Flow Cytometric Expression of CD49d in Newly Diagnosed Chronic Lymphocytic Leukemia and Its Correlation with Established Prognostic Markers

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Abstract

Introduction   Chronic lymphocytic leukemia (CLL) is the commonest hematological malignancy in the West but is relatively uncommon in India. The prognosis of CLL is determined by well-established prognostic markers. CD49d has been emerging as a promising prognostic marker in CLL. CD49d expression in CLL has been found to have an aggressive clinical course, shorter time to first treatment, and poorer prognosis. The aim of this study was to analyze the flow cytometric expression of CD49d in newly diagnosed CLL and to correlate its expression with clinico-hematological parameters.
Materials and Methods   Twenty-five consecutive patients of CLL, diagnosed on flow cytometry, were included in the study. Patients on treatment or those with relapse were excluded. The panel for flow cytometry included the routine markers used for CLL diagnosis along with CD49d. The expression of CD49d was correlated with clinico-hematological parameters in all patients. "R" software was used for the statistical analysis. Fisher's exact test and Wilcox test were used to assess the correlation of CD49d to categorical and continuous data, respectively.
Results   The mean age of the patients was 62.6 ± 12.5 years, and 80% were symptomatic at diagnosis. CD49d expression was found in 44% cases, with a higher proportion being male patients. CD49d and prolymphocyte percentage showed a statistically significant correlation ($p = 0.0007$). We found a statistically significant correlation between CD49d expression and lymphadenopathy and splenomegaly with p-values of 0.033 and 0.0472, respectively. CD49d positivity correlated significantly with a higher Rai stage ($p = 0.0196$) and intermediate and high-risk cases according to Binet staging ($p = 0.033$).
Conclusion   CD49d expression in the present study correlated with a higher prolymphocyte percentage, lymphadenopathy, splenomegaly, and higher Rai and Binet stages. CD49d expression on flow cytometry was reproducible and easy to interpret.

Keywords
► CD49d
► Chronic lymphocytic
► flow cytometry
► leukemia
► prognosis

Introduction

Chronic lymphocytic leukemia (CLL) is a clonal hematological disorder characterized by the proliferation of mature small B-lymphocytes (CD5, CD19, and CD23 positive), in the peripheral blood, lymphoid organs, and bone marrow.[1] It is the most common hematological malignancy in the western world, accounting for 25 to 30% of all hematological malignancies, but is much rarer in the Indian subcontinent, with a reported incidence of 1.7 to 8.8%.[2] The prognosis of CLL is determined by well-established parameters such as Rai/Binet staging, hematological parameters, antigenic expression, and genetic factors.[1,3,4] Relatively newer parameters like immunoglobulin heavy-chain variable region (IGHV) gene mutation status, presence of p53 mutation, ZAP-70 and CD38 expression, and cytogenetic abnormalities have also been found to predict disease progression, therapeutic outcomes, and overall survival in patients with early stages of CLL.[1,5,6]

There is considerable heterogeneity in clinical progression and survival of patients belonging to the same stage of CLL, with some requiring treatment immediately, while the others do well without treatment for long durations after diagnosis.[3,4] This unpredictability in clinical advancement has led to active research to determine disease markers which can identify patients who are likely to follow an aggressive course and, therefore, require treatment earlier. CD49d is one such marker, which has been postulated as a promising predictor of disease progression in CLL. It is expressed in around 40% cases of CLL and has been associated with an aggressive clinical course, reduced overall survival, and shorter time to first treatment.[7] The current study was conducted to study the expression of CD49d in patients with CLL and to correlate its expression with the other well-established clinico-hematological parameters.

Materials and Methods

The present study was an observational, prospective study conducted at a tertiary care center in Uttarakhand, India, from January 2019 to March 2020. Twenty-five patients diagnosed with CLL on flow cytometry were included in the study. The study was started after due approval from the Institutional Ethics Committee. Informed written consent was obtained from all the patients included in the study. All flow-cytometry-proven new cases of CLL were included in the study. Patients who were on treatment or patients who had relapsed were excluded from the study.

Clinical details of these patients including presenting symptoms, age, gender, clinical stage (Rai/Binet), lymphadenopathy, and organomegaly were recorded. Hematological parameters like hemoglobin (Hb), total leukocyte count (TLC), absolute lymphocyte count (ALC), percentage of lymphocytes, and platelet count were noted. Leishman-stained peripheral smears were examined to record differential leukocyte count, percentage of prolymphocytes, and the presence or absence of atypical morphology. Relevant biochemical laboratory results were also recorded.

Flow cytometry was performed using 3 mL peripheral blood sample collected in an ethylenediaminetetraacetic acid vial. The panel of antibodies studied on flow cytometry included CD45, CD19, CD5, CD23, CD49d, s-Kappa, s-Lambda, ZAP-70, FMC-7, CD38, CD20, CD34, and HLA-DR. The sample preparation method as standardized in the laboratory was followed. Data analysis was done using FACS Diva software provided by BD Biosciences. Cells with low side scatter and bright CD45 expression were gated as lymphocytes. The gated cells of interest were studied for the expression of other markers. Immunoexpression of CD19, CD23, and CD5 was assessed, and cases showing co-expression of these markers were diagnosed as CLL.[1] The tumor cells were assessed for the expression of CD49d, FMC7, CD38 (in 13 cases), and ZAP-70 (in seven cases). The marker expression was recorded as negative or positive (dim, moderate, or bright). Reactivity by T lymphocytes was used as an internal positive control for CD49d and ZAP-70.[8-10] ZAP-70 and CD49d were considered positive if immunoexpression was present in more than 20% and 30% of the tumor cells, respectively.[7,11-15] B lymphocytes were used as internal controls for CD19 and CD20.

Statistical analysis: "R" software was used for the statistical calculations. Fisher's exact test was used to assess the correlation of CD49d to categorical data, like gender, Rai and Binet staging, lymphadenopathy, splenomegaly, hepatomegaly, and CD38 expression. The Wilcox test was used to assess the correlation of CD49d to continuous data, like age, TLC, ALC, Hb levels, platelet count, and prolymphocyte percentage.

Results

Patient characteristics: The study included 25 consecutive cases of CLL diagnosed on flow cytometry. There were 18 males and seven female patients in the study, with a male-to-female (M:F) ratio of 2.6:1. The age of the patients ranged from 38 to 96 years, with a mean age of 62.6 ± 12.5 years and median age of 65 years. Twenty-four percent of the patients were less than 55 years of age. The majority of the patients (80%) were symptomatic, while only 20% cases were asymptomatic at diagnosis. Lymphadenopathy, splenomegaly, and hepatomegaly were found in 64, 52, and 20% of the cases, respectively.

Clinical staging: According to modified Rai staging, 24% of our cases were in the low-risk group (stage 0), 32% cases in the intermediate-risk group (two cases in stage 1 and six cases in stage 2), and 44% cases in the high-risk group (three in stage 3 and eight in stage 4). As per Binet staging, 32% of our cases were in the low-risk group (stage A), 28% cases in the intermediate-risk group (Stage B), and 44% cases in the high-risk group (stage C). The detailed distribution of the cases according to the staging systems is given in ►Table 1.

Hematological parameters: The mean TLC in the present study was 68,070 cells/μL and mean ALC was 60,741 cells/μL. All the females included in the study had the TLC of less than 100,000 cells/μL. One patient had a lymphocyte doubling time of 3 months. None of the other patients showed

Table 1 Distribution of patients according to modified Rai and Binet staging

| | Number of patients | | | | | | | |
| | Modified Rai staging | | | | Binet staging | | | |
Risk group	Stage	Male	Female	Total	Stage	Male	Female	Total
Low	0	4	2	6	A	4	4	8
Intermediate	Stage 1	0	2	2	B	6	1	7
	Stage 2	5	1	6				
High	Stage 3	3	0	3	C	8	2	10
	Stage 4	6	2	8				
Total		18	7	25		18	7	25

Table 2 The range of hematological values obtained in cases of CLL along with mean and standard deviation (SD)

Parameter	Minimum value observed	Maximum value observed	Mean (SD)
TLC (cells/μL)	11,760	263,290	68,070 (55,388)
ALC (cells/μL)	6,585	255,390	60,741 (54,484)
Hemoglobin (gm/dL)	3.3	13.0	10.66 (2.9)
Platelet (cells/μL)	11,000	273,000	125,932 (71,705)
Prolymphocyte %	1	22	4.88

Abbreviations: ALC, absolute lymphocyte count; TLC, total leukocyte count.

doubling of ALC during the study period. The median Hb in our patients was 11.8 gm/dL (3.3–13.9), and the median platelet count was 132,000 cells/μL (11,000–273,000). The details of the hematological parameters are given in ►**Table 2**.

Morphological features: The majority of the cases (92%) showed the typical morphology of CLL (►**Fig. 1a,b**). Only two patients (8%) showed atypical morphology (►**Fig. 1c**), both of which expressed CD49d, while one also expressed FMC-7. Two patients (8%) were diagnosed as CLL-prolymphocytic leukemia (PLL) (►**Fig. 1d**), with prolymphocyte values of 17% (stage 2/B) and 22% (stage 4/C). Rest of the 23 cases had a prolymphocyte count of less than 10%. There was no case of PLL in our study. Bone marrow examination was done in only 5 out of 25 patients: two cases showed a diffuse pattern of infiltration and two showed a mixed interstitial and diffuse pattern of infiltration. One case showed a nodular and interstitial pattern of infiltration.

Flow cytometric immunophenotyping: Eighty-eight percent of the patients (22 out of 25) expressed the typical immunophenotype of CLL with bright CD19 positivity and moderate to bright co-expression of CD5 and CD23 (►**Fig. 2**). CD49d expression was assessed in all 25 cases, with 44% (11 out of 25) cases showing positivity (►**Fig. 3**). Immunophenotypic aberrancies were detected in 3 out of 25 cases (12%), which were as follows: one case was negative for CD5, two cases showed FMC7 positivity, and one of these had dim CD5 expression.

Correlation of CD49d with Other Parameters

CD49d expression showed a statistically significant correlation with prolymphocyte percentage, with a *p*-value of 0.0007.

There were two cases of CLL-PLL and both were positive for CD49d. A statistically significant correlation was found between CD49d positivity and higher Rai staging, with a *p*-value of 0.0196. While assessing the Binet staging, we classified low-risk cases as one category and intermediate- and high-risk cases together as the second category. CD49d expression was found to have a statistically significant correlation with the intermediate and high-risk cases with a *p*-value of 0.033.
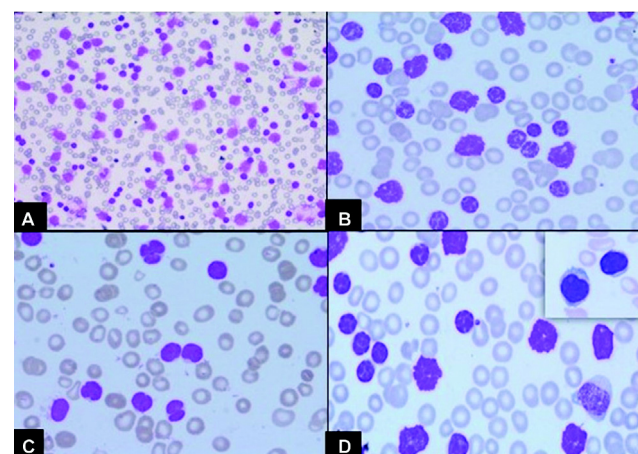
Fig. 1 Peripheral smear showing typical morphology of CLL in **(A)** and **(B):** with **(A)** lymphocytosis, occasional prolymphocytes, and an increased number of smudge cells (×400, Leishman stain), **(B)** smear shows lymphocytosis and many smudge cells (×1,000, Leishman stain). **(C)** Cells showing atypical morphology with nuclear folds and clefts, binucleate cells, cleaved nuclei, buttock-shaped cells and flower-shaped nucleus, in some cells. This case expressed CD49d (×1,000, Leishman stain). **(D)** *CLL/PLL*: Peripheral smears showing few prolymphocytes, inset shows a prolymphocyte.
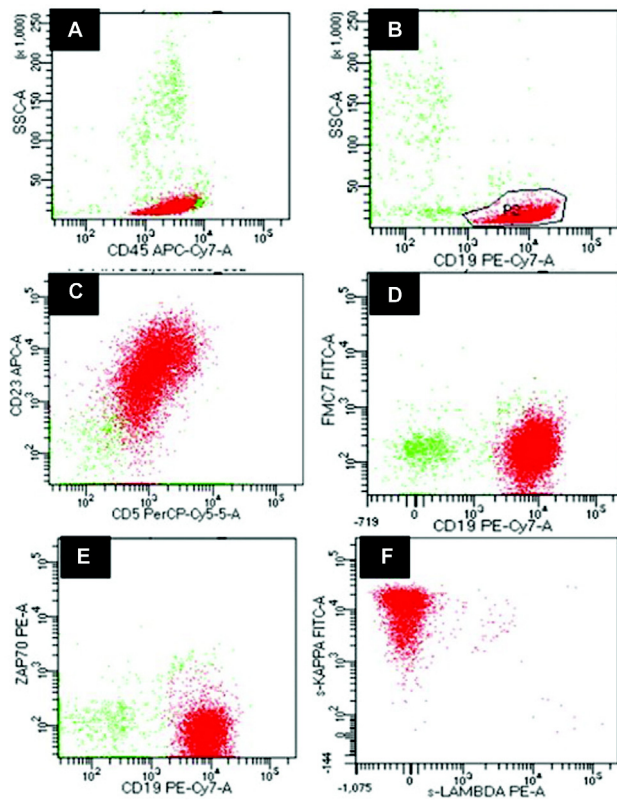
Fig. 2 Flow cytometric immunophenotyping of a classical CLL case. **(A)** Cells of interest (*red*) showing bright CD45 expression and low side scatter. **(B)** Cells of interest have been gated (*red*) using bright CD19 expression and **(C)** low side scatter cells. These cells show co-expression of CD5 and CD23 and are **(D)** negative for FMC7 and **(E)** lack ZAP-70 expression. **(F)** The neoplastic cells show kappa (*light chain*) restriction proving monoclonality.

We found a statistically significant correlation between CD49d expression and lymphadenopathy and splenomegaly ($p = 0.033$ and 0.0472, respectively). Hepatomegaly and CD49d correlation showed a *p*-value of 0.1333; however, the odds ratio was 6.84. CD49d was positive in 14.3% (one out of seven) females and 55.5% (10 out of 18) males, showing that a higher proportion of male patients had CD49d positivity; however, this finding was not statistically significant. CD49d-positive patients had lower median Hb levels ($p = 0.68$) and higher TLC and ALC compared to CD49d-negative patients, however; these were not statistically significant. There was no correlation between CD49d expression and platelet count ($p = 0.84$).

The expression of CD38 could be assessed in only 13 cases, and its correlation with CD49d expression showed a *p*-value of 0.069. Similarly, ZAP-70 expression could be studied in only seven patients. Since the number of patients in whom these two markers could be assessed was small, the statistical association of these two markers with CD49d could not be ascertained.

Molecular findings: Molecular tests were done only in four patients. One patient had CLL with Evan's syndrome, who showed IGHV-unmutated status, TP53 mutation and ATM mutation, CD49d positivity, and ZAP-70 negativity.
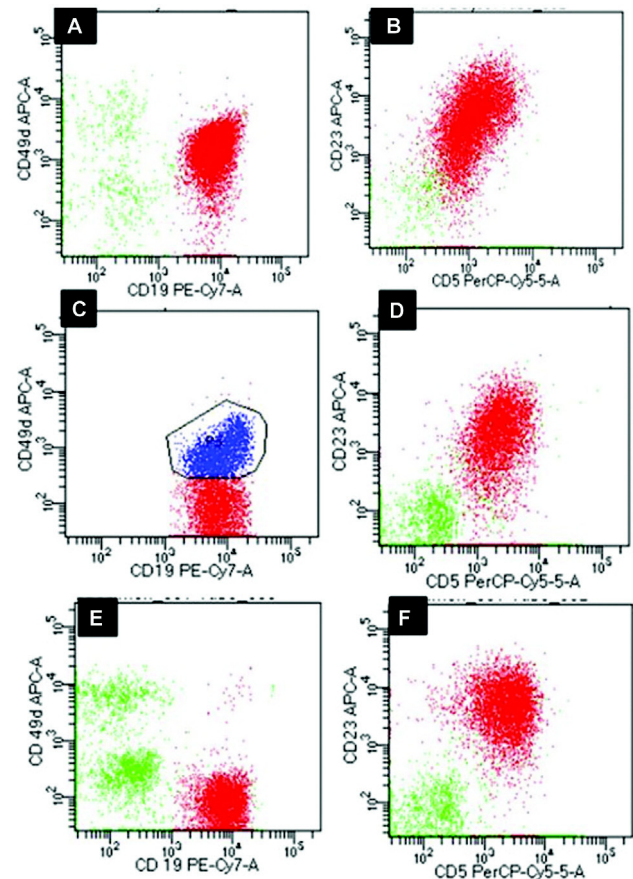
Fig. 3 *CD49d expression in CLL* **(A)** cells of interest (*gated red*) showing bright CD19 expression and positive CD49d expression. **(B)** The same case showing dual positivity of CD5 and CD23 in the neoplastic cells. **(C)** Another case showing bright CD19 expression in the cells of interest, with 35% of these cells showing moderate CD49d expression (blue). **(D)** The same case showing dual positivity for CD5 and CD23 in the neoplastic cells. **(E)** Scatter plots of a case showing bright CD19 expression and negative CD49d expression, while **(F)** the cells co-express CD5 and CD23.

The second patient had trisomy 12 and showed bright positivity for CD49d on flow cytometry. Del 13q14 was positive in two patients, one belonging to stage 0/A with CD49d negativity, while other belonging to stage 2/B with positive CD49d expression. Owing to the absence of cytogenetic details in the majority of the patients, these features could not be assessed for statistical significance.

Discussion

CLL is the most common hematological malignancy in the western world but is much rarer in the Indian subcontinent.[1,2] There is limited literature on the clinico-hematological profile and the biology of CLL in Indian patients. The available Indian data show significant differences from the western patients, with respect to demographic, clinical, and hematological features of CLL. Even though male predilection is well known in CLL, the M:F ratio has been found to be higher in the Indian population. The median age of presentation is lower in India (59–64 years) than that in the western

countries (70 years).[1,2,16–21] While most of the patients from the west are asymptomatic at diagnosis,[1] the proportion of asymptomatic patients in India is reportedly lower, ranging from 7.5 to 22%.[18,20] This finding could be attributed to the well-developed screening programs in the western countries as compared to India. Lymphadenopathy, the most common clinical sign associated with CLL, has been reported in 50 to 90% of patients, both in the west and India.[3,4,18,20] Splenomegaly and hepatomegaly show a worldwide incidence of 25 to 50% and 15 to 25%[3,4] but are found in a remarkably higher proportion of Indian patients, with a reported incidence of 50 to 66% and 40 to 63%, respectively.[18,20] Studies from the west have shown a higher number of cases presenting in the low-risk group,[3,4] while Indian studies have shown a higher proportion of cases in the intermediate- and high-risk groups.[2,18,20] In the present study, the demographic and clinical findings of CLL were in accordance with the published Indian data, except for the trend of hepatomegaly which was found to be much lower.

Western studies on CLL patients have documented a higher median Hb value of 13.5 to 14gm/dL as compared to Indian data (median Hb of 11.3–11.5 gm/dL). The lower Hb values in Indian studies could be attributed to the presence of coexisting nutritional anemia.[2,18,20,22,23] In terms of TLC, ALC, and platelet counts, the findings of our study are similar to the reported Indian data, which report a TLC ranging from 50,000 to 70,000 cells/μL, ALC ranging from 40,000 to 50,000 cells/μL, and median platelet counts of 150,000 cells/μL. The reported incidence of atypical morphology in CLL ranges from 7 to 10%.[21,24,25] We found atypical morphology in only 8% of the cases, which concurs with the reported literature. Atypical morphology in CLL has been found to be associated with underlying trisomy 12.[24,26]

CLL has many prognostic indicators, including clinical features, antigenic, and molecular properties. CD49d is a new marker, which has been gaining eminence as an important marker in CLL.[7,13] Recent studies from the west have reported its utility as an independent prognostic marker in CLL; however, the Indian literature on the same is limited.[1,7,13,14,27] CD49d is an adhesion molecule belonging to the integrin family and is also known as integrin-4 alpha chain. It shows very high expression in normal circulating B cells, moderate expression in T cells, and positivity in monocytes in some cases.[10] The expression of CD49d varies between normal peripheral B cells and neoplastic cells of CLL.[23,28] CD49d expression has been found to be significantly higher in high-risk cases of CLL or in cases showing a faster progression to higher stages.[13,14,22]

The flow cytometric expression of CLL shows a clear-cut positivity or negativity, with very rare cases falling in the borderline zone. This inherent property of CD49d makes it a more suitable prognostic marker, which is easily interpretable, as compared to other markers whose expression values are usually distributed around the cut-off and borderline grey zone.[7,13] We found it very simple to interpret the CD49d expression in our cases and found its interpretation to be reproducible when assessed independently by two different pathologists. An added benefit of CD49d expression is the

stability of its expression, which is retained throughout the course of the disease, thus allowing the reliable evaluation of its expression at any point during the course of the disease before treatment.[7,15]

The expression of CD49d has been reported in around 40% cases of CLL,[7,13,14] and we found its expression in 44% of our cases. There are very few studies on CD49d expression from India. Gogia et al reported CD49d expression in 50% of their cases.[19] Studies published recently have not shown any association between gender and CD49d expression.[7,22,29] Even though we did not find a statistical association between the above, our study clearly shows a higher proportion of male patients showing CD49d positivity.

Various studies have assessed the correlation between CD49d expression and clinical stage in CLL (►Table 3) and have concluded that cases in higher stages show higher CD49d expression.[7,13,14,22] In most studies, stages were assessed taking low-risk stage as one group and intermediate- and high-risk stage together as a second group,[30] which we also followed. We found a statistically significant correlation between CD49d positivity and higher Rai stages and CD49d expression with intermediate and high-risk cases as per Binet staging. Rossi et al found CD49d positivity in the early stage of CLL to be associated with poor prognosis and faster conversion to higher stages.[31] We could not assess this association due to the limited duration of the study and the coronavirus disease 2019 (COVID-19) pandemic, wherein patients could not be followed up. Patients with high CD49d expression have also been found to have a significantly shorter time to develop the doubling of ALC.[13,31]

While studying these correlations in the present study, we observed that none of the cases in low-risk group in Rai staging was positive for CD49d and only one case in Binet staging low-risk group expressed CD49d. However, a linear correlation between the Rai and Binet stages and CD49d expression was not obtained. In other words, there was no increase in the percentage of positive cases as the stages progressed higher from stage 1 to 4, and A to C, respectively. While studying for the presence of linear correlation by categorizing into low, intermediate, and high-risk groups, the statistical significance of correlation between CD49d and Rai stage was maintained ($p = 0.0456$), while that of the Binet stage was lost ($p = 0.0627$). When the Rai stage was assessed by considering all the five stages as different categories, the statistical significance was lost, with the p-value changing to 0.1006. This can be attributed to the fact that the sample size was low and the distribution of patients in different stages was highly varied.

CD49d expression has not been found to have any correlation with age, Hb levels, and platelet counts.[7,22,23,29] In terms of TLC and ALC, there is a varied opinion, with some studies reporting that patients with a higher CD49d expression have lower TLC and ALC[22,23] while others have reported this association to be statistically insignificant.[29] Even though we found CD49d-positive cases to have higher TLC and ALC, these findings were not statistically significant. A higher prolymphocyte percentage in CLL is associated with poorer prognosis.[32] Cases of CLL with increased

Table 3 Comparative studies on CD49d expression in CLL and its correlation with other prognostic parameters

S.no.	Authors	Year	Sample size	Study duration	Median age (y)	Cases expressing CD49d (%)	Correlation of CD49d expression with:			Other findings
							Rai stage	Binet stage	Organo-megaly	
1.	Current study	2021	25	15 mo	65	44%	$p = 0.0196$	$p = 0.033$	LN- $p = 0.033$ SM- $p = 0.0417$ HM- $p = 0.1333$	CD49d correlated with prolympho-cyte % ($p = 0.0007$)
2.	Nematollahi et al[29]	2020	98	3 y	68	52%	$p < 0.0001$	–	–	Significant correlation of CD49d with CD38 expression, Hb levels, and platelet counts.
3.	Gogia et al[19]	2019	510	18 y	60	50.3%	–	–	–	Impact of different factors on overall survival was studied.
4.	Abdel-Aziz et al[35]	2019	41	8 y	59	56.1%	$p = 0.8$	$p = 0.5$	LN- $p = 0.6$ SM- $p = 0.7$ HM- $p = 0.3$	CD49d positivity: independent prognostic factor for shorter pro-gression free survival. No correlation with prolymphocyte % ($p = 0.329$)
5.	Strati et al[23]	2017	797	14 y	63	35%	$p < 0.001$	–	LN- $p < 0.001$	Significant correlation of CD49d with ZAP-70 expression and IGHV mutation status.
6.	Al-Rubaie et al[30]	2016	30	4 mo	60.5	60%		$p = 0.035$	–	CD49d was found to be more sen-sitive than CD38 and ZAP-70 in predicting the intermediate ad-vanced stages.
7.	Ibrahim et al[13]	2015	103	4 y	59.1	55.3%	$p < 0.001$	0.002	SM- $p = 0.001$	CD26 expression also studied. Both CD49d and CD26 were found to be independent prognostic markers.
8.	Gattei et al[7]	2008	303	18 y	63.5	42.9%	$p = 0.02$	–	–	CD49d correlated with CD38, ZAP-70, and IGHV mutation status. CD49d: Independent prognostic factor for overall survival
9.	Shanafelt et al[14]	2008	158	8.5 y	64	28%	$p < 0.0001$	–		Significant correlation of CD49d with ZAP-70 and CD38 expression.
10.	Rossi et al[31]	2008	140	10 y	69	38.6%	–	–	–	Included Binet-A cases only. CD49d positivity: risk factor for progression to advanced stage and lymphocyte doubling.

Abbreviation: HM, hepatomegaly; IGHV, immunoglobulin heavy-chain variable region LM, lymphadenopathy; SM, splenomegaly.

prolymphocytes usually show FMC7 positivity, CD23 and CD5 negativity (or dim expression), and strong SmIg expression.[25,32] An important finding in our study is the statistically significant correlation between CD49d expression and prolymphocyte percentage. Some authors have also reported higher CD49d expression in cases with increased prolymphocyte count and in PLL as compared to CLL.[33,34] Atypical morphology in CLL has also been found to be associated with CD49d expression,[34] which was also seen in our cases.

Our study showed a significant correlation between CD49d expression and lymphadenopathy and splenomegaly. Strati et al also reported an association between lymphadenopathy and CD49d expression.[23] A study by Baumann et al assessed the correlation between splenomegaly and CD49d positivity and found a high percentage of CD49d-positive patients having splenomegaly as compared to the negative group; however, this was not statistically significant.[22] Ibrahim et al found a statistically significant correlation between CD49d and hepatomegaly,[13] while Abdel-Aziz et al did not find any such correlation.[35]

The published literature identifies a strong association between positive expression of CD49d, CD38, and ZAP-70, and these three markers have been found to be independent factors for the prognostication in CLL.[7,14,15,22] Some studies have found CD49d to be the best flow cytometry-based marker to stratify prognosis, since its expression is associated with poorer outcome, irrespective of the CD38 or ZAP-70 status.[13,14,31] These studies have reported that the omission of CD49d from the panel of flow-cytometric antibodies reduces the prognostic ability of the study. They have recommended that CD49d expression analysis should be included in the baseline prognostic assessment of newly diagnosed CLL cases.[14,15,22]

We detected immunophenotypic aberrancies in 12% of the CLL cases. CD5 negativity is the most common antigen aberrancy reported in around 7 to 20% cases of CLL.[36] We found one case of CD5-negative CLL. FMC7 expression in CLL is known to be associated with atypical morphology, trisomy 12, more frequent in CLL-PLL and PLL, and a poorer prognosis, as compared to FMC7 negative CLL.[24,26] Two of our cases showed aberrant immunoexpression of FMC7; one of these belonged to a higher stage (4/C), while the other was categorized as CLL-PLL, with atypical morphology and showed CD49d expression.

Unmutated IGHV is a strong predictive and prognostic indicator in CLL and is associated with a poor prognosis. The positive expression of CD49d has been found to be associated with an underlying IGHV-unmutated status, therefore conferring a poor prognosis to the patient.[7,14,22] CD49d overexpression is also associated with trisomy 12 and an aggressive clinical outcome.[37] This property of CD49d allows its use as a potential surrogate marker for underlying IGHV mutation and trisomy 12, both of which are associated with a poor outcome. Thus, CD49 could serve as an alternative to testing for these molecular abnormalities, which need a more specialized molecular laboratory setup than flow cytometry.

A humanized monoclonal antibody against CD49d (Natalizumab) is already available and is currently approved for the treatment of multiple sclerosis. It is now being tested in clinical studies of multiple myeloma and acute leukemias.[38] CD49d expression has been found in around 40% cases of CLL, and some studies have reported CD49d-positive CLL to have a different behavior than CD49d-negative ones.[13,14] Therefore, exploring the role of CD49d in CLL could have possible therapeutic implications for the use of Natalizumab in these patients in the near future.

Conclusion

The present study reiterates the emerging significance of CD49d as an independent prognostic marker in CLL. CD49d expression in our study correlated with poor prognostic parameters like higher prolymphocyte percentage, lymphadenopathy, splenomegaly, and higher Rai and Binet stages. We also found significant demographic differences in our CLL patients as compared to the published western literature, which were higher M:F ratio, lower age at presentation, higher proportion of symptomatic patients at diagnosis, and patients presenting in higher Rai and Binet stages.

Limitations

The sample size of this study was low since it was a time-bound study. Due to the limited duration of the study and the COVID pandemic, the patients could not be followed up. Also, due to budgetary constraints, molecular and cytogenetic analysis could not be done for all the patients, and hence, the association of CD49d expression with molecular and cytogenetic abnormalities could not be assessed.

Note

This study has been presented as an oral paper at Haematocon 2021. The study was approved by the Institutional Ethics Committee (letter no.: AIIMS/IEC/19/1166).

Authors' Contributions

A.K. contributed to data acquisition, analysis, and literature search; N.S. contributed to the concept, definition of intellectual content, literature search, manuscript preparation, manuscript editing, and manuscript review; A.K.G. contributed to manuscript editing and manuscript review; N.C. contributed to statistical analysis and manuscript review; U.K.N. contributed to clinical details and follow-up of patient and manuscript editing; and H.C. contributed to the definition of intellectual content and manuscript review.

Conflict of Interest
None declared.

References

1 Campo E, Ghia P, Montserrat E, et al. Chronic lymphocytic leukaemia/small lymphocytic lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al., eds. WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues. Revised 4th ed. Lyon,

France:: International Agency for Research on Cancer (IARC);; 2017:216–221

2 Saxena R, Kumar R, Sazawal S, Mahapatra M. CLL in india may have a different biology from that in the west. Blood 2016;128(22):5574

3 Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, Pasternack BS. Clinical staging of chronic lymphocytic leukemia. Blood 1975; 46(02):219–234

4 Binet JL, Auquier A, Dighiero G, et al. A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer 1981;48(01):198–206

5 Oscier DG, Gardiner AC, Mould SJ, et al. Multivariate analysis of prognostic factors in CLL: clinical stage, IGVH gene mutational status, and loss or mutation of the p53 gene are independent prognostic factors. Blood 2002;100(04):1177–1184

6 Boonstra JG, van Lom K, Langerak AW, et al. CD38 as a prognostic factor in B cell chronic lymphocytic leukaemia (B-CLL): comparison of three approaches to analyze its expression. Cytometry B Clin Cytom 2006;70(03):136–141

7 Gattei V, Bulian P, Del Principe MI, et al. Relevance of CD49d protein expression as overall survival and progressive disease prognosticator in chronic lymphocytic leukemia. Blood 2008;111(02):865–873

8 Crespo M, Villamor N, Giné E, et al. ZAP-70 expression in normal pro/pre B cells, mature B cells, and in B-cell acute lymphoblastic leukemia. Clin Cancer Res 2006;12(3 Pt 1):726–734

9 Wiestner A, Rosenwald A, Barry TS, et al. ZAP-70 expression identifies a chronic lymphocytic leukemia subtype with unmutated immunoglobulin genes, inferior clinical outcome, and distinct gene expression profile. Blood 2003;101(12):4944–4951

10 Hemler ME. VLA proteins in the integrin family: structures, functions, and their role on leukocytes. Annu Rev Immunol 1990;8(01):365–400

11 Bosch F, Muntañola A, Giné E, et al. Clinical implications of ZAP-70 expression in chronic lymphocytic leukemia. Cytometry B Clin Cytom 2006;70(04):214–217

12 Rassenti LZ, Huynh L, Toy TL, et al. ZAP-70 compared with immunoglobulin heavy-chain gene mutation status as a predictor of disease progression in chronic lymphocytic leukemia. N Engl J Med 2004;351(09):893–901

13 Ibrahem L, Elderiny WE, Elhelw L, Ismail M. CD49d and CD26 are independent prognostic markers for disease progression in patients with chronic lymphocytic leukemia. Blood Cells Mol Dis 2015;55(02):154–160

14 Shanafelt TD, Geyer SM, Bone ND, et al. CD49d expression is an independent predictor of overall survival in patients with chronic lymphocytic leukaemia: a prognostic parameter with therapeutic potential. Br J Haematol 2008;140(05):537–546

15 Bulian P, Shanafelt TD, Fegan C, et al. CD49d is the strongest flow cytometry-based predictor of overall survival in chronic lymphocytic leukemia. J Clin Oncol 2014;32(09):897–904

16 Molica S. Sex differences in incidence and outcome of chronic lymphocytic leukemia patients. Leuk Lymphoma 2006;47(08): 1477–1480

17 Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin 2020;70(01):7–30

18 Agrawal N, Naithani R, Mahapatra M, et al. Chronic lymphocytic leukemia in India—a clinico-hematological profile. Hematology 2007;12(03):229–233

19 Gogia A, Gupta R, Sharma A, Kumar L, Raina V, Rani L. Chronic lymphocytic leukemia: an Indian experience. J Clin Oncol 2019;37 (15, suppl):e19007–e19007

20 Gogia A, Sharma A, Raina V, et al. Assessment of 285 cases of chronic lymphocytic leukemia seen at single large tertiary center in Northern India. Leuk Lymphoma 2012;53(10):1961–1965

21 Okaly GVP, Nargund AR, Venkataswamy E, Jayanna PK, Juvva CR, Prabhudesai S. Chronic lymphoproliferative disorders at an Indian tertiary cancer centre—the panel sufficiency in the diagnosis of chronic lymphocytic leukaemia. J Clin Diagn Res 2013;7(07): 1366–1371

22 Baumann T, Delgado J, Santacruz R, et al. CD49d (ITGA4) expression is a predictor of time to first treatment in patients with chronic lymphocytic leukaemia and mutated IGHV status. Br J Haematol 2016;172(01):48–55

23 Strati P, Parikh SA, Chaffee KG, et al. CD49d associates with nodal presentation and subsequent development of lymphadenopathy in patients with chronic lymphocytic leukaemia. Br J Haematol 2017;178(01):99–105

24 Criel A, Michaux L, De Wolf-Peeters C. The concept of typical and atypical chronic lymphocytic leukaemia. Leuk Lymphoma 1999; 33(1-2):33–45

25 Frater JL, McCarron KF, Hammel JP, et al. Typical and atypical chronic lymphocytic leukemia differ clinically and immunophenotypically. Am J Clin Pathol 2001;116(05):655–664

26 Matutes E, Oscier D, Garcia-Marco J, et al. Trisomy 12 defines a group of CLL with atypical morphology: correlation between cytogenetic, clinical and laboratory features in 544 patients. Br J Haematol 1996;92(02):382–388

27 Buggins AGS, Levi A, Gohil S, et al. Evidence for a macromolecular complex in poor prognosis CLL that contains CD38, CD49d, CD44 and MMP-9. Br J Haematol 2011;154(02):216–222

28 Baldini L, Cro L, Calori R, Nobili L, Silvestris I, Maiolo AT. Differential expression of very late activation antigen-3 (VLA-3)/VLA-4 in B-cell non-Hodgkin lymphoma and B-cell chronic lymphocytic leukemia. Blood 1992;79(10):2688–2693

29 Nematollahi P, Shakery M, Kefayat A, Goli P. Association of CD49d expression with clinicopathological features of chronic lymphocytic leukemia patients in the Iranian population. Int J Physiol Pathophysiol Pharmacol 2020;12(01):32–39

30 Al-Rubaie HA, Thabit ZA, Jawad AM. CD49d as prognostic marker in B-cell chronic lymphocytic leukemia in correlation with the expression of CD38 ZAP-70 and clinical binet stage. Iraqi Postgrad Med J 2016;15(04):486–492

31 Rossi D, Zucchetto A, Rossi FM, et al. CD49d expression is an independent risk factor of progressive disease in early stage chronic lymphocytic leukemia. Haematologica 2008;93(10): 1575–1579

32 Oscier D, Else M, Matutes E, Morilla R, Strefford JC, Catovsky D. The morphology of CLL revisited: the clinical significance of prolymphocytes and correlations with prognostic/molecular markers in the LRF CLL4 trial. Br J Haematol 2016;174(05):767–775

33 van der Velden VHJ, Hoogeveen PG, de Ridder D, et al. B-cell prolymphocytic leukemia: a specific subgroup of mantle cell lymphoma. Blood 2014;124(03):412–419

34 Criel A, Verhoef G, Vlietinck R, et al. Further characterization of morphologically defined typical and atypical CLL: a clinical, immunophenotypic, cytogenetic and prognostic study on 390 cases. Br J Haematol 1997;97(02):383–391

35 Abdel-Aziz AF, Yahya RS, Abdel-Messih HM, Ata AM. Diagnostic value of CD49d expression in patients with chronic lymphocytic leukemia. Biochem Physiol 2019;8(01):249

36 Cartron G, Linassier C, Bremond JL, et al. CD5 negative B-cell chronic lymphocytic leukemia: clinical and biological features of 42 cases. Leuk Lymphoma 1998;31(1-2):209–216

37 Zucchetto A, Caldana C, Benedetti D, et al. CD49d is overexpressed by trisomy 12 chronic lymphocytic leukemia cells: evidence for a methylation-dependent regulation mechanism. Blood 2013;122 (19):3317–3321

38 Léger OJ, Yednock TA, Tanner L, et al. Humanization of a mouse antibody against human alpha-4 integrin: a potential therapeutic for the treatment of multiple sclerosis. Hum Antibodies 1997;8 (01):3–16