Evaluation of Prognostic Impact of Soluble CD14 in B-Chronic Lymphocytic Leukemia

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Abstract sCD14 is an acute phase reactant; few studies reported its prognostic value in B-CLL patients. This gave us the impetus to conduct this study. This study enrolled 40 newly diagnosed B-CLL Egyptian patients, presented to the Hematology Department of the Medical Research Institute in Alexandria University. The ZAP-70 was determined by flow cytometry whereas serum sCD14 concentration by human sCD14 sandwich ELISA method. The mean serum level of sCD14 was significantly higher among patients with positive ZAP-70, Binet stage C, Rai stage III–IV and high risk CLL prognostic index. It showed a significant positive correlation to the percentage of ZAP-70 expression and significant negative correlation to the hemoglobin concentration. Serum sCD14 concentration could be used to assess B-CLL patients initially as an additional prognostic marker, especially in low resources areas where flow cytometry is not available.

Keywords sCD14 · B-CLL · ZAP-70 · Prognostic · Correlation

Introduction

Chronic lymphocytic leukemia (CLL) is a common leukemia of adults in the Western world, and its incidence increases dramatically with age. It accounts for approximately 30% of all leukemias and approximately 10% of hematological neoplasms [1–3].

Among Arab countries, CLL represents 10% of all registered leukemias in the Saudi Cancer Registry in (2009) [4], 8.6% of cases in Kuwait [5], and 16% of cases in Jordan [6]. In Egypt, CLL was the most common subtype of leukemias [7]. The National Cancer Registry (Aswan profile, 2008) [8] and (Damietta Profile, 2009) [9], reported over 80% of lymphoid leukemias occurring in adulthood were CLL.

Several markers are investigated for prognostic purpose in B-CLL patients, among which is the soluble form of CD14 (sCD14). Soluble CD14 is a novel monocyte-derived survival factor for B-CLL that acts as a co-receptor for Toll-like receptor-4 (TLR-4) to detect bacterial lipopolysaccharide (LPS). Its relevance in B-CLL pathogenesis and its potential usage as a novel therapeutic target is under study [10].

Previous studies focused on the surface or membrane bound CD14 (mCD14), which was found in higher levels among B cell CLL and was associated with advanced disease and shorter overall survival [11]. However, scarce studies are available in the literature concerning sCD14 as prognostic marker in B-CLL patients. This study was carried out to evaluate the prognostic impact of sCD14 among Egyptian patients with newly diagnosed B-CLL.

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Patients and Methods

This study included 40 newly diagnosed B-CLL patients presented to the Hematology Department of the Medical Research Institute (MRI) of Alexandria University. Another 20 age and sex matched normal subjects were added as a control.

This sample size represented the total number of newly diagnosed patients with B-CLL in the MRI hematology clinic, presented from January to December 2015, including those who did not receive any therapeutic intervention and had no objection in participation in this work.

For accurate estimation of sCD14 level, this study excluded CLL patients who have secondary malignancy, alcoholism, chronic liver disease, severe trauma, infection at the time of sampling and positive acquired immunodeficiency syndrome.

All patients were physically examined, then investigated for complete blood count, LDH, B₂M levels; flow cytometry using chronic panel, where all the included patients had B-CLL immunophenotypic score of 4 or 5. The ZAP-70 was determined by flow cytometry and serum sCD14 concentration was determined by using human sCD14 sandwich enzyme-linked immunosorbent assay (hycult biotech). Clinical staging (Binet Rain staging) and the CLL prognostic index which is based on the presence of risk factors were performed too (Table 1) [12, 13].

For the analysis of ZAP-70 expression, blood samples were incubated in anti-CD5-FITC and anti-CD19-PerCP-Cy5.5 (Immunotech, Beckman Coulter) for 15 min; red cells were lysed using IO Test 3 lysing solution (Beckman Coulter, Fullerton, USA). ZAP-70 is an intracytoplasmic antigen, so its detection requires an extra step of permeabilization. After centrifugation at 200 g, fixation and permeabilization of cells were carried out using 4% paraformaldehyde and 2% Tween 20, Intraprep kits reagent (Immunotech, Beckman Coulter); anti-ZAP-70—PE (clone SBZAP; Beckman Coulter, Fullerton, California, USA) was then added and cells were incubated for 15 min, cells

were washed with PBS, and analyzed by flow cytometry. After gating on B cells (CD5+/CD19+), percentages of ZAP-70-positive CLL cells were determined with negative threshold cutoff values set using ZAP-70-stained normal B cells, as well as isotype control-stained CLL cells. The cut-off point for ZAP-70 positivity in CLL cells was more than 20%.

Data were processed by the Statistical Package for Social Sciences (SPSS) for Windows, version 21. It was of normal distribution (parametric), so that parametric tests were conducted (t test for two means, one way ANOVA for more than 2 means, and the Pearson correlation test) with the 95% confidence interval.

Results

The studied patients included 28/40 (70.0%) males and 12/40 (30.0%) females with a male to female ratio of 2.4:1. Their age ranged from 46 to 76 years with a mean of 58.95 ± 7.9 years. The mean age was not statistically significantly differing between both sexes ($p > 0.05$; Table 2).

The level of sCD14 ranged from 2.8 to 12.7 µg/ml among the studied patients with a significant higher mean (7.94 ± 2.74 µg/ml) when compared to the control group (2.51 ± 0.56 µg/ml; Table 2).

In the studied B-CLL patients; the mean sCD14 was significantly higher among ZAP-70 positive patients, Binet stage C patients, Rai stage III–IV patients and those with high risk CLL prognostic index patients (Table 3).

Correlation test was conducted between sCD14 and hematological parameters, ZAP-70, B₂M, and LDH. Serum sCD14 showed significant positive correlation to the percentage of ZAP-70 expression ($r = 0.542$, $p = 0.0001$; Fig. 1), and significant negative correlation to hemoglobin concentration ($r = -0.504$, $p = 0.001$; Fig. 2). There is no significant correlation observed with other hematological parameters or serum markers.

Table 1 Prognostic index based on the presence of risk factors

Characteristics	Point contribution			
	0	1	2	3
Age (years)	–	<50	50–65	>65
β ₂ M (mg/L)	<ULN	1–2 × ULN	>2 × ULN	–
ALC (×10 ⁹ /L)	<20	20–50	>50	–
Sex	Female	Male	–	–
Rai stage	0–II	III–IV	–	–
Number of involved nodal groups	≤2	3	–	–

ULN Upper limit of normal, β₂M beta 2 microglobulin, ALC absolute lymphocytes count

Score: 1–3 (low risk), 4–7 (intermediate risk), ≥8 (high risk)

Table 2 Sex, age and serum soluble CD14 in the studied patients and control

Item	B-CLL patients (n = 40)	Control (n = 20)	p value
Sex (male)	28 (70.0%)	14 (70.0%)	1.00
Age (years)	58.95 ± 7.9 (46–76)	54.8 ± 8.7 (40.0–64.0)	0.069
sCD14 (μg/ml)	7.94 ± 2.74 (2.8–12.7)	2.51 ± 0.56 (1.6–3.9)	0.0001*

* Statistically significant

Table 3 Serum soluble CD14 in B-CLL patients according to some CLL parameters

Parameters	sCD14 (μg/ml)	p value
ZAP-70		
Positive (n = 11)	9.90 ± 2.16	0.004*
Negative (n = 29)	7.19 ± 2.59	
Binet staging		
A (n = 8)	4.9 ± 1.8	0.0001*
B (n = 27)	8.0 ± 1.7	
C (n = 5)	10.6 ± 1.5	
Rai staging		
0–I (n = 10)	6.25 ± 2.65	0.0001*
II (n = 16)	6.97 ± 2.23	
III–IV (n = 14)	10.24 ± 1.69	
Prognostic index		
Low risk (n = 5)	4.4 ± 2.06	0.0001*
Intermediate (n = 27)	7.92 ± 2.35	
High risk (n = 8)	10.17 ± 2.1	

* Statistically significant

Fig. 1 Positive correlation between serum sCD14 and ZAP-70 expression in B-CLL patients

By using the ROC curve as a sensitive indicator for sCD14 level in relation to ZAP-70 positivity, it was found that the area under the curve was of high statistical significance (equals 0.77). Such higher area under the curve denotes that serum sCD14 is a sensitive variable in predicting ZAP-70 positivity among B-CLL patients (Fig. 3).

Fig. 2 Negative correlation between serum sCD14 and the hemoglobin concentration in B-CLL patients **Fig. 3** ROC curve for detection of sCD14 as a sensitive predictor for ZAP-70 positivity. Diagonal segments are produced by ties

By conducting the multiple regression analysis for all the studied predictors in patients with B-CLL as regard to ZAP-70 being a dependent variable (criterion), it was found that the significant predictors in patients with

Table 4 Multiple regression analysis for predictors in the studied patients with B-chronic lymphocytic leukemia

Predictors	Standardized beta coefficients	<i>t</i>	<i>p</i>
Hemoglobin concentration (g/dl)	−0.372	−2.373	0.024*
Total leukocytic count ($\times 10^9/L$)	0.826	1.770	0.086
Platelets count ($\times 10^9/L$)	−0.110	−0.766	0.450
Absolute lymphocytes count ($\times 10^9/L$)	−0.423	−0.920	0.365
$\beta 2$ -microglobulin (mg/dl)	−0.169	−1.098	0.280
Lactate dehydrogenase (IU/L)	0.158	1.218	0.232
sCD14 ($\mu g/ml$)	0.276	2.94	0.012*

The test performed by using the enter method ($F = 5.97$, $p < 0.0001$)

Adjusted R square = 0.75

* Statistically significant

B-CLL were serum sCD14 ($p = 0.012$) and the age of the patient ($p = 0.024$). Other variables did not show significant predicting effect in the studied B-CLL patients (Table 4).

Discussion

CD14 is a charming molecule with multiple functions and associations. It is now widely recognized that CD14, in innate immunity, acts as a pattern recognition receptor for a variety of ligands; including apoptotic cells, fungi, bacterial products and microbial cell walls [14–16]. CD14 is considered the homing receptor for the endotoxin (LPS) of gram-negative bacteria [17, 18]. Other possible physiological roles of CD14 is its association with IL-2 signaling, at least in monocytes [19].

The soluble form of CD14 (sCD14) appears either after shedding of the membrane bound CD14 or is directly secreted from intracellular vesicles. It is secreted by the liver and monocytes in low concentrations as an LPS-responsiveness to other cells that do not express CD14 [20].

In normal human blood, the normal concentration of sCD14 is 2–3 $\mu g/ml$. Levels up to 10 $\mu g/ml$ may be associated with a range of human inflammatory diseases, whether of infectious, autoimmune, or even traumatic origin [21–23]. In this study, the normal control showed sCD14 range from 1.6 to 3.9 $\mu g/ml$ meanwhile B-CLL patients showed higher level, up to 12.7 $\mu g/ml$.

Circulating sCD14 is regarded as an acute phase protein, because in response to inflammation and infection, IL-6 induces hepatic sCD14 expression [24]. Therefore, it will increase in acute and chronic infectious and inflammatory conditions. It is well known that inflammation is involved in the pathogenesis of variable chronic lymphoid malignancies of B-cell type [25], among which B-CLL [26]. Previous studies postulated that B-CLL with high sCD14

levels, reflect the maximum monocyte activation [27–29]. Other studies described the presence of a potential source of sCD14 (follicular dendritic cells) in the lymph nodes and bone marrow of B-CLL patients [30, 31].

In this study, the level of serum sCD14 in B-CLL patients showed significant association to the disease progression. Advance stages (Binet C and Rai III–IV) as well as high risk CLL prognostic index showed significant higher mean sCD14 in compare to early stage disease or to low and intermediate risk CLL prognostic index.

The finding of high sCD14 in serum of patients with CLL was first reported in Italy in 1996 [32], and then in 2010 by the German Cancer Research Center, Heidelberg [10]. In both studies, higher sCD14 was significantly related to advanced CLL disease. These are coinciding with the finding of the current study. Even those with positive ZAP-70 expression, which is considered as a surrogate marker for IGHV mutational status [33], showed higher serum sCD14 levels.

One of the most important genetic parameters to establish the prognosis of patients with B-CLL is the mutational status of variable regions of Ig heavy chains (V_H) genes. ZAP-70 is a surrogate marker for this mutational status with higher level of expression indicates a worse prognosis. ZAP-70 is now used as a prognostic marker for B-CLL instead of this mutational status [34, 35].

sCD14 could be used as an alternative marker for ZAP-70 expression. It demonstrated a significant positive correlation to the percentage of ZAP-70 expression and could be measured by ELISA facilitating the evaluation of B-CLL patients, especially when flow cytometry is not available. The significant negative correlation between sCD14 and hemoglobin concentration in the studied B-CLL patients emphasized the prognostic value of serum sCD14. An increasing sCD14 concentration is associated with a decreasing hemoglobin concentration reflecting advance stage of the disease.

Conclusion and Recommendation

This study concluded that serum sCD14 concentration at presentation of B-CLL patients could be used as an additional prognostic marker for disease progression. Thus we recommend measuring it especially in low resources areas where flow cytometry is not available.

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Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in this study involved B-CLL patients and the control group were in accordance with the ethical standards of the Institutional and/or National Research Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent An informed consent was obtained from all individual participants (patients and control) included in this study.

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