Clinical Significance of Soluble CD27 in Chronic Lymphocytic Leukemia

[1]Mohamed Mabed, MD, PhD; [2]Loaie El-Helw, MD; [1]Tarek Abouzeid, MD; [1]Emad Azmy, MD; [2]Mohamed A. Ebrahim, MD; [2]Sameh Shamaa, MD.

[1] Division of Hematology, Oncology Center, Mansoura University, Egypt.
[2] Division of Medical Oncology, Oncology Center, Mansoura University, Egypt.
loaieelhelw@hotmail.com, mohmabed@mans.edu.eg

Abstract: Background: The clinical course of patients with B Cell Chronic Lymphocytic Leukemia (B-CLL) is quite variable, with many patients surviving for prolonged periods without any therapy, whereas others succumb rapidly despite aggressive treatment. **Aim of work:** We-therefore-studied the prognostic value of soluble CD 27 (sCD 27) and in relation to the level of β_2- microglobulin in fifty consecutive patients with B-CLL. **Results:** They were 32 males and 18 females, with a median age of 54 years. A significantly higher level of sCD 27 in patients with CLL has been detected with a mean ± SD of 443.69 ± 346.71 U/ml compared to control level of 196.0±33.3 U/ml (P 0.01). Similarly, a significant higher level of serum β_2- microglobulin in patient with CLL has been detected compared to controls (4.516 ± 1.16 µL/ml vs. 0.6±1.3 µL/ml; P 0.02). Moreover, a significant positive correlation was detected between the pretreatment levels of sCD 27 and β2-microglobulins (r =0.423, P = 0.002). A significant higher pretreatment level of sCD27 and serum β_2-microglobulins was detected in patients who didn't achieve complete response (CR) compared to those who developed CR. Also, they were significant prognostic indicators for both progression free and overall survival rates. **Conclusion**: The sCD 27 is considered to have a predictive and prognostic value in patients with CLL.
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1. Introduction

The clinical course of patients with B Cell Chronic Lymphocytic Leukemia (B-CLL) is quite variable, with many patients surviving for prolonged periods without any therapy, whereas others succumb rapidly despite aggressive treatment. [(1)] In general, it is estimated that one third of CLL patients never require therapy, one third need treatment as soon as they are seen, and one third have disease progression over the years and require therapy at some point. [(2)] Although the two major staging systems have provided valuable information in addressing this clinical heterogeneity [(3, 4)], several other parameters have been proposed as additional factors to the current staging systems to differentiate prognostic subsets. [(5-9)]

A number of studies have demonstrated the importance of CD38+ as a prognostic marker. [(10)] However, there is controversy as to the number of cells required to denote positively. Although Damle and colleagues, initially found a correlation between the presence of an IgVH gene mutation and CD38 (< 30% CD38+ cells), this has not been confirmed by others. In all of these studies, CD38+ has been associated with shorter survival and correlates with increasing Rai stage, intrathoracic and abdominal lymphadenopathy, short doubling time, increased β2-microglobulin levels and atypical morphology. [(11)]

Similarly, ZAP-70 has been investigated as a prognostic marker in CLL. [(12)] There is a 70 to 90% correlation between ZAP-70 expression and absence of IgVH mutations, regardless of whether ZAP-70 is measured by flow cytometry (>20% cells positive), western blot analysis or immunohistochernistry. ZAP-70 positivity correlates moderately with CD38 positivity and the presence of poor-risk cytogenetics, i.e., del 11q22-q23, del 17pl3, and trisomy 12. Moreover, unlike CD38, the ZAP-70 status appears to be stable over time. Kröber et al, have demonstrated that discordant cases may have poor prognostic features including deletions of 17p13 or 11q22-q23, or IgVH3-21 expression. Thus, some patients with a mutated IgVH3-21 gene express high levels of ZAP-70 and do poorly. [(12)] It is unclear why ZAP-70-positive CLL patients have a poor prognosis although it has been related to the magnified signaling by the immunoglobulin cell receptor in ZAP-70-positive CLL cells. [(13)]

β_2-microglobulin is a serum marker that correlates with tumor burden and disease stage in patients with CLL. It was a significant prognostic indicator for response to therapy, time to treatment failure and overall survival in many studies [14-17].

CD 27, a transmembrane homodimer belongs to the nerve growth factor (NGF) receptor super family is typically expressed on leukemic CD5+ cells in B-

CLL and found in soluble form in the serum of CLL patients. [18] We herein study the prognostic value of soluble CD 27 and its impact on clinical course and outcome in patients with B-CLL in comparison to β_2-microglobulin.

2. Patients and methods

Fifty consecutive B-CLL patients were enrolled in the study, from 2010 to 2014.

All fulfilling the recommended diagnostic morphological and immunophenotypic criteria. Proper staging according to both Binet, and Rai staging systems was done. The patients were treated with Fludarabine-based protocols. The response to treatment was recognized both clinically and laboratory. Soluble CD 27 levels at initial and after completing six cycles of treatment were correlated to clinical staging, response to treatment, and other prognostic factors.

2.1. Measurement of soluble CD 27:

Soluble CD 27 level was assessed by using Human sCD27 Instant ELISA (BMS286INST) kits. An anti-human sCD27 coating antibody was adsorbed onto micro wells. Human sCD27 present in the samples or standard binds to antibodies. A biotin-conjugated anti –human sCD27 antibody bounded to the human sCD27 was captured by the first antibody. Streptavidin-HRP was bounded to the biotin conjugated anti- human sCD27. Following incubation, unbound biotin conjugated anti- human sCD27 and Streptavidin-HRP were removed during a wash step and a substrate solution reactive with HRP was added to the wells. A colored product was formed in proportion to the amount of human sCD27 present in the sample. The reaction was terminated by the addition of acid and adsorbance was measured at 450 nm. A standard curve was prepared from 7 human sCD27 standard dilutions and human sCD27 sample concentration determined. A panel of 40 sera samples randomly selected from apparently healthy donors (28 males and 12 females) was tested for human sCD27 and β_2- microglobulin and used as controls. Their ages ranged from 25 to 52 years with a median of 46.5 $\pm$ 4.3 years.

2.2. Measurement of β_2- microglobulin:

β_2- microglobulin level was measured by the use of ORG 5BM Beta 2 Microglobulin (Orentec Diagnostica GmbH). Highly purified anti- human-β2-microglobulin antibodies are bound to microwells. β2-microglobulin, if present in diluted serum, bind to the respective antibody. Washing of the microwells removed the unspecified components. Horseradish peroxidase (HRP) conjugated anti-human-β2-microglobulin immunologically detected the bound patient β2-microglobulin forming a conjugate/β2-microglobulin/antibody complex. Washing the microwells removed the unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzed to form a blue colour. The addition of an acid stopped the reaction forming a yellow end product. The intensity of the yellow colour was measured photometrically at 450 nm. The amount of colour was directly proportional to the concentration of β2-microglobulin present in the original sample.

2.3. Statistical Analysis:

Data were analyzed using SPSS program (Standard version 10, 1999). Quantitative data were presented in the form of mean and standard deviation (mean ± SD). A one-way analysis of variance (ANOVA) across the different groups was carried out for each set of variables. The chi-square test was used for categorical data. Pearson's correlation was used for the correlations of different parameters that were assumed to have a normal distribution. Statistical significance was set at $p \leq 0.05$. For evaluation of the impact of prognostic factors on progression free survival (PFS) and overall survival (OS) a univariate cox regression analysis was preformed. OS was calculated from the first day of treatment to the day of last follow-up or the patient's death. PFS was the time from the start of treatment to the time of disease progression. For detection of independent prognostic factors multivariate forward stepwise cox regression analysis was performed.

3. Results:

The patients were representative of a population of patients with B-CLL at Mansoura University Cancer Center, Egypt and included 50 newly diagnosed patients. They were 32 males and 18 females, with a median age of 54 years (range from 42 to 61 years) and a hemoglobin level ranged from 3.9 – 14.2 gm/ dl (mean value of 10.4 ± 2.5 gm/dl). The white cell count ranged from 15.8 – 420 $x10^3/\mu l$ with a mean of 108.9 ± 87.3 $x10^3/\mu l$ and the lymphocytes ranged from 25 to 96% (mean 78 $\pm$ 15.9 %). The platelets ranged from 41 to 460 $x10^3/\mu l$ (mean 148.1 $\pm$ 78.3 $x10^3/\mu l$). Most of the patients (40%) were found to have Rai stage II, while most of them (48%) were found to be Binet stage C [table 1].

Table 1: Patient's characteristics

Parameter		range	mean ± SD
No. patients	50		
Age, years		42-61	54.5 ± 5.3
Male, no. (%)	32 (64%)		
WBCs x 10^3/µL		15.8-420	108.9 ±87.3
Lymphocytes (%)		25-96	78±15.9
Platelets x 10^3/ µL		41-460	148.1± 78.3
Hemoglobin g/L		3.9-14.2	10.4 ± 2.5
Rai stage, no (%)			
0	- (-)		
I	2 (4)		
II	20 (40)		
III	17 (34)		
IV	11 (22)		
Binet stage, no (%)			
A	6 (12)		
B	20 (40)		
C	24 (48)		

The pretreatment serum levels of soluble CD 27 and β2- microglobulin compared to the controls were shown in table 2. The detected human sCD27 levels of the controls ranged between 70.8- 335.5 U/ml, with a mean level of 196.0±33.3 U/ml. A significantly higher level of sCD 27 in patients with CLL has been detected with a mean ± SD of 443.69 ± 346.71 U/ml (Range 96.5 – 1616 U/ml; P 0.01). Similarly, a significant higher level of serum β_2- microglobulin in patient with CLL has been detected compared to controls (4.516 ± 1.16 µL/ml vs. 0.6±1.3 µL/ml; P 0.02).

Table 2: Pretreatment levels of soluble CD 27 and serum β_2-microglobulins in all studied patients.

sCD27 (U/ml)	control	P	β_2-microglobulins (µL/ml)	control	P	
Range	96.5 – 1616	70.8- 335.5	0.01	2-7	0-3	0.02
Mean ± SD	443.69 ± 346.71	196.0±33.3		4.516 ± 1.16	0.6±1.3	

Correlations between the pretreatment serum level of β_2-microglobulins and soluble CD 27 and other parameters in all studied patients have been shown in table 3. A significant positive correlation was detected between the pretreatment levels of soluble CD 27 and β2-microglobulins (r =0.423, P = 0.002). A significant negative correlation was detected between sCD27 and hemoglobin level (r =- 0.436, P =0.002). However, circulating CD 27 did not correlate significantly with WBCs count, ESR or LDH levels [table 3].

Table 3: Correlations between the pretreatment serum level of β2 microglobulin and soluble CD 27 with other parameters in all studied patients (50 patients).

Parameter	β_2-microglobulins	sCD 27 before
ESR	r = 0.162	r = 0.231
	P = 0.262	p = 0.107
LDH	r = 0.255	r = 0.187
	P = 0.074	p = 0.2
Hb level	r = - 0.231	r = - 0.436
	P = 0.110	p = 0.002
WBCs	r = 0.121	r = 0.03
	P = 0.407	p = 0.8
β 2 microglobulin	r = 0.423	
	p = 0.002	
sCD 27	r = 0.423	
	p = 0.002	

Correlation with response to treatment:

Eleven (22%) patients developed complete response (CR), while 56%, 4%, 18% developed partial response (PR), stable disease (SD), and progressive disease (PD) respectively. The pretreatment levels of soluble CD 27 and β_2-microglobulins were predictive for the overall response (ORR) including patients with complete and partial response (r=0.678, P=0.001; r=0.383, P=0.006, respectively) [table 4]. At the same time, a significant higher pretreatment level of soluble CD27 and serum β_2-microglobulins was detected in patients who didn't develop CR compared to those who developed CR [table 5]. The mean value ± SD of sCD 27 in patients who showed complete response was 98.845 ± 31.89 U/ml compared to 344.713 ± 294.891 U/ml in non responders (P = 0.01). The pretreatment level of β_2-microglobulin was 1.666 ± 1.576 µL/ml in complete responders compared to 3.054 ± 1.609 µL/ml in patients who did not show CR (P = 0.001).

Table 4: Correlation of pre-treatment soluble CD27 and serum β_2-microglobulins levels with overall response rate (ORR)

Parameter	ORR	
Pearson's correlation	p	
sCD 27	r = 0.678	0.001
β_2-microglobulins	r = 0.383	0.006

Table 5: Relation of pretreatment levels of serum β_2-microglobulins and soluble CD27 and response to treatment

CR	no CR	test of significance	
sCD 27 Mean ± SD U/ml	98.845 ± 31.89	344.713 ± 294.891	t = 2.6 p = 0.01
β_2-microglobulin Mean ± SD µL/ml	1.666 ± 1.576	3.054 ± 1.609	t = 3.0 p = 0.001

Comparison between pre and post treatment levels of both soluble CD 27 and β_2-microglobulins is presented in table 6. A significant reduction of pre treatment level of both sCD27 and β_2-microglobulins compared to post treatment levels has been detected (443.7 ± 346.7 U/ml vs 283.1 ± 255.2 U/ml, P 0.001 and 4.5 ± 1.2 µL/ml vs 2.6 ± 1.5 µL/ml, P 0.001, respectively).

Table 6: Comparison between pre and post treatment levels of soluble CD 27 and β_2-microglobulins

Parameter (Mean ± SD)	Pretreatment level (Mean ± SD)	Post treatment level	P
soluble CD 27 (U/ml)	443.7 ± 346.7	283.1 ± 255.2	0.001
β_2-microglobulins (µL/ml)	4.5 ± 1.2	2.6 ± 1.5	0.001

Correlation with survival:

Soluble CD27, β_2- microglobulin, Coomb's test, RAI and Binet staging systems were investigated as predictors for survival. Univariate analysis of variables predictive of progression free survival in all studied patients showed only sCD 27 and β_2-microglobulins to be statistically significant (P = 0.008, P = 0.037 respectively) [Table 7]. By multivariate analysis, sCD27 and β2-microglobulins remained statistically significant (P = 0.04, 0.03 respectively) [Table 8]. By univariate analysis of the overall survival in all studied patients, both sCD 27 and β2-microglobulins were statistically significant (P =0.039; 0.007 respectively) [Table 7] and they remained statistically significant in a multivariate analysis (P = 0.001; 0.001 respectively) [Table 8].

4. Discussion:

In the last years many prognostic factors emerged and have been tried to find their correlations with the survival of patients with CLL**.** The use of these markers to stratify patients in clinical trials, to help assess the need for therapy and to help to select the type of therapy continues to evolve.[19] sCD27 is a trans membrane homodimer belonging to the nerve growth factor receptor super family. It is typically expressed on leukemic CD5+ cells in B- cell CLL and found in a soluble form in the serum of CLL patients

and it can be a reliable marker of tumor mass in these patients. [18] The expression of CD27 on malignant B cells was investigated in physiologic antigen-independent and -dependent B-cell development. In normal lymphoid tissue CD27+ B cells were only found in the peripheral blood and in germinal centers. With the exception of pro-B and the majority of pre-pre-B acute lymphocytic leukemias and of myelomas, CD27 expression of variable intensity was detected on almost all immature and mature malignant B cells tested. Moreover, very high amounts of the soluble 28- to 32-kD form of sCD27 were detected in the sera of patients with B-cell malignancies. The highest levels of sCD27 were observed in patients with CLL and low-grade non-Hodgkin's lymphomas. Most importantly, both in transversal and longitudinal studies, a strong correlation between sCD27 levels in the serum and tumor load was detected. [20]

Table 7: Univariate analysis of variables predictive of progression free survival and overall survival in all studied patients (50).

	Progression free survival			Overall survival		
Parameter	**Odds Ratio**	**P**	**95% CI**	**Odds Ratio**	**P**	**95% CI**
s CD 27	1.02	0.037	1.001 – 1.003	1.09	0.039	1.003 – 1.007
β_2-microglobulins	2.2	0.008	1.2 – 3.9	2.19	0.007	1.3 – 3.7
Coomb's test	0.85	0.75	0.2 -2.5	0.81	0.73	0.19 -2.4
RAI staging	0.65	0.5	0.18 – 2.3	0.62	0.5	0.16 – 2.2
Binet staging	1.1	0.8	0.3 - 4.4	1.2	0.7	0.2 - 4.3

Table 8: Multivariate analysis of variables predictive of progression free survival and overall survival in all studied patients (50)

	Progression free survival			Overall survival		
Parameter	**Odds Ratio**	**P**	**95% CI**	**Odds Ratio**	**P**	**95% CI**
sCD 27	1.01	0.04	0.9 – 1.3	1.03	0.001	1.01 – 1.05
β_2-microglobulins	2.07	0.03	1.6 – 11.8	2.6	0.001	1.3 – 11.1

In this study, we evaluate the prognostic impact of sCD27 in CLL patients to find out its correlation to treatment response and its relation to other prognostic factors such as β_2-microglobulins. A strong positive correlation was detected between sCD27 and β_2-microglobulins. Moreover, the pretreatment levels of sCD 27 and β2-microglobulins were statistically higher than their controls and they were predictives for the overall response rate. Also, they were significant prognostic indicators for both progression free and overall survival rates.

These results are in agreement with Ho et al, 2008 who stated that sCD27 is predictive for the overall survival as it reflects tumor burden and disease activity. The same authors demonstrated the important role of sCD27-CD70 signaling among bone marrow mast cells and demonstrate a novel mechanism of action for sCD27 as a regulator of two principal TNF family members (APRIL and CD40L) whose role as growth and survival factors has previously been established in CLL and other B-cell malignancies. [21] Actually, the interaction between CD27 and its ligand, CD70, has been implicated in regulating cellular immune responses to cancer.[22] B cells from most patients with CLL express both membrane-bound CD27 (mCD27) and soluble CD27. Expression of sCD27 inhibits CD27-dependent T-cell or CLL-cell activation mediated by its ligand, CD70. Metalloprotease inhibitors can block the production of sCD27, which can interfere with mCD27-CD70 interactions that induce expression of immune costimulatory molecules on CLL B cells. These data suggest that targeting CD70 and sCD27-CD70 interactions may produce important clinical benefits for patients with B-cell malignancies such as CLL and Waldenström's macroglobulinemia (WM) as well as autoimmune-related disorders wherein elevated sCD27 levels have been reported**.** Conceivably, treatment of CLL cells with metalloprotease inhibitors may enhance their potential for stimulating cellular immune recognition of leukemia-associated antigens. **(18)**

Similar to our results, Kara et al, 2007 explored levels of sCD27, interleukin (IL)-8 and IL-10 in B-CLL to correlate their levels with disease stage and prognosis. sCD27 was a significant prognostic factor for B-CLL. **(23)** Also**,** the clinico-biological implications of increased serum levels of sCD27 in an unselected series of B-CLL patients were investigated by Molica et al, 1998. There was a close relationship between sCD27 and soluble TNF-alpha, another molecule belonging to the NGF receptor superfamily. Changes in sCD27 level correlated with clinical stage, beta2-microglobulin and LDH. **(24)**

During the last decade, disease-associated parameters have been indentified which reflect the biology of the disease as well as give prognostic information. Along this line, different chromosomal aberrations have been shown to be related to different courses of the disease with 17p and 11q deletions being associated with a shorter overall survival while cases with a 13q deletion as sole abnormality have a relatively favorable overall survival. [25] The immunoglobulin variable heavy chain (IgVH) mutation status has been demonstrated not only to subdivide CLL according to the pathogenetic basis but to also result in a clinically powerful parameter. [26-28]

Conclusion:

Our study provided evidence that sCD 27 has a predictive and prognostic value in patients with CLL as the serum β2-microglobulin. The association of sCD27 expression with the IgVH mutation status is clearly warranted to support the evaluation of this antibody in the setting of clinical trials to further define its role and place in routine diagnosis and management of patients with CLL.

Conflict of interest:

The authors declare that they have no conflict of interest.

Corresponding Author:
Mohamed Mabed, MD, PhD
Division of Hematology, Oncology Center,
Faculty of Medicine, Mansoura University, Egypt
Tel: 00201068595165 Fax: 002050 2370103
E mail: loaieelhelw@hotmail.com
mohmabed@mans.edu.eg

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