CD49d as Prognostic Marker in B-Cell Chronic Lymphocytic Leukemia in Correlation with the Expression of CD38, ZAP-70 and Clinical Binet Stage

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ABSTRACT:

BACKGROUND:

Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with highly variable clinical course and outcome. A number of clinical and biological features have been used to separate patients with CLL into subgroups with different prognoses. CD49d, CD38 and ZAP-70 expressions have shown to independently predict prognosis in CLL.

OBJECTIVE:

To assess the expression of CD49d in CLL patients and correlates them with CD38 and ZAP-70, and with clinical Binet staging.

PATIENTS AND METHODS:

This study was conducted on 30 newly diagnosed CLL patients. Diagnosis was based on lymphocyte count of > 5×10^9/L and immunophenotyping. The expression of CD49d, CD38 and ZAP-70 were investigated using four-color flow cytometer.

RESULTS:

The expression of CD49d, CD38 and ZAP-70 were detected in 60%, 56.7% and 30% of patients, respectively. The correlations between the expression of CD38 and both CD49d and ZAP-70 were both statistically significant (P= 0.002). A higher significant correlation was found between CD49d and ZAP-70 (P= 0.001). There was a statistically significant relationship between CD49d expression and Binet staging (P =0.035), while no significant relations were found between both CD38 and ZAP-70 and Binet staging (P >0.05). CD49d was more sensitive (76.5%) than the other two markers in prediction the intermediate and advanced stage with accuracy of 70%.

CONCLUSION:

CD49d expression was higher than that of CD38 and ZAP-70 and was significantly correlated with both of them. The adverse prognostic impact of CD49d was demonstrated by higher expression levels in intermediate and advanced-stage patients. CD49d has the highest sensitivity and accuracy with considerable specificity when compared with CD38 and ZAP-70, rendering CD49d a reliable biomarker for prognostication of CLL.

KEY WORDS: chronic lymphocytic leukemia, CD49d, CD38, ZAP-70, binet staging.

INTRODUCTION:

Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with highly variable clinical course and outcome characterized by the accumulation of a monoclonal population of small, mature-appearing CD5+ B lymphocytes in the peripheral blood (PB), bone marrow, and lymphoid tissues.[1] A number of clinical and biological features have been used to separate patients with CLL into subgroups with different prognosis and requirement of different therapeutic approaches. CD49d, CD38 and ZAP-70 expressions have been proposed as easily investigated markers (by flow cytometry) that have shown to independently predict prognosis in CLL.[2] CD49d, an adhesion molecule mediating cell-to-cell and cell-to-extracellular matrix interactions, represents a novel and the most reliable immunophenotypic marker regarding prognosis and independent of other markers such as IGHV mutational status.[3] CD38 has a pivotal role in initiating and modulating a series of input signals from the microenvironment, and its expression is a measure of cell division and a reflection of growth in vivo.[4] ZAP-70 is a

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tyrosine kinase protein and is of relevance in T-cell signaling. B cells of CLL may variably express this marker, but its positivity is one of the prognostic factors for predicting the course of the disease.[5]

AIM OF THE STUDY:

is to assess the expression of CD49d in newly diagnosed CLL patients and correlates them with the known prognostic markers, CD38 and ZAP-70, and with clinical Binet staging.

PATIENTS, MATERIALS, AND METHODS:

This cross-sectional study was conducted on 30 adult, newly diagnosed, CLL patients from December 5, 2014 to April 15, 2015. The patients were attending the Hematology outpatient clinic at Oncology Teaching Hospital of the Medical City. Data were collected for each patient using questionnaires form including: name, age, sex, the presence of lymphadenopathy, splenomegaly and hepatomegaly. Clinical staging by Binet staging system was used. From each patient a 5 ml venous blood sample was collected in K_2-EDTA tubes and analyzed for complete blood picture by automated hematology analyser (Cell-DYN, RUBY, Abbott Diagnostic, USA). CLL cases have been diagnosed in the Medical City Teaching Laboratories and Flow cytometry unit of Nursing Home Hospital based on typical lymphocyte morphology and absolute lymphocyte count (ALC) in the PB and immunophenotyping using six-color BD FACSCalibur™ flow cytometer (all patients had ALC > 5×10^9/L with CLL score > 3). The samples were then investigated for the expression of the surface marker antigens CD49d, CD38 and ZAP-70 using four-color flow cytometer (Partec Cyflow® Cube 6, Germany).

Identification of cells was performed using FSC/SSC parameters:

- CD38 antigen expression was considered to be positive when the percentage of positive cells was $\geq$ 7 %.[6]
- CD49d antigen expression was considered to be positive when the percentage of positive cells was $\geq$ 30 %.[7]
- ZAP-70 antigen expression was considered to be positive when the percentage of positive cells was $\geq$ 20 %.[6]

Statistical Analysis:

Nominal variables were expressed as frequency (number) and percentage. The continuous variables were presented as mean, standard deviations (SD), median and range accordingly. Pearson's chi-square and Fisher exact tests were used to assess the association between the categorical data. Spearman's rho non-parametric correlation test was used to predict correlation between the parameters of the patients. Validity parameters: sensitivity, specificity, accuracy, positive and negative predictive values were calculated to compare between the Binet staging and CD49d, CD38 and ZAP-70. *P* value < 0.05 was considered significant.

RESULTS:

The current study included 30 patients with diagnosis of CLL (25 males and 5 females). The mean age was 60.5 ± 10.8 (mean ± SD) years and a range of 45-85 years.

Distribution of CLL patients according to the Binet staging. Out of the 30 patients, 43.3% (13/30) were stage A, 10% (3/30) were stage B and 46.7% (14/30) were stage C.

CD markers expression. CD49d expression was detected in 18/30 patients (60%) while CD38 expression was detected in 17/30 patients (56.7%) and ZAP-70 expression was detected in 9/30 patients (30%), as illustrated in Figure 1.

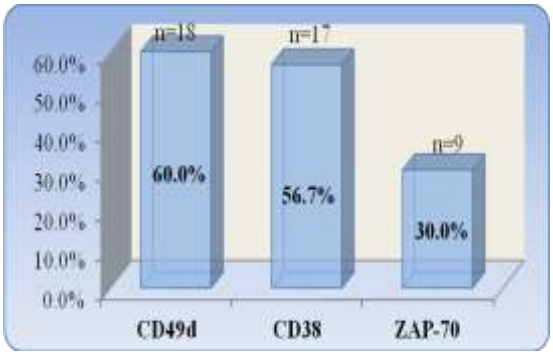

Figure 1: Percentage of positively expressed CD markers in 30 CLL patients.

The mean of positive expression of CD49d was 44.74% with a range 30%-75% while for CD38 was 17.62% with a range 9%-29% and ZAP-70 was 26.97% with a range 21.5%-39% (Table 1).

Table 1: Mean and range of the studied CD markers expression of the 30 CLL patients.

Marker expression (no.)		Mean% ± SD	Range%
*CD49d	Positive (18)	44.74 ± 15.1	30 - 75
	Negative (12)	6.9 ± 7.12	0.5 - 26
**CD38	Positive (17)	17.62 ± 7.68	9 - 29
	Negative (13)	1.68 ± 1.54	0 - 6
***ZAP-70	Positive (9)	26.97 ± 7.29	21.5 - 39
	Negative (21)	2.49 ± 2.5	0 - 10

*CD49d ≥30% is considered positive; ** CD38 ≥7% is considered positive; *** ZAP-70 ≥20% is considered positive.

Markers correlations and relationships. There were significant correlations between all CD markers according to their percentage of expression. The correlations between the expression of CD38 and both CD49d and ZAP-70 were both statistically significant (*P* value= 0.002). A higher significant correlation was found between CD49d and ZAP-70 with *P* value= 0.001 (Table 2).

Table 2 :Spearman's rho correlation between the markers in CLL patients, n=30.

Parameters		CD49d	CD38
CD38	r	0.547	
	P value	0.002	
ZAP-70	r	0.576	0.541
	P value	0.001	0.002

Also there was statistically significant relationships between the positive and negative expressions of CD49d and CD38; CD49d and ZAP-70; and CD38 and ZAP-70 with *P* values <0.001 (Table 3), 0.004 (Table 4) and 0.042 (Table 5), respectively.

Table 3: Comparison of the expressions between the CD49d and CD38 markers, n=30.

		CD49d		Total No. (%)
		Negative No. (%)	Positive No. (%)	
CD38	Negative	11 (84.6)	2 (15.4)	13 (100)
	Positive	1 (5.9)	16 (94.1)	17 (100)
Total		12 (40)	18 (60)	30 (100)
Pearson's chi-square= 19.03 *P* value <0.001				

Table 4: Comparison of the expressions between the CD49d and ZAP-70 markers, n=30.

		CD49d		Total No. (%)
		Negative No. (%)	Positive No. (%)	
ZAP-70	Negative	12 (57.1)	9 (42.9)	21 (100)
	Positive	0 (0)	9 (100)	9 (100)
Total		12 (40)	18 (60)	30 (100)
Fisher's exact test= 8.6 *P* value =0.004				

Table 5: Comparison of the expressions between the CD38 and ZAP-70 markers, n=30.

		CD38		Total No. (%)
		Negative No. (%)	Positive No. (%)	
ZAP-70	Negative	12 (57.1)	9 (42.9)	21 (100)
	Positive	1 (11.1)	8 (88.9)	9 (100)
Total		13 (43.3)	17 (56.7)	30 (100)
Fisher's exact test= 5.4 *P* value=0.042				

Relationship of CD markers expression to Binet staging. There was a statistically significant relationship between CD49d expression and Binet staging (advanced and early stage) with *P* value =0.035, while no significant relations were found between both CD38 and ZAP-70 and Binet staging with *P* value >0.05 (Table 6).

Table 6: Comparison of Binet staging of CLL patients with CD markers expression, n=30.

Markers	Binet staging		Total (n=30) No. (%)	*P* value
	Intermediate & advanced stage (B+C) (n=17) No. (%)	Early stage (A) (n=13) No. (%)		
CD49d				
Positive	13 (76.5)	5 (38.5)	18 (60)	0.035*
Negative	4 (23.5)	8 (61.5)	12 (40)	
CD38				
Positive	12 (70.6)	5 (38.5)	17 (56.7)	0.078 *
Negative	5 (29.4)	8 (61.5)	13 (43.3)	
ZAP-70				
Positive	6 (35.3)	3 (23.1)	9 (30)	0.691 **
Negative	11 (64.7)	10 (76.9)	21 (70)	
*Pearson's chi-square, **Fisher's exact				

Validity of CD markers expression as prognosis predictors in relation to Binet staging. CD49d was more sensitive (76.5%) than the other two markers in prediction the intermediate and advanced stage (stage B+C) "poor prognosis stages" with accuracy of 70% while ZAP-70 is more specific than others (76.9%) but associated with the least sensitivity (35.3%) and accuracy (53.3%), as shown in Table 7.

Table 7 :Values of validity of CD markers tests as prognosis predictors in comparison to Binet stage of the30 CLL patients.

Markers	Sensitivity	Specificity	*PPV	**NPV	Accuracy
CD49d	76.5%	61.5%	72.2%	66.7%	70%
CD38	70.6%	61.5%	70.6%	61.5%	66.7%
ZAP-70	35.3%	76.9%	66.7%	47.6%	53.3%

*PPV, positive predictive value; **NPV, negative predictive value.

DISCUSSION:

The current study revealed that among 30 newly diagnosed CLL patients, 60% of them were CD49d antigen positive that was close to the result of Uzay A et al.[8] 2012 in Turkey who reported that CD49d was expressed in 52% of CLL cases. But this study result was higher than the 47% reported by Gattei V et al.[2] 2008 in Italy, the 39% reported by Zucchetto A et al.[13] 2013 in Italy, the 38% reported by Bulian P et al.[9] 2014 in Italy. And this may be explained by the ethnic differences and the larger sample size of the other studies. CD49d expression showed a bimodal distribution, with most patients shown either very high or very low levels of expression. The mean % (range) of CD49d positive expression was 44.74% (30-75), while for negative expression was 6.9% (0.5-26). This fact minimized the number of cases with borderline CD49d expression clustered around the cutoff, making CD49d a pragmatic choice of biomarker for reliable prognostication of CLL.[9]

For CD38 in this study, it was expressed in 56.7% of CLL cases which was higher than that reported by Hassanein NM et al.[10] 2010 in USA (32.4%), D'Arena G et al.[11] 2007 in Italy (29%), Wiestner A et al.[12] 2003 in UK (30%), Hus I, et al.[13] 2006 in Germany (33.3%). These differences may be due to the choice of different cut-off value for the number of CD38+ve cells as these studies used a 20-30% cut-off in contrast to this study which used a 7% cutoff for CD38 expression as the largest studies to-date found that a cutoff percentage of 7% was best at separating different prognostic groups.[12,14] Maximally selected log-rank statistics were performed by Krober A et al.[14] 2002 to evaluate a possible cut-off value for CD38 expression, with respect to changes in the survival time distribution and time to disease progression and found that the estimated CD38 expression level yielding the best separation of two subgroups with different survival probabilities was 7%.

For ZAP-70 expression, in this study 30% showed ZAP-70 expression in agreement with D'Arena G et al.[11] 2007 in Italy (36%), Del Principe MI et al.[15] 2006 in Italy (36%), Hus I et al.[13] 2006 in Germany (36.5%). This study result was lower than reported by Uzay A et al.[8] 2012 in Turkey (79.4%) and Schroers R et al.[16] 2005 in Germany (46.8%).

Regarding the correlation between CD49d and other prognostic parameters (CD38 and ZAP-70) when considered as continuous variables, the percentage of CD49d expression was significantly and positively correlated with the percentages of CD38 expression ($r = 0.547$, $P = 0.002$) and ZAP-70 expression ($r = 0.576$, $P = 0.001$). The strongest statistically significant relation was between CD49d and CD38 with P value <0.001, followed by CD49d and ZAP-70 ($P = 0.004$) and lower but still significant association between CD38 and ZAP-70 ($P = 0.042$). This result is consistent with that obtained by Gattei V et al.[2] 2008 in Italy, Shanafelt TD et al.[17] 2008 in USA and Zucchetto A et al.[18] 2006 in Italy.

Binet clinical staging of CLL patients in this study revealed that 46.7% of patients were at stage C, while 43.3% of patients were within stage A and the rest 10% were within stage B. The previous Iraqi studies by Jasim HN et al. 2010[19], Ja′afar AM et al. 2010 [20] and Mohammed S et al. 2013,[21] showed 34%, 63.3% and 64.7% patients were within stage C, respectively. These variations are probably due to different sample sizes. In contrast to the western countries that showed lower percentage of patients that fell within stage C reaching 9% and higher percentage of patients within stage A reaching 60%,[2,9] which may be attributed to the regular checkup of their patients.

This study showed that there was a significant relationship between CD49d expression and clinical stage of CLL (Binet staging) with P value = 0.035. This result was in agreement with Zucchetto A et al.[22] 2006 in Italy, Shanafelt TD et al.[17] 2008 in USA and Gattei V et al.[2] 2008 in Italy. Advanced-stage disease is associated with high tumor mass, which in turn is associated with extensive organ and tissue invasion by CLL cells. Thus, in patients with advanced-stage disease there should be more production of integrins and ligands, as expected.[8]

The actual prognostic value of high CD49d expression, which was expressed in 38.5% of stage A patients at the time of diagnosis in this study, would become more interesting and firmly established if these patients are shown to have more rapid disease progression on follow-up. For CD38 and ZAP-70, there was no significant relationship with Binet staging (P >0.05). This result is consistent with that of Assem M et al.[23] (Egypt, 2009), and Sorour A et al.[24] (Egypt, 2007). In contrast, most of the studies worldwide found a significant relationship, e.g. D'Arena G et al.[11] (Italy, 2007), Schroers R et al.[16] (Germany, 2005), and Del Principe ML et al.[15]

(Italy, 2006). The reason for this discrepancy may be due to different study population and low number of cases in the current study.

Regarding the assessment of the validity of CD makers as poor prognosticators of CLL cases in relation to Binet staging, we found that CD49d is highly sensitive (76.5%) and specific (61.5%) with the highest accuracy (70%) than other CD markers, but unfortunately, no other studies were found for comparison.

CONCLUSION:

CD49d expression on CLL B-cells was detected in more than half of patients and higher than the expression of CD38 and ZAP-70. CD49d expression was significantly correlated with other poor prognostic parameters CD38 and ZAP-70. The adverse prognostic impact of CD49d is consistent with the high expression levels of CD49d in intermediate and advanced Binet stage patients. CD49d has the highest sensitivity and accuracy with considerable specificity when compared with CD38 and ZAP-70, thus CD49d is a pragmatic choice of biomarker for reliable prognostication of CLL. Close follow-up is recommended for patients with Binet stage A showing CD49d positive expression to monitor disease progression.

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