RESEARCH ARTICLE

Editorial Process: Submission:01/09/2023 Acceptance:06/19/2023

Prognostic Value of Serum CD200 Concentrations in Chronic Lymphocytic Leukemia

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Abstract

Background: little is known regarding the prognostic value of soluble CD200 (sCD200)in chronic lymphocytic leukemia patients. Therefore, the objective of our study is to address the prognostic value of sCD200 antigen concentration on CLL patients outcome. **Methods:** Determination of serum sCD200 was done using ELISA kit in 158 CLL patients at diagnosis before start of therapy beside 21 healthy controls. **Results:** sCD200 concentration levels was significantly higher in CLL patients as compared to healthy controls. High sCD200 was associated with poor prognostic markers (high expression of CD38+% and ZAP70+, high LDH, high risk Rai stages, unfavorable cytogenetic finding, time to first treatment (TTT) as well as patients outcome (P<0.001 for All). sCD200 at cut-off value (752.5 pg/ml) could predicts TTT with specificity 83.4%. **Conclusion:** Determination of sCD200 concentration levels at diagnosis could be used as a prognostic biomarker in CLL patients.

Keywords: CLL- Prognosis- sCD200

Asian Pac J Cancer Prev, **24 (6)**, 1943-1948

Introduction

Chronic lymphocytic leukemia (CLL) is very heterogeneous disease at both the clinical and the biological levels. It is typically occurs in old age patients. While a subgroup of CLL patients have an indolent clinical course for many years, another one require immediate treatment. Risk stratification of CLL patients at diagnosis facilitates proper clinical decision. Several prognostic scores have been postulated including clinical staging (Binet and Rai stages) as well as international prognostic index (Hallek and Al-Sawaf 2021) .

Recently, CD200 expression has emerged as a useful tool to better discriminate between a typical CLL and mantle cell lymphoma (Hallek et al., 2018). In addition to the usefulness of this marker in the diagnostic setting, little studies have addressed the prognostic significance in CLL patients (Hallek and Al-Sawaf 2021, Fouad et al., 2018).

CD200 is present 2 forms, cellular forms and soluble plasma one. The cellular forms of CD200 glycoprotein is a single-pass, type I, highly conserved membrane protein, belonging to the immunoglobulin superfamily, spanning the membrane once, with the N-terminus on the extracellular side of the membrane. It is composed of two extracellular (one variable and one constant) immunoglobulin-like domains, a single trans-membrane region, and a cytoplasmic tail (D'Arena et al., 2020).

Biochemical analyses of the soluble CD200 (sCD200) demonstrated that this portion contains only the extracellular part of CD200 and did not contain cytoplasmic part, a finding that is consistent with codomain shedding in other products. Previous studies suggested that soluble CD200 levels were correlated with tumor burden. (Aref et al., 2015; Aref et al., 2017, Aref et al., 2020; Wong et al., 2016, D'Arena et al., 2021).

sCD200, by means of the interaction with its receptor, induces the suppression of T-cell mediated responses, limiting inflammation in a wide range of inflammatory diseases. The inhibition of macrophage function, induction of regulatory T-helper cell type (Th1) to Th2 cytokine profile switch, suppression of natural killer cell function, and inhibition of tumor-specific T-cell immunity have been all experimentally demonstrated (D'Arena et al., 2020).

This study aimed to address the prognostic significance of sCD200 concentration levels at diagnosis in CLL patients.

Materials and Methods

Subjects

In a prospective study included 158 CLL patients (100 male; 58 female); at MUOC, Mansoura, Egypt. In the period from January 2015- January 2020. The CLL diagnosis was based on morphological assessment of blood and bone marrow smear, absolute Lymphocytes blood count ($\geq 5.0X10^9/L$), and immunophenotyping

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(CD5+/CD19+; CD23+, FMC7-, sIgM-, CD200+, Kappa or Lambda restriction) using flowcytometry.

The study protocol was approved by IRB of Mansoura Faculty of Medicine. All included patients were subjected for full history taking, clinical assessment (Rai staging) and FISH cytogenetic detection of 17p del, 13q del, 11q del, Trisomy 12. For all included CLL patients sCD200 was estimated before start of therapy as well as for 21 normal controls of matched age and sex . The healthy controls recruited from blood bank donors. All patient data including age, complete hemogram, and course of the disease were collected from the patient's medical record sheet.

Methods

Venous blood samples from patients and controls were collected in blank tube and left to clot at room temperature. The serum samples were separated by centrifugation and then were poured in 1.5 ml aliquot and stored at -80°C until analysis.

CD200 Human ELISA Kit (Invitrogen by Thermo Fisher Scientific)

The serum concentration of sCD200 was determined using enzyme linked immunosorbent assay (ELISA) kit, that was obtained from Thermo Fisher, USA. The procedure was done according manufacturer protocol.

Procedure

In 96-well plate human (the wells coated by CD200 antibody), we added 100 µL of provided standards; patients and controls serum samples.

- The plate wells was covered and incubate for 2.5 hours at room temperature with gentle shaking. Then the wells solution were discard and washed 4 times with 1X Wash Buffer. Using automatic washer. After the last wash, any remaining wash Buffer by was removed by invert the plate and blot it against clean paper towels.

- Then we added 100 µL of prepared biotin conjugate to each well.

- Then the plated was incubated for 1 hour at room temperature with gentle shaking.

- Then we discard the solution. Repeat the washing 3 times.

- Then we added 100 µL of prepared Streptavidin-Horseradish peroxidase (HRP) solution to each well.

- After that the plate was incubated for 45 minutes at room temperature with gentle shaking.

- Then we discard the solution and 3 times washing were done

- Then we added 100 µL of Tetramethylbenzidine (TMB) substrate to each well. The substrate will begin to turn blue.

- Then we Incubated the plate for 30 minutes at room temperature in the dark with gentle shaking.

- After that we added 50 µL of Stop Solution to each well. The solution in the well changes from blue to yellow.

- Then we read the absorbance at 450 nm. The reading was done within 30 minutes after adding the Stop Solution. The minimum detectable dose of human CD200 is 20 pg/mL.

Statistical analysis

Statistical analysis was performed using the Statistical 12 software. Mann-Whitney- U test was used to compare sCD200 concentration in individual patient groups. The data are presented as median and range. The χ^2 and Fisher's exact tests were used to compare individual groups of patients with respect to clinical and demographic characteristics. $P<0.05$ was considered to indicate a statistically significant difference. Multivariate Cox proportional hazards were fitted to assess associations between patients' characteristics and time-dependent variables. For the multivariate analysis, covariates were selected based on the significance in univariate analysis. TTT is the considered the time interval between the date of CLL diagnosis and the date of first treatment. Kaplan Meier curve was used to study the impact of sCD200 concentration on TTT and the difference between groups were evaluated with the log-rank. ROC curve was used in order to evaluate the best cutoff level that better discriminate between CLL patients with short TTT and those with longer one. The sensitivity, specificity was addressed from ROC curve.

Results

This study includes 158 patients (100 males, 58 females) beside 21 healthy controls matched in age and sex. The clinical and laboratory data of the studied patients are shown in Table 1. The mean patients age was 61.39 ± 10.07 years. According to Rai staging risk score, the studied patients were categorized as 27,8% stage1, 46.8% stage2 and 25.4% stage3. Positive ZAP70, CD38, and high ß2-microglobulin was recorded in 44.3%, 33.5% and 36.1% respectively. The most frequent cytogenetic finding was 13q del (found in 53.8% of patients) (Table 1).

Comparison of studied parameters in CLL patients group with control group revealed that there is significant elevation of sCD200 in patients group as compared to control group (Table 2, Figure 1).

CLL patients group was subdivided into 2 subgroups according to median sCD200 concentration levels (≥ vs <690 pg/ml). CLL subgroup of patients that has high sCD200 showed elevated WBCs, smudge cells count, LDH levels, ß2 microglobulin, ZAP70+, CD38+ and shorter TTT as compared to those with low sCD200. Moreover, CLL patients that has high sCD200 were significantly associated with advanced Rai stages, Poor FISH cytogenetic finding and diffuse pattern of BM lymphocytes (Table 2).

ROC analysis was conducted to identify the optimal cut off levels for prediction of shorter TTT. sCD200 best cut-off value was 752.5 pg/ml. The area under the curve (AUC) was 0.834 (p=<0.001) (Table 3, Figure 2).

Cox regression analysis was conducted for prediction of TTT, using age, gender, WBCs, smudge cells, LDH, sCD200, ZAP70+, CD38+, ß2-microglobulin, and Rai stages risk score as covariates. In multivariate analysis higher sCD200, CD38+, ß2-microglobulin and advanced Ria stages were significantly risk factors for shorter TTT (Table 4).

Survival analysis studies using Kaplan Meir curve

Table 1. The Clinical and Laboratory Data of the Studied CLL Patients

Parameter	
Mean age	61.39 ± 10.07
Gender	
Male	100 (67.0%)
Female	58 (33.0%)
Absolute lymphocytes ($\times 10^9$ /L)at diagnosis, median (range)	74.0 (32.0-320.0)
Smudge cell (%),median (range)	7.0 (1-30)
LDH (IU/L) at diagnosis, median (range)	334 (120-4200)
Rai stages risk system	
low	44 (27.8%)
Intermediate	74 (46.8%)
high	40 (25.4%)
ZAP70%	
Negative	88 (55.7%)
Positive	70 (44.3%)
CD38%	
Negative	105 (66.5%)
Positive	53 (33.5%)
ß2 microglobulin (mg/L)	
Normal	101 (63.9%)
High	57 (36.1%)
FISH	
Normal	31 (19.6%)
Cytogenetic Findings, no(%)	
17p del	11 (7.0%)
11q del	15 (9.5%)
Trisomy 12	16 (10.1%)
13q del	85 (53.8%)
Median time to first treatment (TTT) (month)	15.0 (1-25)
Median sCD200 (pg/mL) at diagnosis	690.0 (36-5200)

was used to address the impact of sCD200 concentration on the TTT in studied cohort of CLL patients. The results displayed that patients with low sCD200 (<752.5) has longer TTT as compared to those with higher sCD200 and the difference is statistically significant (P<0.001) (Figure 3).

Discussion

In the present study sCD200 concentration levels was significantly higher in CLL patients as compared to healthy controls. This finding is similar to that reported in a previous recent study (D'Arena et al., 2021). Moreover, there is association between sCD200 levels and absolute lymphocytes count, advanced Rai stages, β2-microglobulin level, positive CD38%, as well as positive ZAP70%. These results are consistent with findings that reported by D'Arena et al., (2021). In contrast to our findings D'Arena did not find an association between bulky disease or lymphocytosis and sCD200 concentrations level. These is because this study is retrospective study, while our study is prospective one. sCD200 might have a role in tumor growth through engage the CD200 receptor, which in turn can result in increased tumor growth, by means of a negative impact on tumor immune- surveillance (Wong et al., 2012).

The source of soluble forms of sCD200 is the product of either alternative splicing or ectoderm shedding from surface anchored molecule by 12 ADAM proteases (Twito et al., 2013). The biological function of sCD200 was demonstrated by its ability to bind and phosphorylate CD200R1, the major receptor responsible for mediating the downstream immunoregulatory functions of CD200 (Gorczynski, 2005), induces the suppression of T-cell mediated responses, limiting inflammation in a wide range of inflammatory diseases, down regulate macrophage function (Moreaux et al., 2008), induction of regulatory

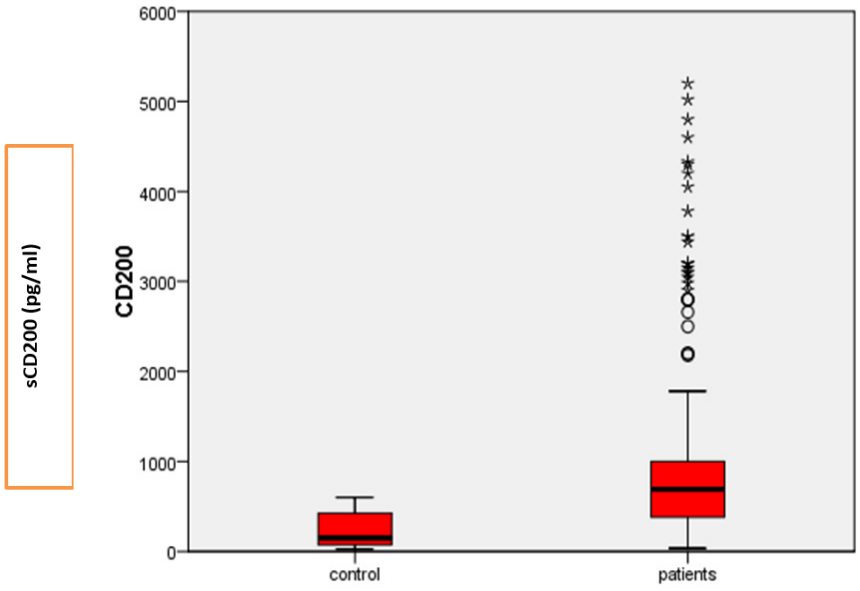

Figure 1. Box Plot Diagram Displayed sCD200 Concentration Levels in Control Group and CLL Patients. The line inside the box and bars represent median and interquartile ranges. The circles and asterisks represent patients with markedly elevated sCD200. The differences between groups was statistically significant (P<0.001).

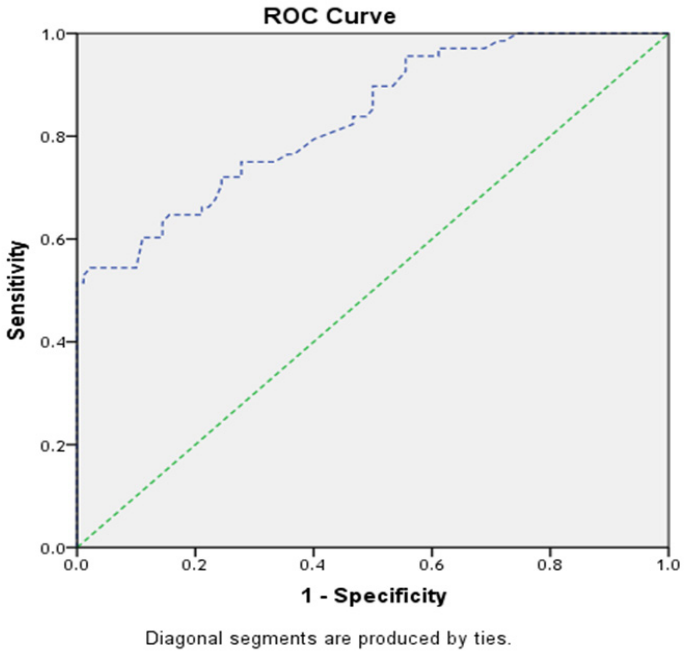

Figure 2. ROC Curve for sCD200 to Identify the Cutoff Levels that Predict the TTT. At sCD200 cutoff level (752.5 pg/ml) could predict TTT with sensitivity 72.1%, and specificity 75.6%.

T-helper cell type (Th1) to Th2 cytokine profile switch, suppression of natural killer cell function, and inhibition of tumor-specific T-cell immunity have been all experimentally demonstrated (Kretz-Rommel et al., 2007). Moreover, data extracted from experimental study proved that plasma derived from CLL patients enhance engraftment of CD5/CD19+ CLL cells in the peritoneal cavity and spleen, while plasma containing low sCD200

Table 2. Comparison of Patient's Findings in a Subgroup of CLL Patients with High versus Those with Low sCD200.

Parameter		sCD200<690 (n=79)	sCD200≥690 (n=79)	P value
Age	Mean ± SD	62.57 ± 9.7	60.4 ± 10.3	0.186
Gender	Male	55 (69.6%)	53 (67.1%)	0.732
	Female	24 (30.4%)	26 (32.9%)	
Absolute lymphocytes ($\times 10^9$/L)	Median (Min-Max)	68.0 (32-122)	78.0 (39-320)	<0.001
Smudge cells (%)	Median (Min-Max)	9.0 (2-30)	7.0 (1-27)	<0.001
sLDH (IU/L)	Median (Min-Max)	270.0 (120-936)	600.0 (128-4200)	<0.001
Rai stages	Low	22 (27.8%)	22 (27.8%)	<0.001
Risk system	iIntermediate	56 (70.9%)	18 (22.8%)	
	High	1 (1.3%)	39 (49.4%)	
ZAP70 (%)	Negative	58 (37.4%)	30 (38.0%)	<0.001
	Positive	21 (26.6%)	49 (62.0%)	
CD38(%)	Negative	70 (88.6%)	35 (44.3%)	<0.001
	Positive	9 (11.4%)	44 (55.7%)	
ß2 microglobulin	Negative	67 (84.8%)	34 (43.0%)	<0.001
(mg/L)	Positive	12 (15.2%)	45 (57.0%)	
FISH Cytogenetic findings; no(%)	Normal	14 (17.7%)	17 (21.5%)	<0.001
	17p del	0 (0.0%)	11 (13.9%)	
	11q del	1 (1.3%)	14 (17.7%)	
	Trisomy 12	2 (2.5%)	14 (17.7%)	
	13q del	62 (78.5%)	23 (29.1%)	
TTT (month)	Median (Min-Max)	23.0 (2-25)	4.0 (1-24)	<0.001
Status	Live	79 (100.0%)	52 (65.8%)	<0.001
	Dead	0 (0.0%)	27 (34.2%)	

Mann-Whitney tests, Chi-Square test, independent sample T test; significant (P value < 0.05), sLDH (serum lactic dehydrogenase)

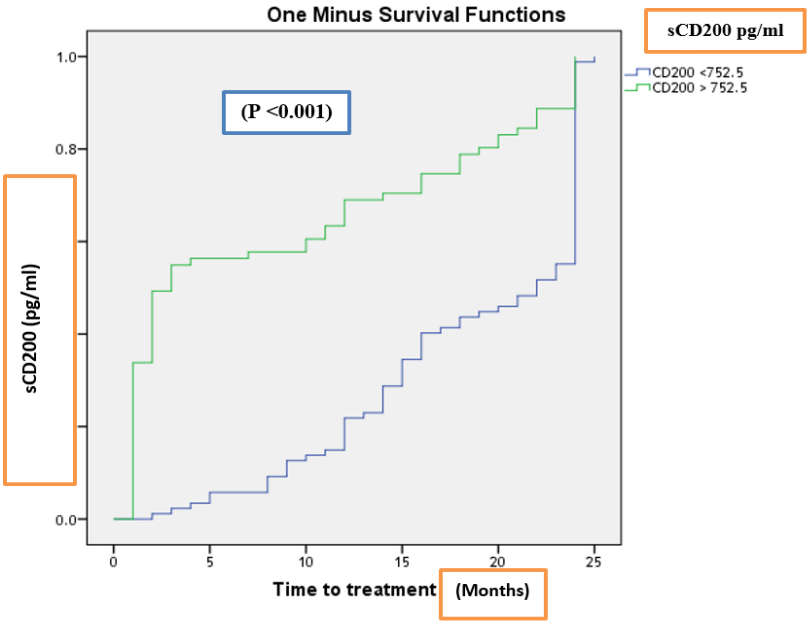

Figure 3. TTT (months) for CLL Patients with Low sCD200 vs. those with High sCD200. CLL patients with high sCD200 have significantly shorter TTT as compared to those with lower one (P<0.0001).

Table 3. Performance Characteristics of sCD200 for Prediction of TTT Using ROC Curve

	AUC	SE	p	95% CI	Cut off	Sensitivity (%)	Specificity (%)
sCD200 (pg/ml)	0.834	0.032	<0.001	0.772-0.896	752.5	72.10	75.60

Sensitivity=[a/(a+c)]×100; Specificity=[d/(b+d)]×100; (a) true positive, (b) false positive, (c) False negative, (d) True negative

from health individuals associated with low engraftment of CLLs. The CLL cells that engraft in experimental animals express the same immunophenotypic markers at the same levels similar to original CLL cells, suggesting no selection for survival of unique subpopulations occurred in vivo under these conditions (Gorczynski et al., 2009; Wong et al., 2010).

Cox regression analysis revealed that high sCD200:concentration level has Hazard ratio (HR) 1.002 (CI : 1.001-1.003). Likewise there is association between sCD200 concentration and other known prognostic factors namely ZAP70, ß2-microglobulin, CD38%, smudge cells. Although the sCD200 HR in Cox regression analysis is low as compared to other prognostic markers (Clinical stages, ZAP70%, CD38%) when tested in multivariate analysis, still it can discriminate significantly between 2 subgroups of CLL patients, one with short TTT and those with long TTT. These findings suggest that sCD200 concentration level at diagnosis could be used as risk prognostic marker for CLL stratification. Similar finding was reported by D'Arena et al., (2020).

Using ROC curve to address the sCD200 cutoff that can discriminate between a subgroup of CLL patients with short TTT as compared to the remaining CLL patients.

Table 4. COX Regression Analysis for Prediction of TTT in CLL Patients

		Univariate analysis				Multivariate analysis			
		P	HR	95% CI		p	HR	95% CI	
Age		0.032	0.975	0.952	0.988	0.966	0.999	0.972	1.028
Gender		0.325	1.281	0.782	2.097				
Absolute lymphocytes (×10^9/L)		<0.001	1.015	1.011	1.019	0.595	1.002	0.995	1.009
Smudge cells(%)		<0.001	1.145	1.108	1.184	0.876	0.995	0.936	1.058
sLDH (IU/L)		<0.001	1.002	1.001	1.003	0.817	1.001	0.999	1.002
sCD200 (pg/ml)		<0.001	1.003	1.001	1.004	0.011	1.002	1.001	1.003
ZAP70 (%)		<0.001	5.806	3.36	10.03	0.593	1.255	0.546	2.882
CD38 (%)		<0.001	17.471	9.759	31.27	<0.001	6.622	2.554	17.164
ß2-microglobulin (mg/L)		<0.001	10.41	6	18.07	0.032	2.419	1.079	5.423
Rai stages	2 vs. 1	0.773	0.894	0.419	1.909				
Risk system	3 vs. 1	<0.001	21.023	14.395	35.576	0.019	18.846	1.627	21.251

HR, hazard ratio; CI, confidence interval; Cox regression was used

The sCD200 cutoff levels was 752.5 pg/ml. This findings are parallel with that recently reported in a retrospective study (D'Arena et al., 2021).

Taken these findings together suggested that administration of an antagonistic anti-CD200 Ab enabled the human immune system to eradicate CD200-expressing cancer cells (Xiong et al., 2020) and subsequently improve CLL patients outcome. The limitation of this study is that serial determination of sCD200 at diagnosis and during CLL course.

In conclusion, The findings in our study approved the prognostic relevance of sCD200 in CLL. We recommend to evaluate sCD200 at diagnosis in order to make better risk stratification of CLL at diagnosis. sCD200 could be a potential antigen for targeted therapy.

Author Contribution Statement

Salah Aref: Conception, Study Design; Ahmed El Tantawy: Clinical Managements of patients, Data Collection and Analysis; Enas Gouda: Laboratory Work, Manuscript writing; Mohamed Ayed: Study design, Laboratory Work, Manuscript writing.

Acknowledgements

The authors would like to acknowledge all the participants for sharing their time and providing consent and information necessary for the successful completion of the study.

Data availability

The data of the current study is available upon request to the corresponding author.

Compliance with ethical standards

Funding This study was not supported by any funding.

Ethical Statement

The study was approved by Mansoura Faculty of Medicine IRB

Informed consent

All subjects included in this study gave informed consent.

Conflict of interest

The authors declare no competing interests.

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