The utility of CD38 as a prognostic factor in patients with chronic lymphocytic leukemia

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Abstract

The current clinical staging systems for chronic lymphocytic leukemia (CLL) failed to predict which patients will experience an indolent or an aggressive course. Therefore several biological parameters need to be added to provide an insight into the prognosis of each patient and thus determine individualized treatment strategies.

Purpose: To estimate the applicability and predictive value of CD38 regarding clinical outcome in patients with CLL.
Patients and Methods: This study analyzed CD38 expression by flow cytometry in thirty newly diagnosed CLL patients and associated their expression with other prognostic factors including certain cytogenetic abnormalities (analyzed by FISH).
Results: Fifteen (50%) patients showed high expression of CD38 above 30% with median of 65.5% (range: 40%-88.3%). High $CD38^+$ B-cell percentages were significantly associated with unfavorable prognostic factors of CLL including male gender, high risk Rai stage, diffuse pattern of bone marrow infiltration, and high levels of LDH (p=0.01, 0.02, 0.02 and 0.04 respectively). No significant differences were detected regarding trisomy 12 and del 13q14 between the CD38-negative patients and the CD38-positive ones.
Conclusion: High percentages of CD38 (>30%) identified a subgroup of patients with aggressive CLL at diagnosis. Therefore this study strongly suggests that analysis of CD38 surface antigen might be added to the current staging systems of CLL being a valuable prognostic parameter that is easily assessed in clinical laboratories.

Introduction

Chronic lymphoid leukemia (CLL) is the most common type of adult leukemia and is characterized by the accumulation of monoclonal B cells typically positive for CD5, CD23, and CD19 and negative for surface CD22 and FMC7(1).
CLL patients have a highly variable clinical course and prognosis. While some patients show a stable disease and never require treatment, others suffer from a much more severe course requiring intensive treatment shortly or immediately after diagnosis (2).

The two major staging systems for CLL have provided valuable information in addressing this heterogeneity; however they have been unable to predict an indolent or aggressive course(3, 4).

For this reason, several parameters such as lymphocyte doubling time (LDT) (5), serum levels of β_2-microglobulin (6), soluble CD23 (sCD23)(7), serum thymidine kinase levels (8), bone marrow histology(9), have been added to the current staging systems to differentiate prognostic subsets.
Also some Chromosome and gene abnormalities have been identified to help in predicting the course of CLL, including 17p deletion, 11q deletion, trisomy 12, 13q deletion, and p53 gene mutations and deletions (10).

Few years ago, researchers described two or three prognostic groups of CLL. This distinction is based on the maturity of the lymphocytes as discerned by the immunoglobulin variable-region heavy chain (IgV_H) gene mutation status (11, 12).

High risk patients have an immature cell pattern with few mutations in the DNA in the IgV_H antibody gene region whereas low risk patients show considerable mutations of the DNA in the antibody gene region indicating mature lymphocytes(12).

However, the ability to sequence IgV genes is not available in most laboratories; hence an easily performed surrogate assay is desirable as an alternative to this expensive and labour-intensive procedure (12).

CD38 has been suggested by many scientists (13, 14, 15), as a surrogate marker and an independent prognostic factor in CLL. CD38 is a surface protein whose expression increases upon normal B-cell activation (16).

The connection between CD38 expression and IgV_H gene mutational status is not well understood. Both markers, however, probably reflect a common feature, such as maturation stage (17).

Patients with less that 30 percent CD38+ B-CLL cells are likely to have mutated IgV_H genes reflecting mature cell pattern and hence better prognosis, while patients with greater than 30 percent CD38+ B-CLL cells are more likely to have unmutated IgV_H genes, indicating less maturation and bad prognosis (13).

The relative ease and convenience of testing for CD38 has prompted us to analyze its expression on CD5+CD19+ leukemic cells, using flowcytometry, and correlate the results with other risk factors to explore the utilility of CD38 as prognostic factor in patients with CLL.

Patients and methods

Thirty newly diagnosed B-CLL patients, presenting to Clinical oncology and Clinical pathology departments, Kasr Al-aini, Cairo University, were enrolled in this study. Ethical approval was obtained from the institutional Ethics committee. Informed consent was obtained from all participants.

The diagnosis of CLL was based on clinical characteristics, peripheral blood, bone marrow morphology, and immunophenotyping (the National Cancer Institute criteria) (18).

Flow cytometric detection of CD38

Flow cytometric analysis of CD38 was performed on fresh blood samples stained with CD5-FITC (BD Biosciences), CD19-PE (BD Biosciences), and CD38-PE (BD Biosciences). Isotype controls were run to distinguish positive from negative cells. CLL cells (CD5+CD19+) were gated, and CD38+ cells were measured in CD5+CD19+ lymphocyte population. A FACSCalibur flow cytometer (BD Biosciences) and Cell Quest software (BD Biosciences) were used to acquire and analyze data. The cut-off point for CD38-positive in CLL cells was >30%.

Rai staging system

Patients were categorized into low, intermediate and high risk groups according to the Rai staging system (3), which divides CLL into 5 stages: 1-Rai stage 0: The blood lymphocyte count over 10,000 lymphocytes/mm^3 of blood (lymphocytosis), 2- Rai stage I: Lymphocytosis plus enlarged lymph nodes, 3-Rai stage II: Lymphocytosis plus enlarged spleen (and possibly enlarged liver), with or without enlarged lymph nodes, 4- Rai stage III: Lymphocytosis plus anemia, with or without enlarged lymph nodes, spleen, or liver, and 5-Rai stage IV: Lymphocytosis plus thrombocytopenia, with or without anemia, enlarged lymph nodes, spleen, or liver.

Stage 0 is considered low risk, Stages I and II are considered intermediate risk and Stages III and IV are considered high risk.

Measurement of LDH

The LDH levels were measured using Dimension RXL blood chemistry analyzer (DADE BEHRING) with detection limits of 190 U/L.

Bone marrow biopsy

Patients were subjected to bone marrow biopsy to provide the pattern of lymphoid infiltration in the marrow specimen which is used as useful prognostic information (diffuse involvement correlates with progressive or advanced disease, while nodular or interstitial patterns predict a better prognosis). The samples were obtained from the back of the hip bone using local anesthesia.

Detection of molecular cytogenetic aberrations by fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) analysis was performed to detect prognostically relevant anomalies of chromosomal regions 13q, and chromosome 12, using the following fluorescent labeled probes: LSI D13S319 (13q14), and CEP12 (centromere 12) (all probes purchased from Vysis, USA). FISH was performed according to previously described protocols (19). The cut-off levels for positive values, determined from samples of 5 cytogenetically normal persons, was 10% and 7% for, del(13q14) and trisomy 12, respectively.

Statistical analysis

Data were statistically described and expressed as mean ± standard deviation (± SD), median, range, frequency and percentage when appropriate. Comparison of quantitative variables between the study groups was done using Mann Whitney *U* test for independent samples. A probability value (p value) less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs Microsoft Excel 2003 (Microsoft Corporation, NY, USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

Results

This study comprised 30 CLL patients. There were 20 males and 10 females with a mean age of 56.4 years ± 7.9. Patients characteristics are shown in table 1. All patients had a lymphocytosis at least 5×10^9/L with >50% B lymphocytes with monoclonal surface immunoglobulin, CD20, CD5, CD19 and CD23 expressions.

The median age of patients with more than 30% $CD38^+$ was almost the same as those with less than 30% $CD38^+$ (median, 55; range, 49-70 vs. median, 53; range, 44-75).

Table 1 Patients characteristics

Characteristics	No of patients (%)
Gender Male (no, %) Female (no, %)	 20 (66.7%) 10 (33.3%)
Age (years) Median (range)	 54.5 (44-75)
Hb (g/dl) Mean ± SD	 9.68 ±2.52
WBCs (x109/l) Median (range)	 74.9(14.1-205)
Platelet (x109/l) Median (range)	 115(62-245)
Lymohocytic count (%) Median (range)	 84 (50-95)
LDH (U/L) Median (range)	 127.5 (89-884)

CD38 expression

In this study, the median percentage of CD38 expression in all patients was 34% (range: 13.8%-88.3%). The patients were considered positive with more than 30% B cells expressing CD38. This threshold value was recommended by National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology. Accordingly 15 patients (50%) out of the 30 cases involved were considered positive with median percentage of CD38 expression of 65.5% (range: 40%-88.3%) (Figure1).

Correlations between positive CD38 (>30%) expression and disease features

High percentages of CD38 (>30%) identified a subgroup of patients with aggressive CLL at diagnosis. These high percentages were significantly associated with unfavorable prognostic parameters of CLL including male gender, high risk Rai stage, diffuse pattern of bone marrow infiltration (Figure 2), and high level of LDH (p=0.01, 0.02, 0.02 and 0.04 respectively)(Table 2).

To evaluate the relation of genomic aberrations and CD38 expression, interphase cytogenetic analyses by FISH were carried out in leukemia cells of 20 patients. Del(13q14) was more frequent in CD38-negative patients than in CD38-positive ones, whereas trisomy 12 was more frequent in the CD38-positive than the CD38-negative patients, however the differences between the two groups regarding both cytogenetic abnormalities were not significant (Table 2).

Table 2: Correlations between positive CD38 (>30%) expression and disease features

Factors	Cases (%)	Positive CD38 (%)	P value
Gender (n = 30)			
Male	20 (66.7%)	13 (65%)	0.01
Female	10 (33.3%)	2 (20%)	
Rai staging(n = 30)			
Low	0	0	
Intermediate	14 (46.7%)	4 (28.6%)	
high	6 (36.6%)1	11 (68.75%)	0.02
Bone marrow biopsy (n = 25)			
Diffuse	13(52%)	10 (76.9%)	0.02
Nodular	12(48%)	4 (33.3%)	
LDH (n = 28)			
Normal	15(53.6%)	5 (33.3%)	
High	13(46.4%)	10 (76.9%)	0.04
Cytogenetic abnormalities 1-del(13q14) (n = 20)			
Absent	16(80%)	7 (43.75%)	0.05
Present	4(20%)	1 (25%)	
2-Trisomy 12 (n = 20)			
Absent	15(75%)	5 (33.3%)	0.08
Present	5(25%)	2 (40%)	

Discussion

CLL is a heterogeneous disease in which some patients undergo a slowly progressive clinical course, but most will eventually enter an advanced phase requiring recurrent treatment (20).

Numerous studies have searched for reliable prognostic markers of predicting the progression and outcome of CLL. Clinical staging systems (3, 4), have been identified as important, independent prognostic factors. However, these systems fail to identify individual patients at risk for progression, especially in the early stage.

Hence, the challenge was to identify other molecular parameters in order to separate patients into different risk groups, so that in some cases a clinical decision for the urgency of a more aggressive therapy is justified.

In the past few years, the absence of mutations in the IgV genes (12), the presence on the cell surface of CD38 (14), and in the cytoplasm of ZAP-70 (21) have been reported as important tools to define progressive cases at an early stage who might benefit from early and intensive therapy.

IgVH mutation status conferred important prognostic information. However IgVH gene sequencing was not available in most laboratories; therefore, surrogates for IgVH mutation were looked for (11).

Damle et al (13), reported that CD38 positivity can predict IgVH gene mutation status in most cases. They found that patients with more than 30% $CD38^+$ expression in B-CLL, contained unmutated V genes, whereas samples expressing less than 30% $CD38^+$ contained all the mutated cases. Accordingly CD38 can be used as a surrogate marker for IgVH mutation and hence a proper prognostic marker.

Our present study identifies a distinct subset of B-CLL, expressing high CD38 percentages above 30%.This threshold value was previously used by Damle et al(13), and Del Poeta et al (14). Accordingly 15 (50%) of our patients were positive for $CD38^+$ expression ranging from 40%-88.3%.

This study confirms the association between CD38-positivity (>30%) and some of the known bad prognostic parameters for CLL. Positive $CD38^+$ was highly correlated with male gender (p=0.01), high levels of LDH (p=0.04), and diffuse pattern of bone marrow involvement (p=0.02).

Also, we compared the clinical stage at the time of diagnosis by using the Rai system as a function of the percentages of $CD38^+$ cells. Higher CD38 percentages were closely associated with the high risk Rai stages (p = 0.02).

These findings are in agreement with several studies which supported the correlation of CD 38 expression with other prognostic markers of CLL (14, 15, 22).

Previous studies also correlated CD38 expression with poor DFS and OS (13, 14, 23). Del Poeta et al (14), reported that patients with lower $CD38^+$ B-cell percentages had significantly longer progression-free survival (P = .00006) and significantly longer overall survival (p< .00001) than patients with more than 30% $CD38^+$expression. Furthermore, in their study the risk of partial or no response to fludarabine increased with increasing CD38 expression (p = .003).

A major pitfall that might hamper a confident prognostic use of CD38 expression is the possibility that CD38 expression may vary over time raising the suspicion that CD38 may be an unstable, hence unreliable, marker(24). However, Ghia et al (25), reported that despite CD38 expression may vary during the clinical course of CLL, these changes do not modify the overall prognostic prediction made at diagnosis.

Within this limitation, D'Arena et al(26), proposed that concomitant evaluation of ZAP-70 and CD38 expression allows better and more accurate separation of CLL patients in prognostic subgroups and suggest that their simultaneous assessment should have a definitive place in the staging systems of CLL.

The role of CD38 in CLL extends beyond being a mere negative prognostic marker. CD38 has also been proposed as a pathogenetic agent in CLL directly involved in determining a more severe clinical course, by controlling a signaling pathway leading to increased survival and proliferation of the neoplastic cells (27).

In conclusion, our study highly suggests that the evaluation of CD38 expression allows the stratification of CLL patients into prognostic subgroups and helps to identify patients who might benefit from early and more intensive therapy.

The facts that CD38 positivity can predict unmutated IgVH gene mutation status in most cases, together with its easy applicability, render CD38 a valuable tool in the routine diagnostics of CLL, and suggest that its assessment should become an integral component of the CLL diagnostic grid.

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Figures

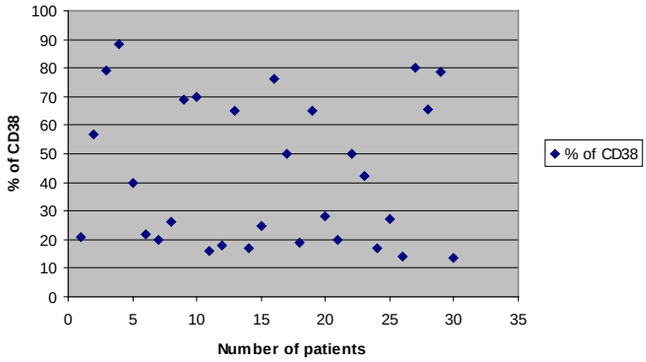

Fig1: Scatter plot showing CD38 percentages in CLL patients:
Each dot represents the percentage of CD38 in the corresponding patient: Fifteen (50%) patients show high percentages of CD38 above 30%.

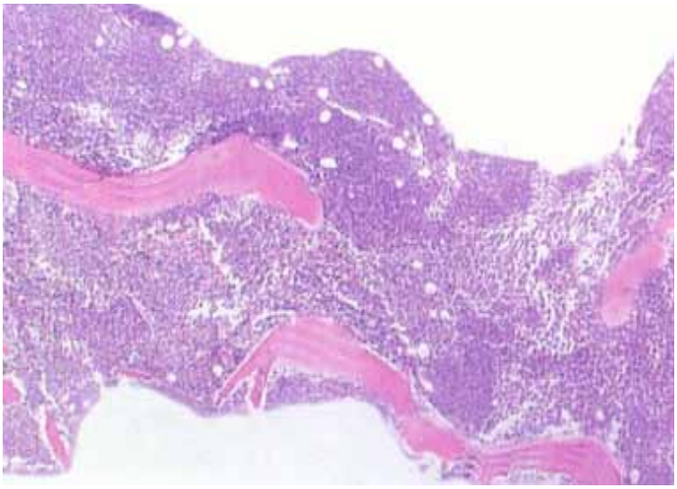

Fig2: Bone marrow biopsy in CLL patient showing a diffuse pattern.