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Role of Leukocyte Associated Immunoglobulin Like Receptor-1 (CD305) in Predicting Clinical Variables of Chronic Lymphocytic Leukemia

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ABSTRACT

Background: Chronic lymphocytic leukemia (CLL) is associated with significant immune system disturbance and clinical outcome heterogeneity. Leukocyte associated immunoglobulin like receptor-1 (LAIR-1) expressed by B-cells was mentioned as an immune inhibitory marker of B cell receptor (BCR)-mediated signaling, controlling cell proliferation pathways.

Objectives: The current study aimed to explore the role of LAIR-1 expression in clinical and pathological heterogeneity of CLL.

Subjects and Methods: This case control study was conducted on 50 individuals including 33 newly diagnosed CLL patients categorized according to Rai staging system into Group 1 (n=18) including stage 0 and I and Group 2 (n=15) including stage II, III and IV as well as a control group (n=17). LAIR-1 expression on B cells was evaluated by Flow cytometry.

Results: LAIR-1 in CLL Group 1 (62.88%) was significantly higher than both CLL Group 2 (7.00%) and control group (6.05%); p=0.002 and 0.001 respectively. While LAIR-1 expression by group 2 CLL and control group was comparable (p=0.882).

Percentage expression of LAIR-1 on B cells was inversely correlated to prognostic marker CD38% (r=–0.357, p=0.042). Rai staging was inversely correlated to LAIR-1% and LAIR-1 mean florescent intensity (MFI) (r=–0.517, p=0 and r=–0.355, p=0.043 respectively) which throws light on LAIR-1 role as a prognostic marker.

Conclusion: LAIR-1 expression is associated with other prognostic parameters in CLL. It might be considered another potential prognostic marker.

Key Words: *CLL – LAIR-1 – CD38.*

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is a neoplasm of functionally incompetent mature B-cell lymphocytes. At presentation, 25%-50% of the patients are asymptomatic. Lymphadenopathy is the most common presentation. Splenomegaly, hepatomegaly, pallor, and petechiae are less common signs and symptoms of CLL/SLL. One of the diagnostic criteria for CLL is the presence of absolute lymphocyte count >5 x 10^9/L **[1]**. The CLL cells display an unusual but characteristic pattern of immunophenotypic features. The typical immunological profile of classic CLL is characteristically strongly co-expressing CD5, and CD23, with CD19 while FMC7 and CD79b may be weakly positive **[2]**.

The CLL is the most prevalent type of leukemia in adults that accounts for 30% of adult leukemia **[3,4]**. Despite a generally good prognosis, the diagnosis of CLL carries a significant psychological burden with emotional well-being scores significantly lower than the general population as well as other individuals with cancer **[5]**.

The clinical staging systems developed by Rai have been widely utilized and accepted as prognostic tools with limitations for predicting early-stage disease progression. Patients are classified into low, intermediate, and high-risk groups on the basis of presence of lymphadenopathy, splenomegaly, anemia, and thrombocytopenia **[6,7]**. The majority of CLL patients do not require treatment at presentation and active monitoring is undertaken until there is evidence of progressive marrow failure, massive lymphadenopathy/splenomegaly, or progressive lymphocytosis with a rapid doubling time **[8]**.

The CLL is associated with significant perturbations of the immune system, and heterogeneity of clinical outcome which is accompanied by variable grades of B cell receptor (BCR) anergy measured as low serum immunoglobulin M (sIgM) levels and signaling capacity **[9]**. This is particularly prominent in mutated immunoglobulin variable-region genes, tumor cells express phenotype of activated B cells resembling B cells undergoing antigenic stimulation **[10]**.

Leukocyte-associated immunoglobulin-like receptor-1 (LAIR-1 or CD305) is a member of the Ig superfamily, and is a type I transmembrane glycoprotein. LAIR-1 acts as an inhibitory receptor expressed by most immune cells. The known LAIR-1 ligands are extracellular matrix (ECM) collagen and the first component of the complement (C1q) **[11]**. The LAIR-1 expression varies during B-cell differentiation. The *in-vivo* role of LAIR-1 in B cells lies in inhibiting BCR-mediated signaling and in controlling kinase pathways involved in cell proliferation **[12]**.

In the present study the B cells expressing LAIR-1 in CLL in Egyptian patients were analyzed using flow cytometer, in order to elucidate the biological role of LAIR-1 in CLL patients.

SUBJECTS AND METHODS

This case control study was conducted in the period July 2016 – July 2017. Thirty three patients found to meet the criteria of CLL were recruited from outpatient clinics and inpatient departments of Al-Zahraa Hospital, Al-Azhar University and samples were processed in Clinical Pathology Department at Al-Zahraa Hospital. They included 26 males and 7 females with an age range of 39 to 80, with a mean of 58±10.5 and a median of 57 years. They were categorized according to Rai staging system into Group 1 (n=18) including (stage 0 and I), and Group 2 (n=15) including higher risk patients (stage II, III and IV) **[6]**. Seventeen age and gender matched controls were included in the study. All procedures performed in the study were in accordance with the ethical standards of the review boards of Clinical Pathology department of Al-Zahraa Hospital, National Research Committee and with the 1964 Helsinki declaration and its later amendments. A written informed consent was obtained from all studied subjects. All patients were subjected to complete history taking, clinical examination and laboratory investigations including complete blood count (Sysmex KX21 Hematology analyzer, Kobe, Japan), blood film, and flow cytometry (FACS Calibur, BD Biosciences, San Jose, USA) immunophenotyping using standard CLL panel to confirm diagnosis. Monoclonal antibodies used for diagnosis of CLL were as follows; CD19APC (cat. no. 345791, lot. no. 6281662), CD5PE (cat. no. 555353, lot no. 4248536), CD79bPE (cat. no. 555679. lot no. 6063678), surface IgM (cat. no. 555782, lot no. 5022938) and anti-kappa FITC/lambda PE (cat. no. 349516. lot no. 5086806); all previous were provided by BD Biosciences, San jose, USA). CD23FITC (Cat no. PNIM 529U, lot no. 45) obtained from Immunotech Bechman Coulter, Marsella, France and FMC7 FITC (cat. no. F7110, lot no. 20014619) obtained from DAKO, Denmark were also included. CD200 (Cat. no. 552475, lot no 6126654) obtained from BD Biosciences, San Jose was added to the panel to confirm the diagnosis of CLL and CD38 FITC (Cat. no. 560982, lot no. 5329747) obtained from BD Biosciences, San Jose was added as a prognostic marker.

Inclusion criteria:

Only newly diagnosed patients with score ≥3 according to modified Matutes scoring system were considered as CLL patients **[13]** and included in the study. In CLL cases with score 3 or patients with presence of atypical morphology, CD200 was added to the panel to confirm the diagnosis of CLL (Fig. 1).

Exclusion criteria:

Patients with CLL receiving treatment and patients with B cell neoplasms other than CLL with Matutes score <3.

Immunophenotyping:

Immunophenotyping was performed on ethylene-diamine-tetra-acetic acid (EDTA) peripheral blood specimens obtained before the administration of any treatment. The samples were analyzed within 24 hours of collection.

Flow cytometry was conducted at the Clinical Pathology Department, Al-Zahraa Hospital, AL-Azhar University using four colors FACS Calibur (BD, Biosciences, San Jose, USA). Cell Quest Pro software (BD Biosciences, San Jose, USA) was used for data analysis. Compensation

setting was established before acquiring the samples using color calibrite beads (BD, Biosciences, San Jose, USA, lot no. 5093879). After adjusting the sample count for acquisition, unstained samples were acquired to detect the sample auto-florescence. Isotype controls, mouse IgG1 FITC /IgG2a PE control (cat. no. 342409) and mouse IgG APC control (cat. no. 550931) were obtained from BD, Biosciences, San Jose for detection of non specific binding.

Then samples were analyzed for the expression of LAIR-1 by PE conjugated Ab (BD Biosciences, San Jose, USA. Cat. no. 550811, lot no. 5329747). The cutoff value of positivity of LAIR-1 as suggested by Perbellini et al., **[12]** was 30%. CD38 FITC (BD Biosciences, San jose. Cat. no. 560982, lot no. 5329747) was added for samples of healthy controls and then analyzed. The optimal concentration for each antibody dye used was detected by titration experiments in all diagnostic and research markers.

Gating strategy: Using forward and side scatter (FS/SS), initial gating was performed on lymphocytes area in the dot plot graph; then within the $CD19^+$ B cell population, the subset of cells expressing LAIR-1 was determined as $LAIR\text{-}1^+$ $CD19^+$ B cells and their percentage evaluated on quadrant histogram (Fig. 2). Data were also expressed as mean fluorescence intensity (MFI) of LAIR- 1 using single histogram (Fig. 3).

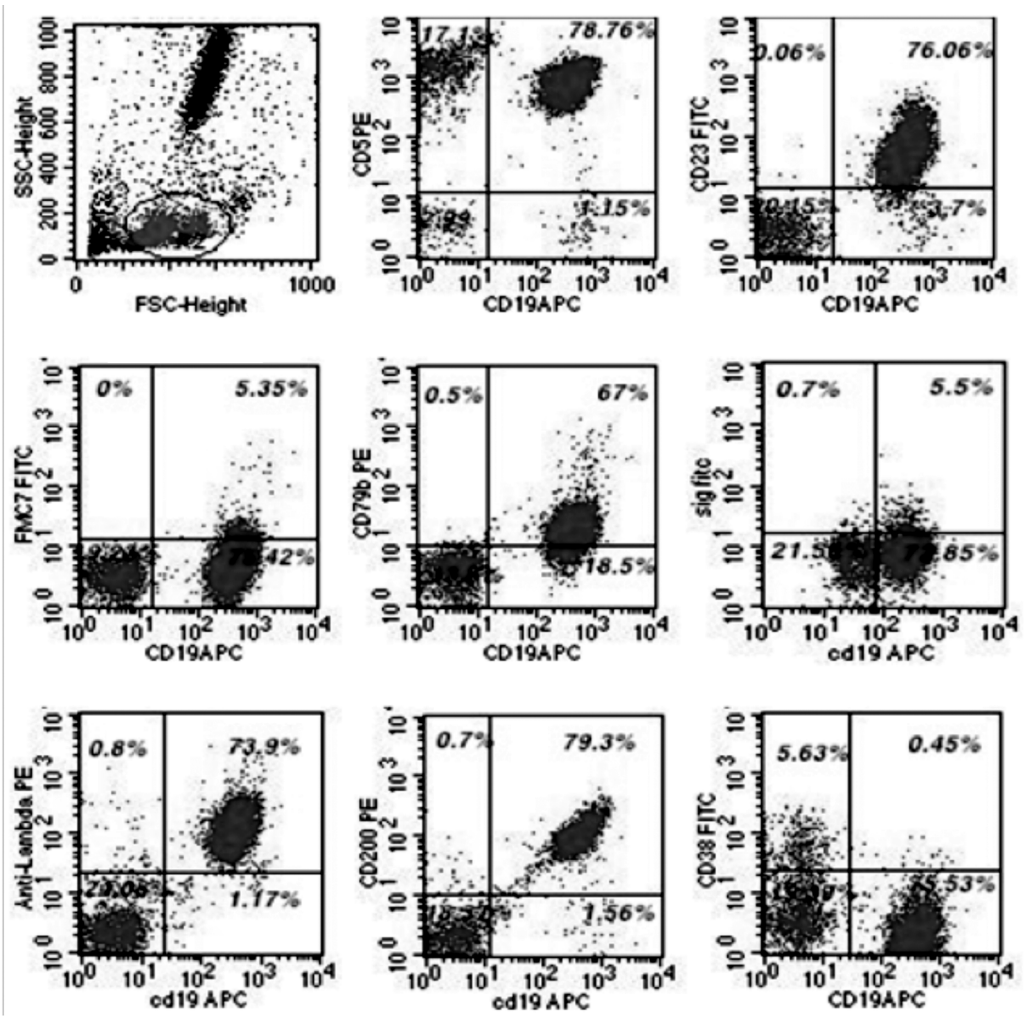

Fig. (1): CLL panel used in the diagnosis according to modified Matutes scoring after initial gating on lymphocyte area on forward scatter/ side scatter (FS/SS). In the given example results showed double positivity for CD5 and CD23, lack of FMC7 expression, dim CD79b expression, lambda restriction (weak expression) and each takes score 1 hence diagnosis of CLL score 5 was reached. CD200 positivity strengthens the diagnosis of CLL, CD 38 (negative in this case) was added for prognostic value.

Statistical analysis:

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 24. Data was summarized using mean, standard deviation, and median in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Mann-Whitney test. For comparing categorical data, Chi square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5. Correlations between quantitative variables were done using Spearman correlation coefficient. *p*-values less than 0.05 were considered statistically significant.

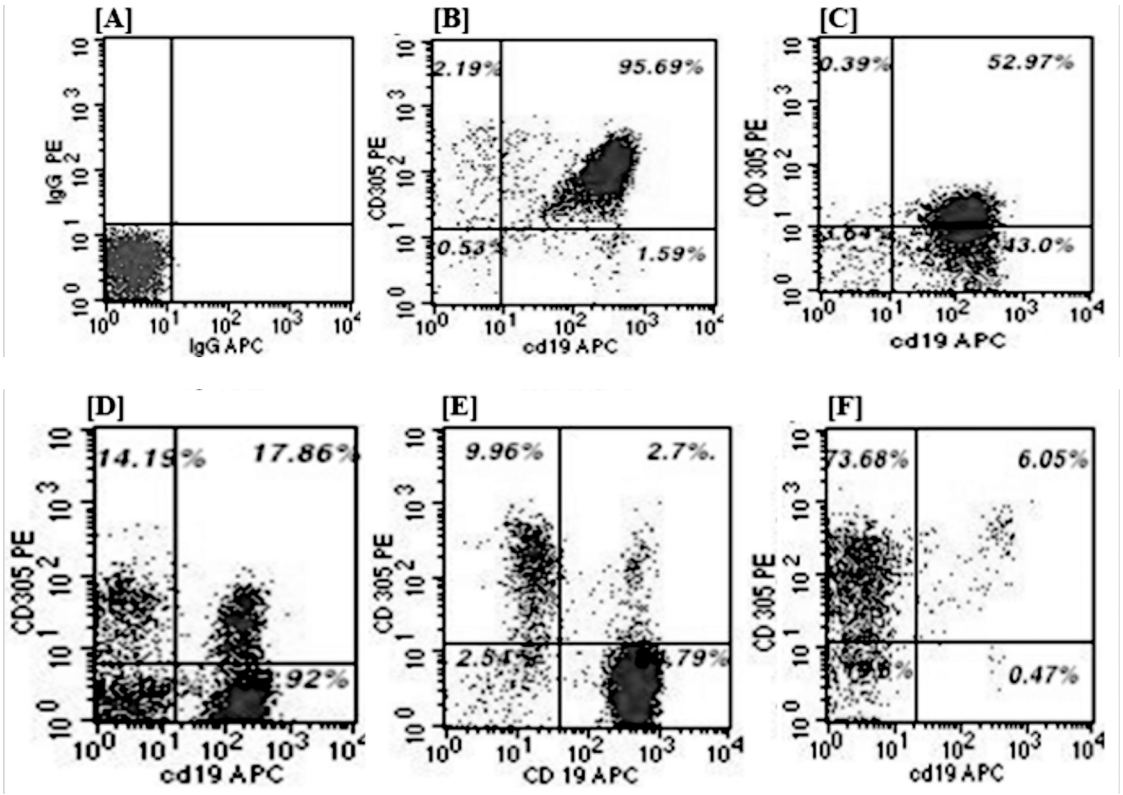

Fig. (2): Expression of LAIR-1(CD305) PE represented on Y axis versus CD19 APC on X axis in a case of chronic lymphocytic leukemia. [A]: Iso type control; [B and C]: Group 1: Rai stage 0 and I samples; [D and E]: Group 2: Rai stage II, III and IV samples; [F]: Control sample.

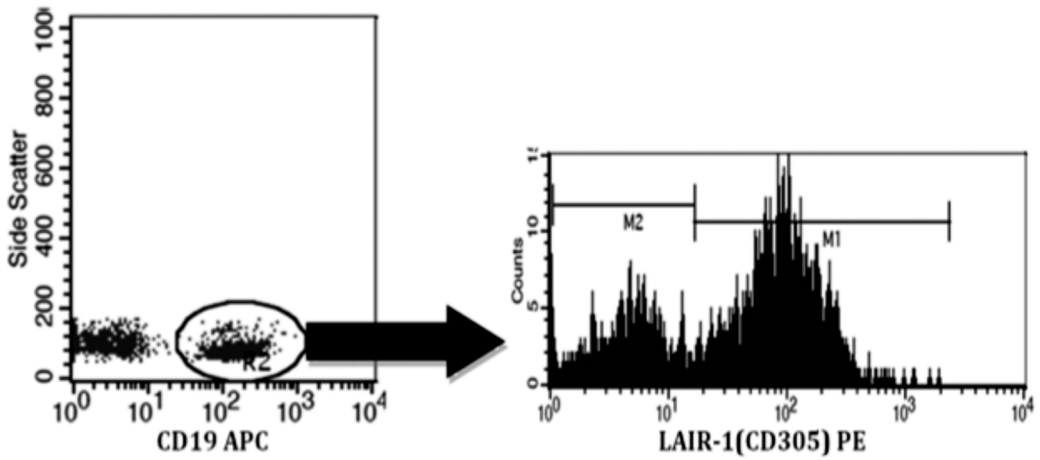

Fig. (3): MFI of LAIR-1+ve cells using a single histogram gated on CD 19 +ve cells. LAIR-1+ve cells MFI was measured in area under M1 marker.

RESULTS

The study was conducted on 33 newly diagnosed CLL patients, categorized according to Rai staging system into Group 1 (n=18) including stage 0 and I, and Group 2 (n=15) including stage II, III and IV as well as control group (n=17). Clinical characteristics of the study cases are illustrated in Table (1).

LAIR-1 expression in the various groups is presented in Table (2). Both % of expression and MFI were significantly higher in group 2 CLL patients as compared to both group 1 and control group (p=0.002 and <0.001 respectively). Group 2 showed significantly higher MFI (p=0.002) but not % expression (p=0.882). CD38% was comparable between Group 1 and 2 (p=0.381) but both showed significantly higher % than the control group (p=<0.001).

LAIR-1 % expression was inversely correlated to CD38% (r=–0.357, p=0.042, Fig. 4) and positively correlated to platelet count (r=0.368, p=0.035). Rai staging was inversely correlated to both LAIR-1 % and LAIR-1 MFI (r=–0.517, p=0.002 and r=–0.355, p=0.043 respectively).

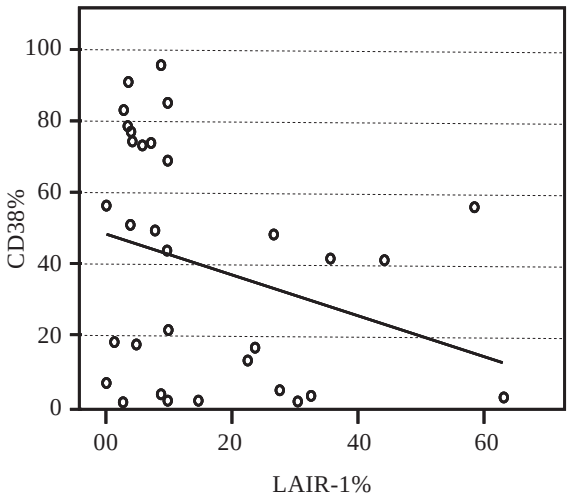

Fig. (4): Correlation between percentages of B cells expressing CD38 and LAIR-1.

Table (1): Clinico-pathological characteristics of 33 chronic lymphocytic leukemia cases.

Parameter	Group 1 (n=18)		Group 2 (n=15)		p-value
	Count	%	Count	%	
Gender:					
F	4	22.2	3	20.0	1
M	14	77.8	12	80.0	
LN:					
P	8	44.4	15	100.0	0.001
HSM:					
P	0	0.0	13	86.7	<0.001
Rai stage:					
0	9	50.0	–	–	
I	9	50.0	–	–	
II	–	–	4	26.7	
III	–	–	3	20.0	
IV	–	–	8	53.3	

Group 1: Rai stage 0, I.
Group 2: Rai stage II, III and IV.
F : Female.
M: Male.
LN: Lymph node.
P : Present.
HSM: Hepatosplenomegaly.

Table (2): Markers expression in chronic lymphocytic leukemia patients, according to Rai staging, compared to control.

Parameter	Controls (n=17)			Group 1 (n=18)			Group 2 (n=15)			p_1	p_2	p_3
	Mean ± SD	Median	Range	Mean ± SD	Median	Range	Mean ± SD	Median	Range			
LAIR-1 %	14.94± 19.22*	6.05	(1.26–55.7)	56.17± 29.57	62.88	(2.03–95.69)	19.87± 23.75	7.00	(1.60–85.10)	< 0.001	0.882	0.002
LAIR-1 MFI	269.07± 116.59	256.0	(110.3–503.0)	73.30± 59.32	45.65	(24.33–236.)	68.99± 69.73	35.86	(22.03–256.0)	< 0.001	< 0.001	0.002
CD38%	2.22± 0.95	2.40	(0.25–3.30)	15.03± 18.63	7.50	(0.20–64.00)	16.38± 14.28	10.00	(0.25–45.0)	< 0.001	< 0.001	0.381

* Mean ± SD, Median (Range).
p_1: Group 1 vs. control.
p_2: Group 2 vs. control.
p_3: Group 1 vs. Group 2.
Group 1: Rai stage 0, I.
Group 2: Rai stage II, III and IV.
LAIR-1: Leukocyte-associated Ig-like receptor-1.
MFI : Mean fluorescence intensity.

DISCUSSION

In CLL several immunophenotypic markers have been identified as significant and independent prognostic variables. However, while attractive because their detection is inexpensive and feasible in most laboratories, only few have been validated by independent series. LAIR-1 is one of the suggested prognostic markers studied **[12]**.

LAIR-1 is broadly expressed on nearly all immune cells, such as T-cells, B-cells, and natural killers **[11]**. For B-cells, LAIR-1 is expressed during ontogenesis; it inhibits the activation of immune cells using two immune receptor tyrosine-based inhibitory motifs located in the cytoplasmic tail of the receptor **[12]**. The current study aimed to explore the role of LAIR-1 expression in clinical heterogeneity of CLL.

Our study showed that LAIR-1 expression was significantly higher in Group 1 including patients with Rai stage 0 and I as compared to Group 2 which included higher risk stages (p=0.002). This is in agreement with previous studies reporting association of LAIR-1 expression with early stage low risk and its lack in advanced high risk CLL **[5,12,14]**.

The previous data could be attributed to the role of LAIR-1 in the modulation of BCR signaling pathways implicated in CLL-cell activation, with its inhibitor capacity being completely lost or significantly reduced when CLL cells do not express LAIR-1 **[14]**. The collagen which is the LAIR-1 ligand is produced by lymph node-derived mesenchymal stromal cells and is able to inhibit B-cell functions through LAIR-1 engagement **[15]**.

Previously it was reported that the extracellular matrix (ECM) anomalies were involved in promoting the progression of a solid tumor. Accordingly, LAIR-1 may be involved in tumor development **[16]**. As collagen, is an important component of ECM, and has been confirmed to be the high-affinity ligand for LAIR-1 **[17]**. ECM anomalies deregulate behavior of stromal cells, facilitate tumor-associated angiogenesis and inflammation, and thus lead to generation of a tumorigenic microenvironment. The presence of abnormal ECM leads to failure of stem cell properties to maintain and undergo symmetric cell division leading to overexpansion of cancer pool. This could explain the aggression seen in high risk CLL lacking LAIR-1 expression leading to spread of clonal population to lymph nodes, liver and spleen **[16]**.

In the current study CD38% was significantly higher in both Group 1 and Group 2 as compared to control (p=<0.001). CD38 originally defined as T cell activation marker plays a role in CLL; in vitro activation through CD38 drives CLL proliferation and chemotaxis via a signaling pathway that includes ZAP-70 and ERK1/2. Consequently, CD38 appears to be a global molecular bridge to the environment, promoting survival/proliferation over apoptosis which supports proliferation and survival of B cells on their way to and after neoplastic transformation **[18]**.

In the current study, CD38 expression in Group 1 and Group 2 was comparable (p= 0.381). This is in disagreement with a previous study which reported that CD38 expression identifies two subgroups of CLL patients with different clinical outcomes; this distinction was based on the percentage of $CD38^+$ leukemic cells within a CLL clone. In the majority of studies, the threshold was considered as ≥30% $CD38^+$ clonal members **[19]**. The 2 patient subgroups that result from this cut-off point was considered to differ clinically in several ways, including bias toward male gender, number of leukemic cells with atypical morphology, extent and level of adenopathy, lactate dehydrogenase and ß2-microglobulin levels, and absolute lymphocyte counts **[18, 20]**.

Previous studies also reported that CD38 expression indicated the proliferative activity of members of the leukemic clone at the time of analysis. Therefore, it was considered as "real-time" indicator of the level of leukemic proliferation and thereby actual or potential clonal evolution, which ultimately determines the clinical course and outcome for an individual patient. So the more $CD38^+$ cells in a clone, the greater the number of dividing cells and hence the greater the chance for occurrence of new DNA lesions, enhanced clonal aggressiveness, and worse clinical outcome as it was generally accepted that $CD38^+$ patients will have a shorter progression-free interval, require earlier and more frequent treatments, and ultimately die sooner **[20, 21]**. Also this data was in disagreement with other studies that proposed

CD38 as a promising and effective target for antibody-mediated therapy in currently incurable leukemia **[18]**.

This discrepancy could be explained by the fact that in our study both the intermediate group (Rai stage II) and the high risk group (Rai stage III and IV) were pooled in one group (Group 2) which might have contributed to the decrease of the values of CD38% in the pooled group. But it is worth mentioning that even if the current study did not reach the statistical significance, still the level of CD38% was higher in Group 2, the higher risk groups; lack of statistical significance might be attributed to the small sample size. However, the reliability of CD38 as a prognostic marker was questioned by some authors **[22,23]**.

In the present study Rai staging was inversely correlated to both LAIR-1% and LAIR-1 MFI. Also percentage of B cells expressing LAIR-1 was positively correlated to platelet count. This is in agreement with Poggi et al., **[14]** who reported the lack of LAIR-1 expression in high-risk CLL which resulted in the absence of a negative signal regulating kinase activation and cell division and demonstrated that the intensity of expression of LAIR-1 in low- and intermediate-risk CLL was associated with the CLL stage and progression of the disease. Their findings and ours potentiate the role of LAIR-1 as a prognostic marker in CLL.

The LAIR-1 % of expression and MFI being conversely correlated with CLL staging matches the studies performed on LAIR-1 in solid tumors **[24]**. Also Wang et al., study on cervical cancer found that the over-expression of LAIR-1 reduced tumor proliferation and increased tumor apoptosis of carcinoma cells; in addition the LAIR-1 expression in the cancer cell nucleus was associated with tumor size, pathological differentiation, classification and clinical stage. These data suggest that cancer patients with a high level of LAIR-1 expression may not experience disease progression **[11]**.

The current study detected that B cells percentage expressing LAIR-1 was inversely correlated to prognostic marker CD38%. This is in agreement with Perbellini et al., **[12]** who observed a significant association between LAIR-1^+ and $CD38^+$ patients. This finding throws light on LAIR-1expression as a good prognostic marker as CD38 was validated to predict adverse prognosis in CLL cases by previous studies **[25-27]**.

In Conclusion, our results strengthen the appeal of LAIR-1 having an active role in the pathophysiology of CLL. LAIR-1 expression in CLL cells carries the potential of being a prognostic marker. Low LAIR-1 expression in advanced CLL patients might be a contributing factor in the process of tumorigenesis of CLL which might qualify it as a potential therapeutic measure. However, larger studies are needed to tie LAIR-1 with other genetic prognostic factors to validate its role.

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Nil.

Conflicts of interest:

There are no conflicts of interest.

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