

CD49d and CD26 in chronic lymphocytic leukemia: their correlation with clinical Binet staging and clinical parameters

Talib Mohammed Ameen¹, Haithem Ahmed Al-Rubaie²

¹Al-Diwaniya Health Office, Al-Diwaniya, Ministry of Health, Iraq

²Department of Pathology, College of Medicine, University of Baghdad, Baghdad, Iraq

Email: talib_mohamad@yahoo.com

Abstract

Chronic lymphocytic leukemia (CLL) is most common in western countries forming about one-third of all leukemias in adults aged above 50 years. Some patients have indolent course and a survival very similar to the general population whereas others have a highly aggressive disease and a rapidly fatal outcome. CD49d and CD26 expressions have shown to independently predict prognosis in CLL. The study aimed to assess the expression of CD49d and CD26 in newly diagnosed CLL patients and find their correlation with clinical Binet stage, and other clinical parameters.

Patients, materials and methods: This study was conducted on 51 newly diagnosed CLL patients based on lymphocyte count $> 5 \times 10^9/L$ and immunophenotyping. The expression of CD49d, and CD26 were investigated using eight-color flow cytometer.

Results: The expression of CD49d and CD26 were detected in 56.9 %, 68.8 % of CLL patients, respectively. The correlation between CD49d expression and CD26 expression was statistically significant ($p < 0.001$) with high concordance rate between them. The positive expression of both CD49d and CD26 had statistically significant association with clinical Binet staging ($p < 0.001$, and 0.001, respectively) and their combined positive expression had also statistically significant association with clinical Binet stage of the disease ($p < 0.001$) with the highest combined positive rate was associated with stage C then followed by B in comparison with stage A.

Conclusion: Positive CD49d and CD26 expressions were significantly correlated with advanced clinical Binet stage and associated with frequent lymphadenopathy and their combined positive expression had highly significant correlation with advanced clinical Binet stage and therefore their co expression could be beneficial as reliable prognostic indicator in CLL patients.

Keywords: chronic lymphocytic leukemia, CD49d, CD26, Binet staging.

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is common in western countries while it is less frequent in Asia and Middle Eastern countries (Ruchlemer R, Polliack A. 2013). The disease occurs rarely in patients younger than 30 years and most patients with CLL are older than 60 years (Kantarjian H, O'Brien S. 2012). The clinical course and prognosis among CLL patients are highly variable and extremely heterogeneous. (Bosch F, Montserrat E. 2002). Therefore, there is a big demand to establish strong prognostic markers

for this disease to assess the disease progression and to predict the need for early treatment (Cramer P, Hallek M. 2011).

CD49d and CD26 have been proposed as easily examined markers by flowcytometry that could be used as independent predictors for prognosis and disease progression in CLL patients (Gattei V, et al. 2008), (Ibrahim L, et al. 2015).

Address for correspondence: Talib Mohammed Ameen, Al-Diwaniya Health Office, Al-Diwaniya, Ministry of Health, Iraq, Email: talib_mohamad@yahoo.com

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CD49d, an adhesion molecule belonging to integrin family, plays a crucial role in leukocyte trafficking, activation, and survival. It mediates cell-to-extracellular matrix and cell-to-cell interactions, through binding with fibronectin and vascular cell adhesion molecule1, respectively (Brachtl G, et al. 2014).

In addition to these adhesion functions, CD49d can also serve as a signaling receptor that influences B-cell survival via upregulation of Bcl-2 family members. Thus, in contrast to CD38, the biological functions of CD49d and the CD49d/CD29 integrin combination (VLA-4) are well defined, with a principal involvement in bone marrow homing and retention of hematopoietic cells, processes important to CLL pathophysiology ((Brachtl G, et al. 2014).

The overexpression of CD49d ($\geq 30\%$ cells stained positive) represents one of the best and most reliable flow-based independent prognostic markers, beside unmutated IGHV and TP53 dysfunction, for predicting reduced time to first treatment (TTFT) and overall survival (OS) (Dal Bo M, et al. 2016).

CD 26 is widely expressed on T cells, B cells and natural killer (NK) cells as well as on epithelial, endothelial and acinar cells of a variety of tissues. Only low levels of CD26 are found on resting lymphocytes, its expression being strongly upregulated following activation. In addition to the integral membrane form, a soluble form of CD26 occurs in serum (Gorrell MD, et al. 2001). Although CD26 expression is very low in B-cells, it is greatly up regulated following activation of these cells (Bühling F, et al. 1995). In CLL patients, CD26 expression is significantly correlated with CD49d and CD38 expression (Cro L, et al. 2009, and Ghannam DME, et al. 2014). This study aims to assess the expression of CD49d and CD26 in newly diagnosed CLL patients and their association with Clinical Binet stage and clinical parameters.

PATIENTS, MATERIALS AND METHODS

This prospective study was conducted on 51 newly diagnosed adult CLL patients from November 2020 to September 2021. The patients were attending at the Hematology outpatient clinic in Baghdad Medical City. Data were collected for each patient including: name, age, sex, the presence of lymphadenopathy (LAP), splenomegaly and hepatomegaly. Clinical Binet staging system was used. The diagnosis of CLL cases was based on typical lymphocyte morphology and absolute lymphocyte count (ALC, $> 5 \times 10^9/L$) in the peripheral blood and immunophenotyping (score > 3) using six-color BD FACSCalibur™ flow cytometer.

The blood samples were then investigated for the expression of the surface marker antigens CD49d and CD26 using

eight-color flow cytometer (BD FACS Canto II Flow Cytometer, Becton Dickinson and Co.). Phycoerythrin (PE)/Cyanine7-conjugated anti-CD19 monoclonal antibodies (mAbs) (BioLegend, San Diego, CA, USA) were used for gating of B-CLL cells. FITC (fluorescein isothiocyanate)-conjugated anti-CD26 mAbs and PE-conjugated anti-CD49d mAbs (BioLegend, San Diego, CA, USA) were used to analyze the target CD markers. The expression of each marker was reported as percentage of CD 19+ CLL cells displaying specific fluorescence intensity. The best cutoff points for CD49d and CD26 expressions were 30% and 10%, respectively (Cro L, et al. 2009), (Zucchetto A, et al. 2006).

The research was approved by the Research Ethics Committee, College of Medicine, University of Baghdad and was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants.

STATISTICAL ANALYSIS

Data were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) version 16 and Microsoft Office Excel 2007. 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. Agreement was done using Kappa agreement statistics. Spearman's rho non-parametric correlation test was used to predict correlation between the parameters of the patients.

RESULTS

The current study included 51 newly diagnosed CLL patients (31 males and 20 females, with male to female ratio of 1.5:1). The mean (\pm SD) age was 59.42 ± 11.75 years and a range of 23 to 80 years and a median age of 61 years.

According to Binet stages; 30 (58.8%) patients were in stage A, 7 (13.7%) in stage B and 14 (27.5%) in stage C.

At presentation, 10 patients (19.6 %) were diagnosed incidentally during the routine examination, while in contrast, 41 (80.4 %) patients were symptomatic. B-symptoms, LAP and splenomegaly were the most common presenting features, seen in 55.6%, 53.7.1% and 42.6% of patients, respectively, whereas hepatomegaly was reported in 25.9% of patients.

CD26- and CD49d- positive expressions were observed in 31/51 (68.8%) and 29/51 (56.9%) of patients, respectively (Table 1).

Table 1: Mean and range of the studied CD markers expression of the 51 CLL patients.

Marker	Expression (n)	Mean ± SD (%)	Range (%)
CD 26	Positive (31)	20.27 ±11.83	10.00 - 57.00
	Negative (20)	4.84 ±2.65	1.10 - 9.90
CD 49d	Positive (29)	65.89 ±26.75	30.20 - 99.80
	Negative (22)	10.93 ±6.74	2.40 - 25.00

The expression of CD26 was increasing with advancing disease stage with highly significant association ($p = 0.001$), and the expression of CD49d was high in stage C in comparison with those in stage A and B manifesting highly significant association ($p < 0.001$) as shown in Table 2.

Table 2: CD26 and CD49d expression according to clinical Binet stage of the disease.

CD marker	Expression	Total n= 51 (%)	Stage A (n= 30)	Stage B (n= 7)	Stage C (n= 14)	P-value*
CD 26	Positive	31 (68.8%)	12 (40%)	6 (85.7 %)	13 (92.9 %)	0.001 †
	Negative	20 (39.2%)	18 (60 %)	1 (14.3 %)	1 (7.1%)	
CD 49d	Positive	29 (56.9 %)	10 (33.3 %)	6 (85.7 %)	13 (92.9 %)	< 0.001 †
	Negative	22 (43.1%)	20 (66.7%)	1 (14.3%)	1 (7.1%)	

* Statistical analysis was done using Chi-square test; † More than 20% of cells have expected count of less than 5. The concordance rate between CD 49d and CD 26 expression in negative results was seen in 37.3% of cases and in 54.9% of cases in positive results. The kappa statistic revealed a strong agreement according to McHugh, 2012 (Table 3).

Table 3: Concordance rate between CD 49d and CD 26 expression.

		CD49d		
		Negative	Positive	Total
CD26	Negative	19 (37.3 %)	3 (5.9 %)	22
	Positive	1 (1.9 %)	28 (54.9 %)	29
	Total	20	31	51
Observed agreement (Po)		0.92		
Expected agreement (Pe)		0.51		
Kappa agreement		0.84		

There was a statistically significant correlation between CD49d and CD26 expressions ($p < 0.001$) (Table 4).

Table 4: Spearman’s rho correlation between the markers in CLL patients, n=51

Parameters		CD49d
CD26	r	0.841
	P value	< 0.001

Combined expression of both CD26 and CD49d in association with stage of disease showed positive results in 59.6 % of patients and highest combined positive rate was associated with stage C followed by stage B then stage A (Table 5).

Table 5: Association of co expression of CD26 and CD49d with CLL clinical Binet stage.

Combined expression	Total n = 47 (%)	Stage A	Stage B	Stage C	P-value*
		n = 28	n = 7	n = 12	
Positive	28 (59.6 %)	10 (35.7 %)	6 (85.7 %)	12 (100 %)	< 0.001 †
Negative	19 (40.4 %)	18 (64.3 %)	1 (14.3 %)	0 (0.0 %)	

* Statistical analysis was done using Chi-square test; † More than 20% of cells have expected count less than 5.

Spearman’s rho correlation between the studied markers and the clinical features showed significant correlation of both CD26 and CD49d with B-symptoms and LAP (Table 6).

Table 6: Spearman’s rho Correlation of the studied markers to clinical features.

Marker	B-symptoms		LAP		Splenomegaly		Hepatomegaly	
	r	p	r	p	r	P	r	p
CD 26	0.436	0.001	0.369	0.008	0.082	0.566	0.044	0.759
CD 49d	0.361	0.009	0.369	0.008	0.153	0.284	0.092	0.520

r correlation coefficient

DISCUSSION

In addition to the crucial role of flowcytometry immunophenotyping in the diagnosis of CLL, it also provides an important prognostic information and assessment of disease progression in different stages of the disease.

Positive CD26 expression was shown in 68.8 % of cases and this result was higher than the result observed by Ghannam DME, et al. 2014 in Egypt (50%), and Cro L, et al. 2009 in Italy (42%) and this variation may be attributed to the difference in the sample size in each study and to the ethnic and geographic differences. The mean percentage of positive CD26 expression was 20.27% and the mean percentage for negative CD 26 expression was 4.84%, this result was comparable to that observed by Sulda ML, et al. 2010 in Australia. There was a bimodal distribution of high and low expressions of this CD marker with a minimum number of cases that had borderline levels of expression which clustered around the cutoff value ($\geq 10\%$). Additionally, there was statistically significant association of the expression of CD26 with Binet clinical stages ($p = 0.001$). Therefore, all these results could make this biomarker as a good and reliable prognostic indicator for disease progression in CLL patients (Cro L, et al.2009), (Sulda ML, et al. 2010).

CD26 showed significant positive correlation between its positive expression with B symptoms ($p = 0.001$) and LAP ($p = 0.008$), and this result in contrary to that of Ibrahim D, et al.2021 in Egypt that showed insignificant correlation between positive CD26 expression and LAP among CLL patients ($p > 0.05$), but as in current study showed insignificant correlation between positive CD26 expression with splenomegaly and hepatomegaly.

Positive CD 49d expression was shown in 56.9% of cases and this result is close to the that reported by an Iraqi study done by Al-Rubaie HA, et al. 2016 that showed 60% positive expression, and also close to that of Uzay A, et al. 2012 in Turkey who reported that CD49d was positively expressed in 52% of CLL cases, whereas this result was higher than those reported by Gattei V, et al. 2008, Zucchetto A, et al. 2006, and Bulian P, et al. 2014 in Italy (47%, 39%, and 38%, respectively). These variations may be explained by the difference in the sample size of each study and due to geographic and ethnic differences also. The mean percentage of positive CD49d expression was 65.89% while for the negative expression was 10.93%. This bimodal distribution with either very high or very low levels of expression minimize the number of patients with borderline levels of expression that clustered around the cutoff value ($\geq 30\%$) and this result was in agreement with the study done in Iraq by Al-Rubaie HA, et al. 2016 and Bulian P, et al. 2014 in Italy. In addition, the positive expression of CD49d had statistically significant association with Binet clinical stage C of the disease ($p < 0.001$) suggesting CD49d as a reliable and independent biomarker in prognostication of CLL patients (Bulian P, et al.2014), (Dal Bo M, et al.2016).

CD49d showed significant positive correlation between its positive expression with B symptoms ($p = 0.009$) and LAP ($p = 0.008$). The association between CD49d positive expression and LAP was shown by many studies as that by Strati P, et al.2017 in USA which demonstrate that CD49d expression was associated with nodal disease at the time of presentation in patients with newly diagnosed CLL. In European study done by Baumann T, et al.2016 observed that high CD49d expression was associated with a more ‘lymphomatous’ phenotype (i.e. lower blood lymphocyte count and more frequent LAP) and explained that higher expression of CD49d which is a subunit of the VLA-4 integrin receptor, could enhance its binding to VCAM1. And this differential homing pattern could in turn allow CD49d-

positive cells to easily populate proliferation centers located in lymph nodes and bone marrow (Baumann T, et al.2016).

In this study, concordance rate between CD49d and CD26 expression was high in both positive and negative expression (47/51) with strong agreement. In addition, the 51 evaluated CLL cases showed a highly significant correlation between CD49d and CD26 expressions and this result was in agreement with Cro L, et al. 2009 in Italy and Ibrahim L, et al. 2015 in Egypt. Furthermore, the combined expression of CD26 and CD49d showed statistically highly significant association with Binet stage C, this high concordance rate and highly significant association between these CD markers may predict poor prognosis if one predictive factor is borderline (Cro L, et al. 2009). In addition, many studies observed that CD49d and CD26 are surface CD markers whose expression can be easily investigated, highly reproducible, stable over time and stable in frozen samples (Gattei V, et al. 2008), (Cro L, et al. 2009).

CONCLUSIONS

Both CD26 and CD49d expressions were detected in more than half of CLL patients involved in this study and both markers were showed statistically significant correlation with each other and with advanced clinical Binet stage and frequent LAP with high concordance rate between them favoring their adverse prognostic impact in CLL patients and their combined use is valuable especially when one marker is borderline.

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