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Soluble CD163 impact as a prognostic biomarker in chronic lymphocytic leukemia

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Abstract

Chronic lymphocytic leukemia (CLL) is a malignant blood disorder in which there is an excess of white blood cells (lymphocytes) in blood and lymphoid tissues. CLL patients experience different clinical behaviors with diversity in disease course and outcome. Accordingly, prognostic markers are crucial for employing appropriate therapy protocols. CD163 (cluster of differentiation 163) is a monocyte/macrophage receptor. Soluble CD163 (sCD163) is an emerging prognostic player in the field of hematopoietic neoplasms. This study aimed to assess the prognostic potential of sCD163 as a serological marker in CLL. The_study included 41_CLL patients and 44 apparently normal healthy volunteers as controls. Expression of CD38 and cytoplasmic ZAP-70 in CLL cells was assessed using flow cytometry. Beta 2 microglobulin (B2M), sCD23, and sCD163 serological markers were measured by ELISA. Serum levels of sCD163 were statistically significantly higher in CLL cases compared to controls (p=0.000). sCD163 levels were positively correlated with absolute lymphocyte count, sCD23, and B2M levels (p= 0.027, p=0.01, and p=0.004, respectively). In conclusion, levels of sCD163 in CLL is a promising prognostic tool for evaluating disease progression.

Keywords: CD163, CLL, ELISA

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Introduction

The chronic lymphocytic leukemia (CLL) disease which most frequently affects older persons is brought on by failed apoptosis and is characterized by subsequent progressive accumulation of functionally incompetent lymphocytes in blood, bone marrow, and secondary lymphoid organs.[1, 2] In the context of clinical presentation of CLL patients, quite

heterogeneous patterns are encountered with a significant portion of patients experiencing an indolent course without requiring treatment regimens for years while others rapidly deteriorate, hence new markers figuring out prognostic path are crucial in clinical management decisions.[3]

Some markers such as mutational status of the variable region of immunoglobulin (Ig) heavy chain gene (IgVH), beta 2 microglobulin (B2M), sCD23, CD38, cytoplasmic zeta–associated protein 70 (cytoZAP-70), and chromosomal aberrations of 11,12,13,17 have been shown to be relevant to CLL prognosis.[2]

Scavenger receptor CD163, a monocyte/macrophage-specific membrane marker, is a member of the cysteine-rich family type B. It consists of an extracellular domain composed of 1048 amino acid residues, a single transmembrane segment, and a cytoplasmic tail with many splice variants. Soluble CD163 protein (sCD163) appears in plasma and other body fluids after cleavage of the CD163 extracellular domain on the membrane of monocytes and macrophages via proteases.[4] sCD163 is upregulated in a large range of inflammatory diseases,[5] and its increased levels were reported in several hematopoietic neoplasms such as Hodgkin Lymphoma.[6] Furthermore, sCD163 was strongly linked to the prognosis of multiple myeloma.[7] The innate immune system's CLL-associated macrophages, also known as tumor-associated macrophages (TAM), contribute to elevated CD163 expression.[8] Our study aimed to determine the role of sCD163 as a trustworthy prognostic marker throughout the CLL disease course.

Subjects and Methods

The study protocol was reviewed and approved by the Medical Research Ethics Committee of the National Research Centre (registration number: 17147). Prior to being included in the study, all participants were informed about the research aims and their verbal consents obtained.

Study design and sample collection

In the present study, we measured serum levels of sCD163 in a cohort of 41 CLL patients recruited from the National Cancer Institute's outpatient clinic between the years 2019 and 2020. The World Health Organization classification of hematological and lymphoid tissues was used to diagnose CLL cases.[9] Full history taking and thorough clinical examination of the patients were accomplished. Also, 44 apparently healthy normal volunteers were enrolled as a control group.

Peripheral venous blood samples were withdrawn under complete aseptic conditions from all participants. A portion of the blood was anticoagulated with EDTA for complete blood count (CBC) and flow cytometric analysis of CD38 and cytoZAP-70 expression. The rest of the blood was left to clot, centrifuged and sera were separated and stored at -20ºC until used for quantitative estimation of sCD163, sCD23, and B2M.

The serum concentration of sCD163 (M130 antigen) was determined using an ELISA assay by a commercially available kit (Cat. no. EHCD163, Thermo Scientific, USA), according to the manufacturer's instructions. Similarly, human sCD23 was determined by a commercially available ELISA kit (Catalog number: BMS 227-2, Invitrogen, Thermo Fisher Scientific, GmbH, Germany), according to the manufacturer's instructions. And B2M was determined by commercial ELISA kits (Catalog number: ORG 5BM from ORGENTEC, Diagnostika GmbH, Germany), according to the manufacturer's instructions.

Flow cytometric analysis was performed using the Beckman Coulter Navios Flow Cytometer (USA). Monoclonal antibodies for prognostic and diagnostic evaluation (Beckman Coulter Immunotech, USA) included: CD19-PC7 (Catalog number: IM3628U); CD5-FITC (Catalog number: IM0468U); CD10-PC5.5 (Catalog number: B16490); CD38-PE (Catalog number: IM2371U) and cytoZAP-70-PE (Catalog number: B57658). Monoclonal antibodies (Agilent Technologies, Inc., California, USA) included: CD20-FITC (Catalog number: F0799); anti-kappa-FITC (Catalog number: F0434); and anti-lambda-PE light chains (Catalog number: R0437).

Statistical analysis

The SPSS statistical software (version 16; IBM Corp.) was used to analyze and examine the data. For categorical data analysis, frequency (count) and relative frequency (%) are shown along with the mean and standard error of the quantitative data. The non-parametric Kruskal-Wallis and Mann-Whitney U tests were used to compare quantitative variables[10]. The Chi-square test was used to compare categorical data. When the anticipated frequency was less than five, Fisher's exact test was applied instead [11]. Spearman's correlation coefficient was used to analyze correlations between quantitative variables. Statistically significant differences were considered at $p<0.05$.

Results

Our study comprised 41 CLL patients and 44 control subjects. The age of our selected cases ranged from 42 to 83 years with a mean value of 57.8±9.8 years, and a male to female ratio of 1.56/1. All CLL patients showed positive expressions for CD5, CD19, CD20, CD23 and negative expression for CD10. Descriptive clinical, hematological, and biochemical data of CLL patients are shown in Table 1.

The studied patients were presented at different stages with Binet stage C and high-risk Rai stages III and IV. Comparison of serological markers between CLL patients and controls is demonstrated in Table 2. Figure 1 shows the difference of sCD163 levels between patients and controls.

Table 1. Descriptive clinical, hematological, and biochemical data of the 41 CLL patients.

Variables	CLL patients data
Age (years); range (mean±SD)	42-83 (57.8±9.8)
Sex; M/F	25/16
Smoking; no (%)	13 (31.7)
Course:	
Indolent; no (%)	13 (31.71)
Aggressive; no (%)	20 (48.8)
New cases; no (%)	8 (19.5)
Binet Stage	
A; no (%)	6 (14.6)
B; no (%)	11 (26.8)
C; no (%)	24 (58.5)
Rai stage at diagnosis, n (%)	
I and II (intermediate risk)	17 (41.5)
III and IV (high risk)	24 (58.5)
Clinical findings, n (%)	
Lymphadenopathy	30 (73.2)
Splenomegaly	28 (68.3)
Hepatomegaly	17 (41.5)
Hb (g/dL); Mean±SE	11.2±0.28
TLC ($x10^3$/µl); Mean±SE	66.2±11.8
Abs. lymph. ($x10^3$/ µl); Mean±SE	60.28±18.2
Kappa light chain positive expression (%)	65.85
Lambda light chain positive expression (%)	34.15
CD38, % range (mean±SE)	0.1-63 (15.6±2.9)
cytoZAP-70, % range (mean±SE)	0.1-46.9 (5.7±1.9)

Hb: hemoglobin; TLC: total leukocytic count; Abs. Lymph. Absolute lymphocytic count; SE: standard error.

Table 2. Comparison between CLL patients and controls as regards serological markers.

Variables	CLL patients (n=41)	Control (n=44)	*p* value
sCD163(pg/ml) mean ± SE	1.8 E2 ± 20.2	40.5 ±2.6	<0.0001
sCD23(U/ml) mean ± SE	1.29 E4± 1.39	54.1±8.1	<0.0001
B2M (µg/ml) mean ± SE	6.3± 0.6	2.8±0.08	<0.000

B2M: beta 2 microglobulin; SE: standard error; *$P \leq 0.05$ is significant.

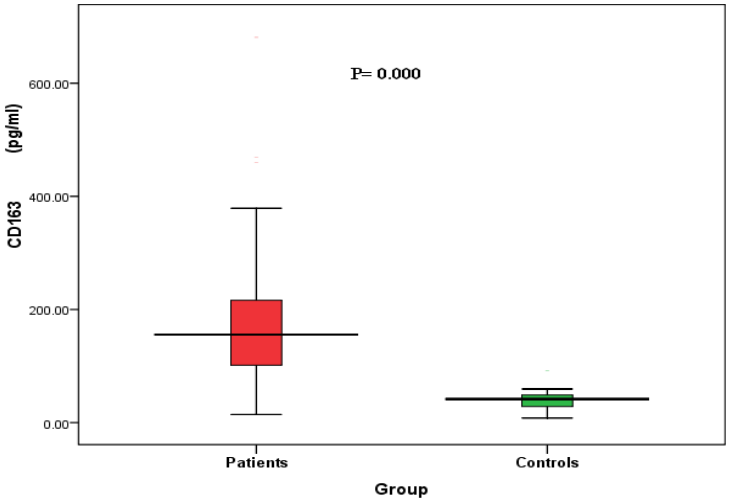

Figure 1. Comparison of sCD163 levels (mean±SE) between CLL patients and apparently healthy controls. *$P \leq 0.05$ is significant.

Correlation analysis was performed to explore any relationship between biomarkers' levels as presented in Table 3. Serum levels of sCD163 showed a significant linear positive correlation with absolute lymphocyte count (r=0.404, p=0.027) and with other prognostic markers, sCD23 (r= 0.409, p=0.01) and B2M (r=0.440, p=0.004). As regards the relation to B2M as a dependent variable, both linear and logistic relations to sCD163 levels were observed as demonstrated in Figure 2 (p=0.004). sCD163 levels were neither related to immunophenotypic markers (CD38 and cytoZAP-70) nor to the presence of lymphadenopathy, advanced disease stage, or aggressive course.

Table 3. Correlation studies between sCD163 and other prognostic variables.

	Abs. lymph.	sCD23	B2M
sCD163; r/p value	0.404/0.027*	0.409/0.01*	0.440/0.004*

Abs. lymph.: absolute lymphocytic count, *$P \leq 0.05$ is significant.

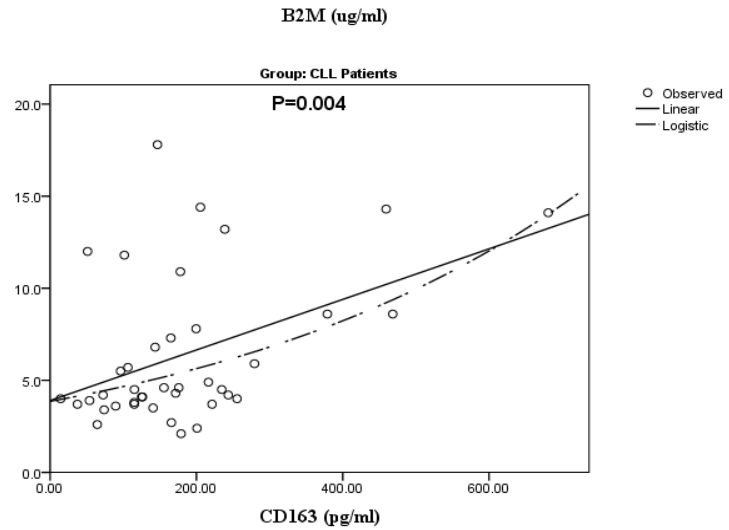

Figure 2. Linear and logistic relationship between CD163 and B2M variables in CLL patients (p=0.004).
B2M: beta 2 microglobulin. *$P \leq 0.05$ is significant.

CLL patients were categorized into two subgroups based on the median B2M level >4.5 μg/ml subgroup (51.2% of patients) and <4.5 μg/ml subgroup (48.8% of patients). There was a significant difference in sCD163 levels between both subgroups (p=0.012), as shown in Figure 3. CLL patients with sCD163 levels higher than 92.4 pg/ml (the maximum concentration detected in the control subjects) were observed in 78% of CLL patients and showed B2M levels more than 4.5ug/ml in 95.2 % of such patients (p=0.01) as shown in Table 4.

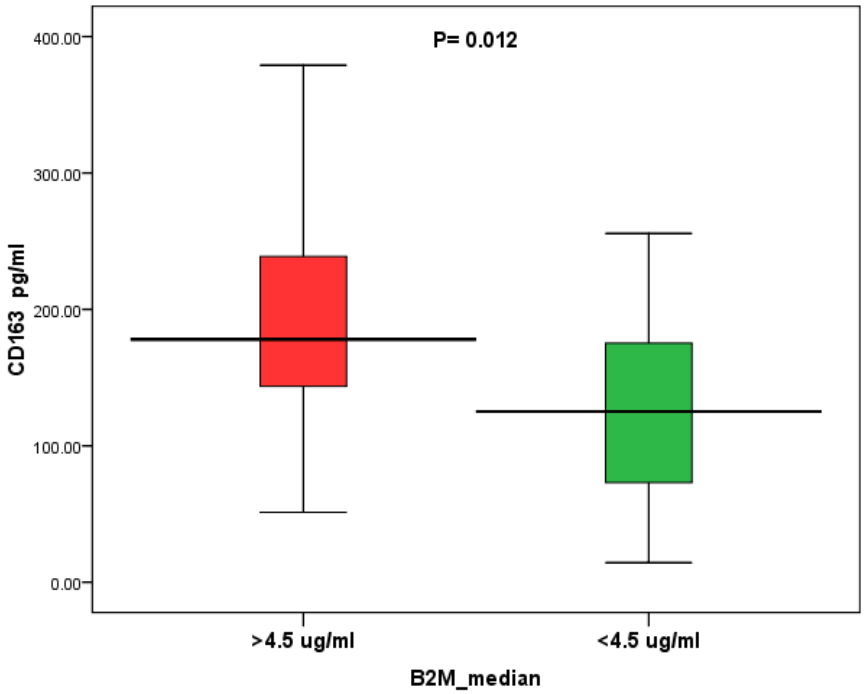

Figure 3. Comparison between sCD163 levels in CLL patients' groups based on B2M subgroups.

B2M: beta 2 microglobulin. *$P \leq 0.05$ is significant.

Table 4. Association between sCD163 levels and B2M subsets in CLL patients.

	sCD163>92.4 pg/ml	sCD163<92.4 pg/ml	Odds ratio (95% CI)	*p* value
B2M>4.5 μg/ml; n/%	20/95.2	1/4.8	0.784-31.892	0.01
B2M<4.5 μg/ml;n/%	12/60	8/40	0.255-0.720	

B2M: beta 2 microglobulin; CI.: Confidence interval. *$P \leq 0.05$ is significant.

Discussion

This study aimed to assess the serum levels of sCD163 in patients with CLL to determine its possible role as a prognostic parameter. In the present study, some of the reliable prognostic markers in CLL were investigated to identify their association with sCD163. A significant increase in sCD163, sCD23, and B2M levels was detected in CLL patients compared to control subjects. Previous research studies indicated that increased levels of these soluble markers in cancer are linked to poor prognosis.[12, 14-16] Sagatys and Zhang, 2012, reported that the survival of CLL cells depends on microenvironmental factors and many soluble antigens have been demonstrated to be significantly predictive to short time of disease progression.[16]

In the current study, a significant association was also observed between sCD163 and increased lymphocytic count which is related to tumor burden. An early study found that a diffuse packed pattern of lymphocyte infiltration was associated with a more advanced stage of CLL.[16] Moreover, sCD163 was significantly correlated to other serological conventional prognostic biomarkers, sCD23 and B2M. A previous study by Sarfati et al., 1996, showed that elevated sCD23 levels can predict a shorter time to disease progression and a shorter overall survival.[17] In addition, sCD163 levels demonstrated significant linear and logistic relation to B2M, a finding that is consistent with previous investigations on multiple myeloma [7]. Elevated B2M levels were previously observed in CLL patients with high tumor burden and massive bone marrow lymphocytic infiltration.[18, 19] B2M was considered an independent prognostic factor in multiparameter scores for risk stratification in CLL.[20]

In the present work, higher B2M levels were observed in 63.32% of CLL patients with a median level of 4.5 μg/ml than normal controls. In this context, patients were stratified into two subgroups based on the median B2M level detected in the CLL patients to determine the sCD163 variation. A significant difference in sCD163 levels between these two subgroups (p=0.012) was detected with a significant association between high levels of both markers (p=0.01). These results agreed with a previous study that showed elevated serum sCD163 levels in cancer patients, significantly associated with poor outcomes.[21] A previous study by Mantovani et al., 2017, linked TAMs to neoplastic progression through encouraging genetic instability.[22] In CLL TAMs modulate tumorigenesis, enhance cell proliferation, and inhibit the apoptotic pathways.[24] Uncommitted macrophages were reported to polarize into either tumor-promoting macrophage with a high CD163 expression or pro-inflammatory macrophages with a high inducible nitric oxide synthase expression.[22-24] sCD163 is considered a highly specific monocyte/macrophage marker for M2 macrophages (M2 type, referred to as alternatively activated macrophages) and can be targeted in future therapeutic research.

In the current study, sCD163 was not related to an advanced disease stage or aggressive disease course. These findings can be explained by their expected variation due to therapeutic interventions indicated for these patients. Vajavaara et al., 2021, measured sCD163 levels in patients with diffuse large B-cell lymphoma before, during, and after treatment. They demonstrated that sCD163 levels decreased in response to therapy and when elevated can be predictive of an unfavorable outcome. Their findings suggested that sCD163 represents an easily assessable useful biomarker for therapeutic monitoring.[25]

In conclusion, the significant contribution of macrophages to CLL carcinogenesis suggests that sCD163 should be considered as a prognostic biomarker. In this study, its significant elevation in our cohort of CLL patients and its association especially with well-established B2M prognosticator is suggestive of its prognostic impact. Further future studies on therapies targeting the TAM can be highly effective in clinical settings.

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Author Contributions

HMR, HRMA, and NAE conceptualized the study; MHI, MMAM, and WAE helped in designing the experiments and analyzed the data. MES, MAMK, examined & diagnosed the patients, HRMA, wrote the manuscript. HMR NAE, AK revised the manuscript; MHI, MMAM, AK and WAE performed the experiments. The final version was read, revised, and edited by all authors before submitted for publication.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by the Medical Research Ethics Committee of the National Research Centre (registration number: 17147).

Informed consent

Prior to being included in the study, all participants were informed about the research aims and verbal consent obtained.

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