	4.1 – 92.8	39.44 ± 28.77		
	CD 305	Present	6.4 – 98	28.02 ± 28.45	11.829	0.001*
		Absent	10.3 – 99	62.93 ± 38.16		
	CD 26	Present	7.3 – 94.4	69.65 ± 28.12	6.110	0.017*
		Absent	4.5 – 94.6	44.42 ± 38.59		

Table (4): Relation between Rai staging system and CD305, CD26, CD38, ZAP 70 expression.

Rai		Range			Mean	±	S. D	F. test	p. value
ZAP 70	I	0.6	–	23.1	9.79	±	8.58	55.374	0.001*
	II	1.3	–	25.4	9.02	±	7.51		
	III	2.6	–	99	67.49	±	29.50		
	IV	46.7	–	99.6	87.81	±	16.03		
CD 38	I	8.2	–	25.2	16.91	±	6.92	47.243	0.001*
	II	4.1	–	21.3	15.28	±	6.83		
	III	27	–	90	59.86	±	16.01		
	IV	23.4	–	97.2	69.21	±	18.10		
CD 305	I	77.9	–	98.3	91.48	±	8.11	55.125	0.001*
	II	86	–	98.5	93.81	±	4.31		
	III	11.2	–	90.5	24.17	±	25.54		
	IV	6.4	–	99	22.56	±	20.36		
CD 26	I	4.5	–	91.2	15.90	±	28.26	16.705	0.001*
	II	5.1	–	76.7	24.81	±	28.66		
	III	10	–	94.6	75.46	±	25.03		
	IV	5.6	–	94.4	73.69	±	24.81		

Correlation between laboratory data in CLL patients with the studied markers (CD305, CD26, CD38, ZAP 70 expressions):

There was a significant negative correlation between CD305 expression and TLC, ALC, LDH, CD26 expression, CD38 expression and ZAP 70 expression and a significant positive correlation with Hb level and platelet count. There was a significant positive correlation between CD26 expression and TLC, ALC, LDH, CD38 expression and ZAP 70 expression and significant negative correlation with Hb level, platelet count and CD305 expression. There was a significant positive correlation between ZAP 70 expression and TLC, ALC, LDH, CD38 expression and CD26 expression and significant negative correlation between Hb, platelet count and CD305 expression. There was a significant positive correlation between CD38 expression and TLC, ALC, LDH, ZAP 70 expression and CD26 expression and significant negative correlation between Hb, platelet count and CD305 expression. There was no significant correlation between CD305, CD26, CD38, ZAP 70 expression and ESR level (Table 5).

Table (5): Correlation between CD305, CD26, CD38, ZAP 70 expression and studied markers

	ZAP 70		CD 38		CD 305		CD 26	
	r.	p	r.	p	r.	p	r.	P
ZAP 70								
CD 38	0.812	0.001*						
CD 305	- 0.842	0.001*	- 0.809	0.001*				
CD 26	0.644	0.001*	0.632	0.001*	- 0.668	0.001*		
Hb (g/dl)	- 0.722	0.001*	- 0.746	0.001*	0.635	0.001*	- 0.559	0.001*
PLT ($x10^9$/L)	- 0.623	0.001*	- 0.508	0.001*	0.528	0.001*	- 0.477	0.001*
TLC ($x10^9$/L)	0.522	0.001*	0.406	0.001*	- 0.447	0.001*	0.386	0.006*
ALC ($x10^9$/L)	0.514	0.001*	0.571	0.001*	- 0.539	0.001*	0.427	0.002*
LDH (IU/L)	0.740	0.001*	0.728	0.001*	- 0.759	0.001*	0.646	0.001*
ESR (mm/hr)	0.104	0.254	0.205	0.147	0.195	0.187	0.297	0.109

Clinical outcome of CLL patients:

Our study yielded that there was a longer overall survival and disease free survival in patient with high CD305 expression than those with low CD305 expression (Figure 1, 2). Also, there was a longer overall survival and disease free survival in patient with low CD26 expression than those with high CD26 expression (Figure 3, 4).

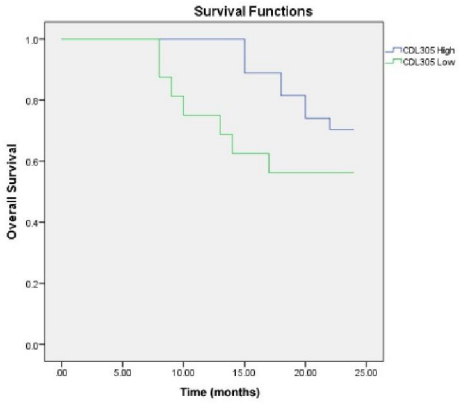

Figure (1): Kaplan-Meier survival curve for overall survival in patient with high and low CD 305 expression.

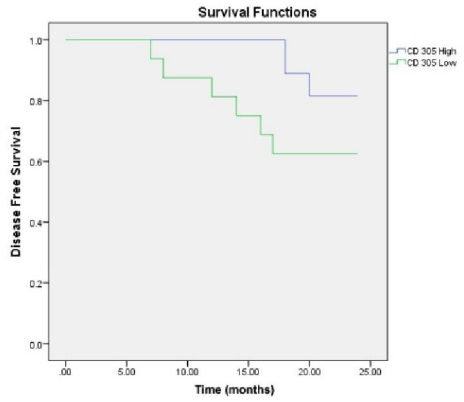

Figure (2): Kaplan-Meier survival curve for disease free survival in patient with high and low CD 305 expression.

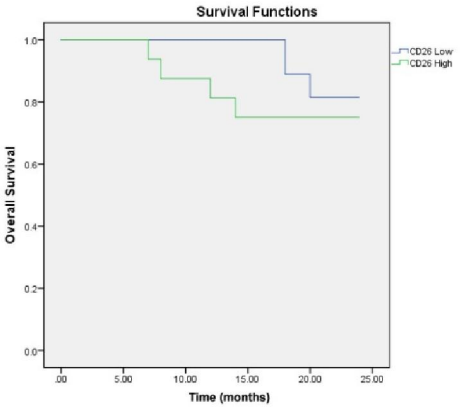

Figure (3): Kaplan-Meier survival curve for overall survival in patient with high and low CD 26 expression.

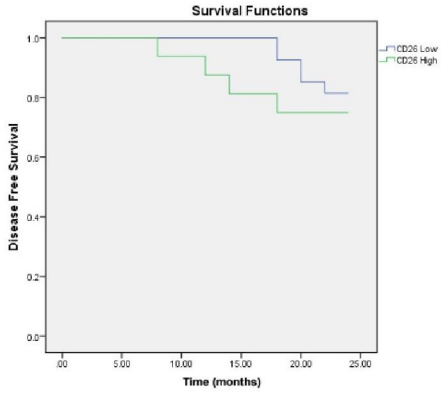

Figure (4): Kaplan-Meier survival curve for disease free survival in patient with high and low CD 26 expression.

4. Discussion:

This study revealed the presence of lymphadenopathy and hepatosplenomegaly in most CLL patients as compared to control group and this was in agreement with **Weirda el al., [8].** Regarding to Rai staging system, most patients were included in high risk group (III, IV) with lymphocytosis together with anemia or thrombocytopenia and heptospleenomegaly this was in contrast with **Wu et al., [9].** Also **Sagatys** and **Zhang, [10]** stated that 80% of newly diagnosed CLL cases were at low risk according to modified Rai staging system.

In the current study, Hb level showed a significant decrease in most CLL patients compared to control group. This finding was in consistent with the results of **Sagatys** and **Zhang, [10].** Regarding platelets, there was a significant decrease in platelets count in CLL patients compared to control group in accordance with **Parker et al., [11].** Total leukocytic count showed significant increase in CLL patients as compared to control group. In agreement with these results **Palumbo et al., [12]** who reported leukocytosis in CLL patients. In addition, a significant increase peripheral lymphocytic count in CLL patients compared to control group which was in accordance with **Perry et al., [13].**

In the current study, serum LDH levels showed significant increase in CLL patients compared to control group. This was in agreement with **Rossi et al., [14]** and **Kipps, [15]** who stated that LDH is one of the most common serum markers increased in CLL. They found that high levels of serum LDH were associated poor outcome and increased risk to develop Richter syndrome. Also increase in ESR levels in CLL patients compared to the control group. It is well known that ESR levels are not specific tool for disease activity. This was in agreement with **Palumbo et al., [12].**

High expression of CD26 was significantly associated with splenomegaly. This was in agreement with **Ibrahim et al., [6]** who reported that higher expression of CD26 was significantly associated with splenomegaly and more advanced Binet and Rai stages of CLL. Also, there was a significant relation between

increased CD26 expression and clinical stage according to Rai (0, I, II, III, IV). This finding was in consistent with the results published by **Ibrahim et al., [6]** and **Matuszak et al., [16].** Increase CD26 expression was found to be significantly correlated with elevated serum LDH activity. These results in accordance with those reported by **Ibrahim et al., [6]**, **Ghannam et al., [17]** and **Matuszak et al., [16].**

In the present study there was a significant relation between increased CD26 expression and ALC. This is in agreement with **Molica et al., [18]** and **Matuszak et al., [16].** As it well known that ALC was an important parameter in diagnosis and follow up. High expression of CD26 was significantly associated significant decrease in platelets count, significant decrease in HB concentration. These findings were in consistent with the results published by **Ibrahim et al., [6].** In addition, high CD26 expression showed significantly higher incidence of hepatomegaly, high total leucocytic count and lower platelets count, high absolute lymphocytic count. This finding was in agreement with **Ghannam et al., [17].**

The present study found a significant correlation between CD26 expression and CD38 expression, CD26 expression and ZAP70 expression. These results in accordance with those reported by **Ghannam et al., [17]** and **Ibrahim et al., [6].** However, **Molica et al., [18]** found a borderline significance for ZAP-70. In contrast to **Matuszak et al., [16]** who stated that there was no correlation between CD26 expression and CD38 expression.

In the present study, high CD305 expression is significantly associated with early stages of CLL (I, II) while low LAIR1 expression is significantly associated more advanced stages (III, IV). This was in agreement with **Rawstron, [19]** and **Sales et al., [20].** There was a significant negative correlation between CD305 expression and CD38 as reported by **Poggi et al., [21]** and **Perbellini et al., [7].** This study found a significant negative correlation between CD305 expression and ZAP 70 expression as reported by **Poggi et al., [21].**

In this study, there was a highly significant difference in the expression of ZAP 70 between patients and control group. This was as reported by **Rassenti et al., [22].** Higher ZAP-70 expression was significantly correlated with more advanced Rai stages, with lymphadenopathies, splenomegaly. These results were in accordance with those reported by **Del Principe et al., [23]** and **Hus et al., [24].** Moreover, in the present study, there was positive correlation between CD38 expression and ZAP70 expression as reported by **Del Principe et al., [23].** High CD38 expression was significantly correlated with advanced Rai stages, lymphadenopathy and splenomegaly. These results were in accordance with those reported by **Hus et al., [24]** and **Del Principe et al., [23].** Also we found that higher CD38 expression was significantly correlated with total leucocytic count. This was in agreement with **Hus et al., [24].**

In this work there was a significant longer survival in patients who had higher CD 305 expression than those who had lower CD 305 expression and a significant longer disease free survival was observed in patients with CD 305 high expression than patients with CD 305 low expression. This was in agreement with **Perbellini et al., [7].** So, It was considered that LAIR1 is an easily applicable and inexpensive marker to predict time to first treatment and disease free survival in patients presenting with CLL. Also, **Sales et al., [20] and Benedetti et al., [25]** said that a high expression has been associated with low-risk disease and with a longer time to first treatment and predicts longer overall survival in CLL. This is in agreement with **Zucchetto et al., [26]** who found that the concomitant high expression of the B-Cell Receptor signaling inhibitor molecules CD150, CD305, and CD307b predicts longer overall survival and longer time to first treatment in CLL.

In the present study there was a significant longer overall survival in patients with low CD 26 expression than patients with high CD 26 expression and a significant longer disease free survival in patients with low CD 26 expression than patients with high CD 26 expression. These results were in accordance with those reported by **Ibrahim et al., [6]** who found that patients with high CD26 expression had significantly shorter time to develop lymphocyte doubling versus those with low CD26 expression. Patients with high CD26 expression had significantly shorter progression-free survival versus those with low CD26 expression. Overall survival showed no significant differences between high and low CD26 groups.

Conclusion:

From this study, it could be concluded that, CD26 expression, CD305 expression together with CD38 and ZAP 70 represent an important adverse prognostic marker and therefore its expression should be routinely investigated for a better prognostic assessment of CLL patients at diagnosis and showed be taken in consideration in designing further therapeutic strategies based on patient specific risk factors.

Acknowledgements

I thank Prof. Dr. Mohamed Kamal Zahrah, Prof. Dr. Sahar Mohy EL-Din Hazzaa and Prof. Dr. Atef Mohamed Tahafor valuable suggestions and continuous encouragement.

Author Disclosure

None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

References:

1. Wierz M, Pierson S, Chouha N, Désaubry L, François JH, Guy Berchem J, Paggetti, Moussay E. The Prohibitin-Binding Compound Fluorizoline Induces Apoptosis In Chronic Lymphocytic Leukemia Cells Ex Vivo But Fails To Prevent Leukemia Development In A Murine Model. Haematologica 2018; 103: e154-157.
2. Adams RLC, Cheung C, Banh R, Saal R, Cross D, Devinder Gill D, Self M, Klein K, Peter Mollee P: Prognostic Value of ZAP-70 Expression in Chronic Lymphocytic Leukemia as Assessed by Quantitative Polymerase Chain Reaction and Flow Cytometry, Cytometry Part B (Clinical Cytometry) 2014; 86B:80–90.
3. Inamdar KV, Bueso-Ramos CE. Pathology of chronic lymphocytic leukemia: an update. Annals of Diagnostic Pathology, 2007; 11: 363-389.
4. Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Döhner H, Hillmen P, Keating MJ, Montserrat E, Rai KR, Kipps TJ. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood 2008; 111: 5446 – 5456.
5. Vural F, Karaca E, Soyer N, Gunduz C, Sahin F, et al. Comparison of CD38, ZAP70 and hTERT expression with known prognostic markers in patients with chronic lymphocytic leukemia during five-year follow-up period. International Journal of Hematology and Oncology 2014; 24: 179-184.
6. Ibrahim L, Elderiny WE, Elhelw L, Ismail M. CD49d and CD26 are Independent Prognostic Markers for Disease Progression in Patients with Chronic Lymphocytic Leukemia. J Leuk 2014; 3: 173-180.
7. Perbellini O, Falisi E, Giaretta I, Boscaro E, Novella E, Facco M, Fortuna S, Finotto S, Amati E, Maniscalco F, Montaldi A, Alghisi A, Aprili F, Bonaldi L, Paolini R, Scupoli MT, Trentin L, Ambrosetti A, Semenzato G, Pizzolo G, Rodeghiero R, Visco C. Clinical significance of LAIR1 (CD305) as assessed by flow cytometry in a prospective series of patients with chronic lymphocytic leukemia. Heamatologica. 2014; 99 (5): 881-887.
8. Weirda WG, O'Brien S, Wang X, Faderl S, Ferrajoli A, Do KA, Cortes J, Thomas D, Garcia-Manero G, Koller C, Beran M, Giles F, Ravandi F, Lerner S, Kantarjian H, Keating M. Prognostic nomogram and index for overall survival in previously untreated patients with chronic lymphocytic leukemia. Blood 2007; 109(11):4679-4695.
9. Wu T, Li Z, Qiu L. Complications of chronic lymphocytic Leukemia: Analysis of 203 cases in China. Leukemia Research 2010; 34(2):64-65.
10. Sagatys EM, Zhang L. Clinical and Laboratory Prognostic Indicators in Chronic Lymphocytic Leukemia, 2012; 19(1):18-25.
11. Parker TL, Strout MP. Chronic lymphocytic leukemia: Prognostic Factors and Impact on treatment. Discov Med 2011; 11(57):115-123.
12. Palumbo GA, Parrinello N, Fargione G. CD200 expression may help in differential diagnosis between matle cell lymphoma and B-cell chronic lymphocytic leukemia. Leuk Res 2009; 33:1212-1216.
13. Perry C, Hazan-Halevy I, Kay S, Cipok M, Grisaru D, Deutsch V, Polliack A, Naparstek E, Herishanu Y. Increased CD 39 expression on CD4+ T lymphocytes has clinical and prognostic significance in chronic lymphocytic leukemia. Ann Hematol 2012; 91(8):1271-1279.
14. Rossi D, Cerri M, Capello D, Deambrogi C, Rossi FM, Zucchetto A, De Paoli L, Cresta S, Rasi S, Spina V, Franceschetti S, Lunghi M, Vendramin C, Bomben R, Ramponi A, Monga G, Conconi A, Magnani C, Gattei V, Gaidano G. Biological and clinical risk factors of chronic lymphocytic leukaemia transformation to Richter syndrome. Br J Haematol 2008; 142(2): 202 – 215.
15. Kipps T. Chronic lymphocytic leukemia and related diseases. In Williams's Hematology, 7th edition. Beutler E, Lichtman M, Coller B, and Kipps T. (eds). MC Graw-Hill Inc 2006; p:1343.
16. Matuszak M, Lewandowskia K, Czyża A, Kiernicka-Parulskaa J, Przybyłowicz-Chaleckaa A, Jarmuż-Szymczaka M, Lewandowskaa M, Komarnicki M. The prognostic significance of surface dipeptidylpeptidase IV (CD26) expression in B-cell chronic lymphocytic leukemia: Leukemia Research 2016; 47:166–171.
17. Ghannam DME, Taalab MM, Ghazy HF, Salam EMA, Fawzy IM. CD26 Expression in Mature B-cell Neoplasms and its Prognostic Impact on B-Cell Chronic Lymphocytic Leukemia. J Blood Disorders Transf 2014; 5: 222-230.
18. Molica S, Digiesi G, Mirabelli R, Cutrona G, Antenucci A, Molica M, Giannarelli D, Sperduti I, Morabito F, Neri A, Baldini L, Ferrarini M. Serum level of CD26 predicts time to first treatment in early B-chronic lymphocytic

leukemia; European Journal of Haematology 2009; 83:208–214.
19. Rawstron AC, Shingles J, de Tute R, Bennett F, Jack AS, Hillmen P. Chronic lymphocytic leukaemia (CLL) and CLL-type monoclonal B-cell lymphocytosis (MBL) show differential expression of molecules involved in lymphoid tissue homing. Cytometry B ClinCytom. 2010; 78B: S42-S6.
20. Sales MM, Ferreira SIACP, Ikoma MRV, Sandes AF, Beltrame MP, Bacal NS, Silva MCA, Malvezzi M, Lorand-Metze IGH, Orfao A, Yamamoto M. Diagnosis of Chronic Lymphoproliferative Disorders by Flow Cytometry Using Four-Color Combinations for Immunophenotyping: A Proposal of the Brazilian Group of Flow Cytometry (GBCFLUX). Cytometry Part B 2017; 92B: 398–410.
21. Poggi A, Catellani S, Bruzzone A, Caligaris-Cappio F, Gobbi M, Zocchi MR. Lack of the leukocyte-associated Ig-like receptor-1 expression in high-risk chronic lymphocytic leukaemia results in the absence of a negative signal regulating kinase activation and cell division. Leukemia. 2008; 22: 980–988.
22. Rassenti LZ, Jain S, Keating MJ, Wierda WG, Grever MR, Byrd JC, Kay NE, Brown JR, Gribben JG, Neuberg DS, He F, Greaves AW, Rai KR, Kipps TJ. Relative value of ZAP-70, CD38, and immunoglobulin mutation status in predicting aggressive disease in chronic lymphocytic leukemia. Blood 2008; 112: 1923-30.
23. Del Principe MI, Del Poeta G, Buccisano F, Maurillo L, Venditti A, Zucchetto A, Marini R, Niscola P, Consalvo MAI, Mazzone C, Ottaviani L, Panetta P, Bruno A, Bomben R, Suppo G, Degan M, Gattei V, Fabritiis P, Cantonetti M, Coco FL, Del Principe D, Amadori S: Clinical significance of ZAP-70 protein expression in B-cell chroniclymphocytic leukemia. Blood. 2006;108: 853-861.
24. Hus I., Podhorecka M., Bojarska-Junak A., Roliński J., Schmitt M., Sieklucka M., Wąsik-Szczepanek E, Dmoszyńska A. The clinical significance of ZAP-70 and CD38 expression in B-cell chronic lymphocytic leukaemia. Annals of Oncology, 2006; 17: 683–690.
25. Benedetti D, Perini C, Tissino E, Dal Bo M, Bulian P, Bomben R, Rossi F, Degan M, Poggi A, Shabani M, Shokri F, Zaja F, Pozzato G, Chiarenza A, Raimondo FD, Del Poeta G, Gattei V, Zucchetto A. The B-Cell Receptor Signaling Inhibitor Molecules CD305 and CD307b Are Markers of Favorable Prognosis in Chronic Lymphocytic Leukemia with Both Mutated and Unmutated IGHV Gene Status. Amer Soc Hematol Blood 2016; 128(22):4358-4361.
26. Zucchetto A, Benedetti D, Caldana C, Tissino E, Dal Bo M, Bulian P, Bomben R, Rossi F, Zaja F, Pozzato G, Chiarenza A, Di Raimondo F, Del Poeta G, Gattei V. The Concomitant High Expression of the B-Cell Receptor Signaling Inhibitor Molecules CD150, CD305, and CD307b Predicts Longer Overall Survival in the Context of Low-Risk Chronic Lymphocytic Leukemia, Blood 2015; 126:1720-1729.

10/30/2019