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Research Paper

Prognostic Value of Soluble CD200 Concentration in Chronic Lymphocytic Leukemia and Its Correlation with Clinical and Laboratory Parameters

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ABSTRACT:

BACKGROUND:

Chronic lymphocytic leukemia (CLL) is a malignancy of mature B-lymphocytes characterized by their accumulation in the bone marrow and peripheral blood. CD200 is an immune checkpoint molecule critical for immune tolerance, and its overexpression has been associated with tumor progression through immune evasion mechanisms, particularly via interaction with CD200R.

OBJECTIVE:

To assess plasma levels of soluble CD200 (sCD200) in newly diagnosed CLL patients compared to healthy individuals. To correlate sCD200 levels with disease stage, hematological parameters, and prognostic markers: β2-Microglobulin (β2M) and Leukocyte Associated Immunoglobulin-like Receptor-1 (LAIR-1).

PATIENTS AND METHODS:

This cross-sectional study was conducted over six months (Dec 2023 – May 2024) at Baghdad Teaching Hospital and involved 68 adult patients newly diagnosed with CLL (age range: 42–83 years) and 20 healthy controls. Diagnosis relied on morphology and flow cytometry. The Binet staging system was used for clinical classification. Plasma levels of sCD200 and β2M were measured via ELISA.

RESULTS:

Plasma levels of CD200 and β2M were significantly higher in CLL patients than in healthy controls (p=0.001). Statistically significant differences across Binet stages were observed in plasma CD200, β2M, Hb, WBC, ALC, and platelet counts (p-values: 0.01–0.001). No significant differences were found for smudge cells (SCs)% and age. CD200 positively correlated with β2M (p=0.02). LAIR-1 expression showed no association with CD200 or β2M levels.

CONCLUSION:

CLL patients exhibited significantly elevated plasma CD200 levels, which increased with disease progression. CD200 also correlated positively with β2M. No association was found with LAIR-1 expression or SCs%.

KEYWORDS: Prognostic, soluble CD200, chronic lymphocytic leukemia, clinical, laboratory parameters.

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INTRODUCTION:

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in the Western world. It is characterized by the clonal expansion of small, mature-appearing CD5-positive B lymphocytes in the blood, bone marrow, lymph nodes, and spleen [(1)]. CLL progression is primarily due to impaired apoptosis and microenvironmental survival signals. These contribute to immune dysfunction, increasing the risk of recurrent infections, which are a leading cause of morbidity and mortality [(2)]. To better predict disease course, models like the Rai, Binet staging system are used in clinical settings [(3)]. Then CLL International Prognostic Index (CLL-IPI) have been established. CLL International Prognostic Index (CLL-IPI) integrates clinical and biological factors such as TP53 status, IGHV mutation, β2M levels, age, and clinical stage for improved risk stratification [(4)]. CD200, a membrane glycoprotein of the immunoglobulin superfamily, is consistently overexpressed in CLL and aids in distinguishing it from similar malignancies like mantle cell lymphoma [(5)]. Notably, CD200 exists in a soluble form (sCD200), which retains only the extracellular domains. Biochemical evidence suggests that

sCD200 results from proteolytic shedding, similar to other soluble immunoregulatory molecules, and may reflect tumor burden in hematologic malignancies [6]. β2-microglobulin (β2M), a component of MHC class I molecules, is elevated in several hematologic malignancies and correlates with tumor load and adverse prognosis [7,8,9]. Leukocyte-associated immunoglobulin-like receptor 1 (LAIR-1/CD305), an inhibitory receptor expressed on most immune cells, has also been explored for its prognostic implications in CLL. Limited studies suggest that reduced LAIR-1 expression is associated with more aggressive disease phenotypes, although its precise prognostic value remains under investigation [10]. While previous studies explored sCD200's prognostic role, few have addressed this in Middle Eastern populations. This study investigates sCD200 and β2M levels, their relationship with clinical stage, and association with LAIR-1 in Iraqi patients, adding to global CLL insights.

AIMS OF THE STUDY:

This study aims to evaluate the plasma level of sCD200 in newly diagnosed CLL patients compared to healthy controls. Additionally, it seeks to correlate sCD200 levels with disease stage, hematological parameters, and key prognostic markers including β2M and LAIR-1.

PATIENTS AND METHODS:

This cross-sectional study was conducted from December 2023 to May 2024 at the Hematology Outpatient Clinic of Baghdad Teaching Hospital, Medical City. A total of 88 participants were enrolled: 68 newly diagnosed, treatment-naïve CLL patients (Group I) and 20 age- and sex-matched healthy controls (Group II), confirmed by complete blood count (CBC) and clinical history.

CLL diagnosis was based on peripheral blood morphology and immunophenotyping using eight-color flow cytometry (BD FACS Canto II, USA) at the National Center for Teaching Laboratories. Inclusion criteria included newly diagnosed CLL cases with a flow cytometric score of 4 or 5. Patients with other hematologic malignancies or a history of prior CLL treatment were excluded.

Sample size was calculated using the Raosoft® sample size calculator (http://www.raosoft.com/samplesize.html), indicating a minimum of 65 participants at a 90% confidence level and a 5% margin of error; accordingly, 68 CLL patients were recruited. This sample size was approved by the Institutional Review Board (IRB-RP No. path38, dated 21/04/2024). The relatively small control group is acknowledged as a study limitation.

Ethical approval was obtained, and verbal consent was acquired in accordance with guidelines for minimal-risk research, ensuring that participants were fully informed about the study's aims and the voluntary nature of participation.

Four milliliters of peripheral blood were collected in K3-EDTA tubes. CBC, reticulocyte count, and blood film examination were performed using a Sysmex hematology analyzer (Japan), following standard staining procedures (Bain & Lewis, 2021). Smudge cell percentage (SC%) was calculated using the formula: smudge cell (SC)% = (SC / [200 lymphocytes + SCs]) × 100.

Reticulocytes were manually counted using New Methylene Blue staining. Clinical staging was determined according to the Binet system [11].

Plasma was separated by centrifugation at 1000×g for 15 minutes and stored at −80°C. Soluble CD200 and β2-microglobulin (β2M) levels were measured by ELISA (ELK Biotechnology, USA) using sandwich-based kits, with sensitivities of 12.9 pg/mL and 0.16 ng/mL, respectively.

Data were analyzed using SPSS version 26.0 and Microsoft Excel 2021. Due to non-normal data distribution, nonparametric tests (Kruskal-Wallis, Mann-Whitney U) were applied. Correlations were assessed using Spearman's test, and the chi-square test was used for categorical variables. A p-value < 0.05 was considered statistically significant.

RESULTS:

This study included 68 newly diagnosed CLL patients with a mean age of 61.8 ± 10.5 years (range: 42–83 years). The majority (33.8%) were in the 60–69 age group (Table 1). Males represented 63.2% of the cohort, yielding a male-to-female ratio of 1.7:1. Clinical staging using the Binet system classified 42.7% of patients as Stage A, 27.9% as Stage B, and 29.4% as Stage C.

Hematological analysis revealed a median hemoglobin (Hb) level of 12.0 g/dL and a median white blood cell (WBC) count of 50.6 $\times 10^9$/L. The absolute lymphocyte count (ALC) had a median of 42.0 $\times 10^9$/L, while the platelet count (PLT) was 170.5 $\times 10^9$/L. The median smudge cell percentage (SC%) was 16.0% (Table 2).

Plasma levels of soluble CD200 (sCD200) were significantly elevated in CLL patients (median: 103.2 pg/mL) compared to healthy controls

(median: 69.2 pg/mL; p = 0.001). Notably, 39.7% of CLL patients exceeded the upper normal limit of 149 pg/mL, and 29.4% of these were in advanced Stage C (Table 3). A statistically significant positive correlation was found between sCD200 levels and age (r = 0.255, p = 0.036). However, no significant correlations were observed between sCD200 and Hb, WBC, ALC, PLT, or SC% (Table 4).

β2-microglobulin (β2M) levels were also significantly elevated in CLL patients (median: 2.40 ng/mL) compared to controls (median: 1.76 ng/mL; p = 0.001) (Table 5). A positive correlation was observed between sCD200 and β2M concentrations (r = 0.276, p = 0.023), suggesting a shared association with disease burden (Figure 1).

Stratification by Binet stage revealed significantly higher levels of sCD200, β2M, WBC, and ALC with advancing disease stage ($p < 0.05$). Conversely, hemoglobin and platelet counts decreased significantly with disease progression. Age and smudge cells (SCs)% did not vary significantly across stages (Table 6; Figures 2,3). Post hoc analysis confirmed significant differences in sCD200 and β2M levels between Stage A and B, and between Stage B and C.

Assessment of LAIR-1 expression indicated that 50% of patients were LAIR-1 positive. No significant differences in sCD200, β2M, or hematological parameters were detected between LAIR-1 positive and negative patients. Furthermore, LAIR-1 expression was not significantly associated with clinical stage (Table 7).

Table 1: Age distribution of CLL patients.

Age group (years)	N	Percentage (%)
<50	10	14.7%
50-59	16	23.5%
60-69	23	33.8%
≥70	19	28%

Table 2: Hematological parameters of CLL patients.

Parameters	Mean	Median	SD	IQR	Range
Hb (g/dl)	11.5	12.0	2.4	3.9	5.0-16.4
WBC ($\times 10^9$/L)	78.8	50.60	80.0	70.4	13-466
ALC ($\times 10^9$/L)	67.4	42.0	74.2	58.6	8.4-465
PLT ($\times 10^9$/L)	199.7	170.50	97.6	111.7	39-518
Smudge Cells (%)	15.5	16.0	3.9	6.0	5.0-21

Table 3: Plasma CD200 Level in CLL patients vs. Healthy Controls.

Statistic	Healthy Controls (n=20)	CLL Patients (n=68)	*P*-value
Median	69.22	103.23	0.001
Mean± SD	76.55± 27.59	208.24± 265.62	
IQR	33	131	
Range	39- 149	24- 1467	

Table 4: Correlation between CD200 and clinical parameters (Spearman Test).

Variables	Correlation Coefficient (r)	*p*- value
Age	0.255	0.036
Smudge Cells (%)	0.227	0.06
WBC ($\times 10^9$/L)	-0.164	0.182
ALC ($\times 10^9$/L)	-0.127	0.304
Hb (g/dL)	0.120	0.331
PLT ($\times 10^9$/L)	-0.004	0.973

Table 5: β2-Microglobulin Levels in CLL patients vs. Healthy Controls.

Statistic	CLL Patients (n= 68)	Healthy Controls (n= 20)	*P* value
Median (ng/mL)	2.399	1.763	0.001
IQR	0.99	0.86	
Range	0.71- 24.55	0.75- 2.59	

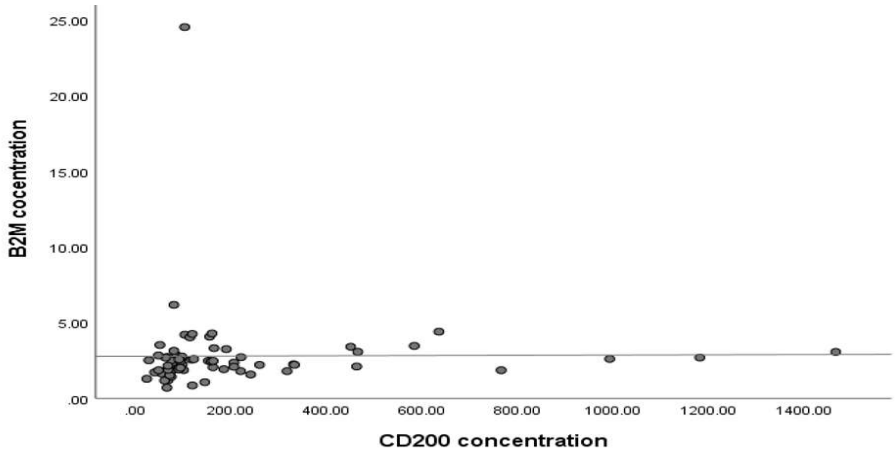

Figure 1: Correlation between plasma CD200 and β2M Level in CLL patients.

Table 6: Key Variable across Binet Stages in CLL Patents.

parameter	Stage A (n=29)	Stage B (n=19)	Stage C (n=20)	*P*- value
Plasma CD200 (pg/mL)	90.91	164.44	110.38	0.010
Plasma β2M (ng/mL)	1.90	2.48	2.90	0.00
Age	62.0	65.0	60.0	0.278
Smudge Cells (%)	17.0	16.0	15.0	0.408
Hb (g/dL)	12.1	12.4	9.7	0.030
ALC×10^9/L	35.0	42.0	88.0	0.021
WBC×$10^{9/L}$	39.0	51.0	101.95	0.027
PLT×10^9/L	178.0	197.0	139.0	0.017

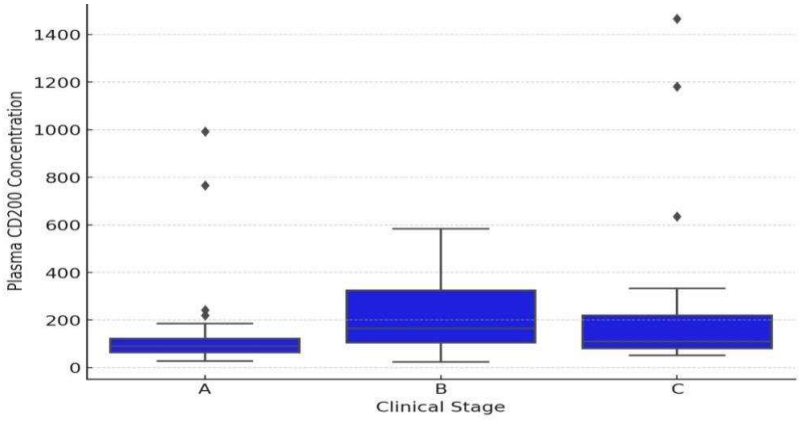

Figure 2: Plasma CD200 across Binet Stage.

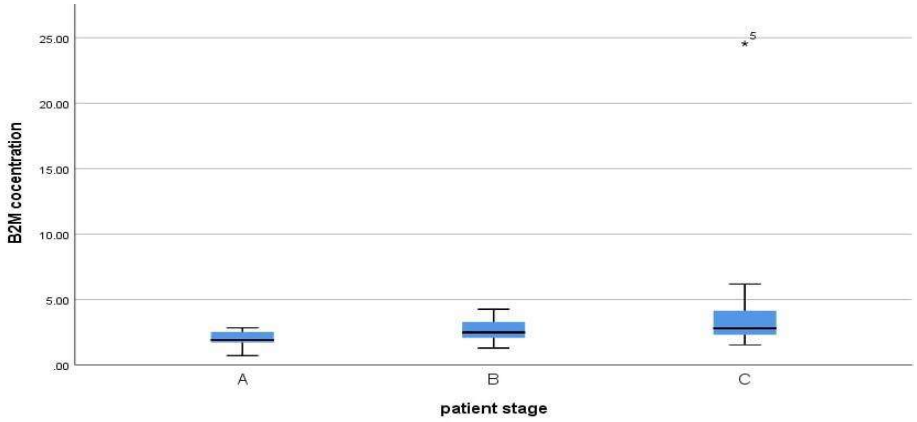

Figure 3: Plasma β2M across Binet Stage.

Table 7: Comparison of Clinical Variables by LAIR-1 Expression.

Parameter	LAIR-1 positive (n=34)	LAIR-1 negative (n=34)	*P*-value
Hb (g/dL)	12.05	11.95	0.787
WBC $\times 10^9$/L	52.0	44.8	0.477
ALC $\times 10^9$/L	43.5	37.4	0.387
PLT $\times 10^9$/L	158.0	200.0	0.090
Binet Stages A:B:C	16:6:12	13:13:8	0.158

DISCUSSION:

Chronic lymphocytic leukemia (CLL) exhibits marked heterogeneity in clinical behavior, ranging from indolent to aggressive courses. This study investigated the prognostic relevance of soluble CD200 (sCD200) and its association with β2-microglobulin (β2M), clinical staging, and hematological parameters in newly diagnosed CLL patients.

In the present study, the mean age of patients was 61.8 ± 10.5 years, aligning with Iraqi studies conducted in 2017 and 2021 [12,13]. The median age of patients (62 years) and male predominance (63.2%) are consistent with regional data from Iraq and Turkey [14,15], though slightly younger than Western cohorts, where the median age at diagnosis is approximately 70 years [16,17]. This demographic trend may reflect population-specific factors or differences in healthcare access and timing of diagnosis. Male predominance was observed in this cohort, with a male-to-female ratio of 1.7:1, in agreement with previous Iraqi and international studies [17,18,19]. This gender disparity may reflect hormonal, genetic, or environmental influences, including potential protective factors linked to X-chromosome-associated genes.

Using the Binet staging system, most patients were classified as Stage A (42.6%), consistent with earlier Iraqi studies [18,19]. However, some studies have reported higher rates of late-stage diagnosis [7,20], possibly reflecting earlier detection in this study due to improved awareness and diagnostic capabilities.

Hematologic findings showed a median hemoglobin (Hb) level of 12 g/dL, comparable to other local studies [15,21], and likely influenced by the higher proportion of early-stage patients. All patients were leukocytic, with median absolute lymphocyte counts (ALC) and platelet counts in line with previous Iraqi data [20]. These findings support the typical hematological profile of CLL and its progression-associated cytopenias.

All patients demonstrated CD200 positivity by flow cytometry. Elevated sCD200 levels in CLL patients compared to controls corroborate findings from Italian (2021) and Egyptian (2023) studies [6,22] and are supported by broader evidence from Canadian and Chinese studies on CD200 in malignancies [23,24]. CD200, an immune checkpoint molecule, is known to inhibit anti-tumor responses via CD200R-mediated immunosuppression. Its overexpression in CLL not only aids in diagnosis but may also reflect tumor burden and immune evasion [23,25].

Our observation that sCD200 levels rise with advancing Binet stage supports its prognostic

relevance, aligning with reports suggesting that soluble CD200 reflects disease progression and immune dysfunction. Interestingly, sCD200 levels were significantly correlated with age, as also reported by the Italian study in 2021 [6]. No significant associations were observed between sCD200 and hematological parameters such as Hb, WBC, ALC, or platelet count, echoing previous findings [6]. However, sCD200 showed a significant progressive increase with advancing Binet stage, reinforcing its association with tumor burden and immune suppression, in agreement with prior studies [6,26].

Statistically significant differences in hemoglobin, platelet counts, WBC, and ALC across Binet stages agree with a previous Nigerian study in 2021 [27], reflecting marrow infiltration and disease burden. No significant differences in age or smudge cells percentage (SCs%) across stages were observed, consistent with Iraqi [13] and Iranian studies [28], but differing from Senegalese data [29]. The lack of variation in smudge cell percentage and age across stages parallels earlier regional findings. As expected, β2-microglobulin (β2M) levels increased with disease stage, consistent with earlier Iraqi research [18], affirming its role as a prognostic marker associated with tumor burden and disease severity. β2M, a well-established tumor burden marker, was significantly elevated and positively correlated with sCD200, confirming reports from Italian and Egyptian studies [6,22]. This further supports the potential utility of sCD200 as a surrogate marker of disease activity. Prior studies have demonstrated β2M's prognostic value in both CLL and multiple myeloma.

Contrary to earlier Egyptian findings [30], which suggested that LAIR-1 expression may influence prognosis, our study found no significant association between LAIR-1 status and CD200, β2M, clinical stage, or hematological parameters. These discrepancies could stem from differences in sample size or assay sensitivity.

Despite these findings, this study has notable limitations. The small control group (n=20) limits the generalizability of comparisons. Additionally, selection bias may have influenced the sample, as all patients were recruited from a single center and restricted to newly diagnosed, treatment-naïve cases.

CONCLUSION:

This study demonstrates that soluble CD200 levels are significantly elevated in patients with chronic lymphocytic leukemia and increase with advancing Binet stages, suggesting a role in disease progression and immune regulation. A significant positive correlation between sCD200 and β2-microglobulin further reinforces its potential as a prognostic biomarker. Hematological parameters such as hemoglobin, white blood cell count, absolute lymphocyte count, and platelet count also varied significantly with disease stage, reflecting disease burden. However, smudge cell percentage and LAIR-1 expression showed no stage-related variation or correlation with sCD200 or β2M levels.

Recommendations:

Further studies involving larger patient and control groups, along with extended follow-up periods, are needed to gather data on progression-free survival (PFS) and overall survival (OS) in patients with high sCD200 levels. Additionally, investigating the correlation of sCD200 levels with other biological and clinical prognostic factors—such as CD38, ZAP-70, and IGHV mutation status—is recommended. Further research is also necessary to evaluate the therapeutic potential of targeting CD200 in CLL, which may offer new insights into treatment strategies.

Conflict of Interest Statement:

The authors declare no conflict of interest.

Authors' Contributions

Dhuha Fawzi Fakhri conducted the research, including study design, patient recruitment, data collection, and laboratory work. Israa Mohammed Baqir Al-Bayaa supervised the study, provided guidance throughout the research process, performed the statistical analysis, and interpreted the results. Both authors contributed to drafting the manuscript and approved the final version.

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