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Correlation between Soluble Cytoadhesion Molecules (sVCAM-1 and sICAM-1) with Prognostic Markers of Disease Activity in Chronic B-Lymphocytic Leukemia (B-CLL)

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ABSTRACT

Purpose: To investigate the potential usefulness of sVCAM-1 and sICAM-1 in morphologically and immunophenotypically confirmed B-CLL as prognostic markers in newly diagnosed patients and to guide in selecting a treatment protocol.

Patients and Methods: The study included 27 newly diagnosed B-CLL patients, in different stages, according to modified Rai classification. Fifteen age and sex matched healthy volunteers were included as a control group. Diagnosis was made in all cases by examination of Leishman-stained peripheral blood and bone marrow smears and was confirmed by phenotypic analysis that was performed on the "Epics profile II" flow cytometer using fluorescein isothiocyanate and phycoerythrin-labeled monoclonal antibodies. Serum levels of sVCAM-1 and sICAM-1 were measured by the ELISA technique. Eighteen out of the 27 patients with elevated sVCAM-1 level were treated with chlorambucil or cyclophosphamide and prednisone. Follow up of the cases was done by serial estimations of sVCAM-1 levels in both treated and untreated patients at 3 month-intervals for one year.

Results: Both sVCAM-1 and sICAM-1 were well correlated with clinical staging, i.e. B-CLL patients representing high risk stages had significantly higher ($p < 0.001$) serum levels of sVCAM-1 and sICAM-1 compared with intermediate risk and low risk patients. However, sVCAM-1 was more superior and closely reflected the tumor burden in B-CLL than sICAM-1 as it was strongly correlated with clinical staging, lymphocyte count and β_2-microglobulin level. In the treated group, during the follow up period, 12 patients showed progressive decline of sVCAM-1 and 10 of them remained alive for the whole time of follow up, whereas the remaining 6 patients showed, more or less, a stationary level of sVCAM-1 and four of them died from disease complications. In the untreated group, 5 patients who had the highest levels of sVCAM-1 progressed rapidly to more advanced stages of the disease and developed complications.

Conclusion: sVCAM-1 is a more sensitive prognostic marker than sICAM-1 as it significantly correlates with tumor burden and serum markers. Moreover, sVCAM-1 measurement is easy, reproducible and accurate and can be used as a laboratory tool for deciding a treatment or no treatment policy for patients in early stages of the disease. This needs to be more confirmed in prospective studies on larger numbers of patients and longer follow-up periods.

Key Words: *B-CLL - sVCAM 1 - sICAM-1 - Prognosis.*

INTRODUCTION

Chronic lymphocytic leukemia of the B cell type (B-CLL) is a disease characterized by the accumulation of monoclonal CD5 + CD23 + B-lymphocytes **[13,27]**. The most commonly used methods for predicting prognosis in B-CLL are clinical staging systems that estimate the static tumor mass **[33]**. Additional information is obtained by studying the kinetics of leukemic cell expansion, i.e. lymphocyte doubling time, lymphocyte and bone marrow morphology and cytogenetic aberrations **[17]**, serum lactate dehydrogenase (LDH), serum thymine kinase 21, serum level of β_2-microglobulin (β_2-m) and more recently, sCD23 **[5]**.

Treatment decisions are difficult, especially in early-stage patients. The advent of new effective treatment options, such as purine analogues and improved predictive tools could prove valuable **[14]**. The variability in the clinical presentations such as lymph node enlargement, spleen or liver enlargement have been suggested to be due to different patterns of adhesion molecule expression and/or distribution in B-CLL **[21,31]**.

Adhesion molecules play a key role in the immune system by promoting cell-cell and cell-stromal interactions and leukocyte trafficking

[10]. Soluble counterparts of adhesion molecules, probably derived through proteolytic cleavage [30] of the corresponding cellular transmemberane molecules, are found circulating in the serum. These molecules were designated soluble VCAM-1 (sVCAM-1) or (sCD106) and soluble ICAM-1 (sICAM-1) or (sCD54) and were found to inhibit the action of the receptor-ligand pairs by binding to and blocking the respective ligands [5,36]. This may represent a mechanism for malignant cells to escape immune surveillance and may be a possible explanation for tumor dissemination and metastasis [19,34].

Vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) are both members of the immunoglobulin (Ig) superfamily [15]. Soluble VCAM-1 is expressed at low levels by bone marrow stromal cells, vascular endothelial cells and follicular dendritic cells [10] and its expression is up-regulated by cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), IL-4 & IL-13 [20]. The sVCAM-1 expression by bone marrow stroma was shown to be important for normal B-cell lymphopoiesis and for sustaining B-leukemic blasts in vitro [18]. An in vitro study suggested that B-cells bind to normal germinal centers via sVCAM-1 [25]. Soluble ICAM-1 is widely distributed among hemopoietic and non - hemopoietic cells. Regulation of its expression is mediated by several cytokines. Up-regulation is mediated by IL-1 and TNF-α, while down-regulation is mediated by IL-4 [35]. It mediates adhesion of monocytes, lymphocytes and neutrophils to activated endothelium. Its presence on bone marrow progenitors and accessory cells suggests that it may play a role in cell-to-cell and cell-stromal interaction during hemopoiesis [10].

Aim of the work:

To study the serum levels of sVCAM-1 and sICAM-1 in morphologically and immunophenotypically confirmed B-CLL and their correlation with tumor burden and other prognostic markers of disease activity, to investigate the potential usefulness of these molecules as prognostic markers in newly diagnosed B-CLL patients, and to guide in selecting suitable treatment protocols.

PATIENTS AND METHODS

This study included 27 newly diagnosed patients of B-CLL. Eighteen patients were males and 9 were females with a mean age of 58±14 years. All patients were attending the clinical oncology departement, Ain Shams University Hospital between December 1998 and March 2001. Patients fulfilled the criteria of the National Cancer Institute- sponsored Working Group guidelines for CLL [9]. Staging of the patients was performed according to the modied Rai classification [28,29]. Fifteen age and gender matched healthy volunteers were included as controls . The control group included 11 males and 4 females with a mean age of 51±23 years. All patients and controls were subjected to:

- History taking and clinical examination laying stress on presence or absence of lymphadenopathy, splenomegaly and/or hepaotmegaly.
- Routine laboratory investigations including-liver and kidney function tests and serum lactate dehydrogenase level using caloremetric methods (Boehringer, Mannheim, Germany).
- Serum β_2-microglobulin (β_2-m) level that was measured by the ELISA technique (Eurogenetis N. V. Technology circle) [15].
- Radiological examination that included chest X-ray and abdominal ultrasonography. MRI and/or CT scan was performed when recommended.
- Complete blood picture: Performed by Coulter Counter T660 (Coulter Electronics LTd, Luton, Beds) and bone marrow aspiration (for patients only) with examination of Leishman-stained peripheral blood and bone marrow smears. Diagnosis was confirmed in all cases by phenotypic analysis.

Immunophenotyping for all patients was performed on the "Epics Profile II" flow cytometer using fluorescine isothiocyanate and phycoerythrin (FITC and PE)-labeled monoclonal antibodies (mAbs) that were purchased from (Coulter). CD20 or 22 and CD19 were used for confirmation of B-cell lineage and CD5 and CD23 were used to differentiate between B-CLL and mantle cell lymphoma. Other markers, like kappa (k), lambda (λ) and surface immunoglobulin (sIgM) were also used for further differentiation and/or confirmation. The expression was considered positive for any surface marker when ≥ 20% of the cells were positive for that marker, with clonal expression of k or λ.

Assay of sVCAM-1 and sICAM-1 was done for all the patients and controls by the ELISA

technique (R&D Systems Europe, Oxon, UK) [6,12].

All patients were subjected to serial estimations of sVCAM-1 every 3 months for 1 year, during which 9 patients received no treatment and 18 patients were treated by combination of chlorambucil (0.9 mg/kg divided on 5 days every 21-28 days) or cyclophosphamide (12-15 mg/kg divided on 7 days every 21-28 days) and glucocorticoids (40 mg/m^2 daily). Glucocorticoids were especially indicated if the patient developed autoimmune anemia or thrombocytopenia and its dose was reduced to 0.5 mg/kg/day in old or fragile patients and in patients with hepatic insufficiency. Some patients needed erythropoietin treatment for reticulocytopenia not responding to treatment. The treatment continued until response or myelosupprssion was observed.

Statistical analysis:

Data were presented for the hematological parameters (hemoglobin, total leucocytic count, platelets and lymphocytes in peripheral blood and bone marrow) and soluble factors sVCAM-1 and sICAM-1 as mean ± standard deviation (SD). p values < 0.05 were considered significant. Kruskal-Wallis ANOVA and Mann-Whitney U test (non parametric tests) were used to compare serum sVCAM-1and sICAM-1 with the clinical classification system of Rai. The Spearman's correlation coefficients were used to determine the association of sVCAM-1 and sICAM-1 to other prognostic clinical and serum markers.

RESULTS

This study was performed on 27 newly diagnosed B-CLL patients attending the clinical oncology departement, Ain Shams University Hospital in different stages of the disease (according to the modified Rai classification) [28,29]. Fifteen age and sex matched healthy subjects were included as controls. The clinical and hematological data of both patients and control groups are presented in Table (1).

Among the studied patient group, 18 (66.7%) of the cases were males and 9 (33.3%) were females. Their ages ranged from 39 to 72 with a mean age of 58 years. Twenty-three patients (85.1%) had lymph node enlargement and 17 (62.9%) had hepatomegaly and/or splenomegaly. According to the modified Rai staging system, 3 patients (11.1%) were in stage 0, 8 patients (29.6%) in stage I and 8 patients (29.6%) in stage II. Five patients were classified as stage III, while the remaining 3 (11.1%) patients were in stage IV.

There was a highly significant difference ($p < 0.001$) between the serum levels of sVCAM-1 in patients (1710±580 ng/ml) and in controls (670±228 ng/ml). The same highly significant difference was found regarding sICAM-1levels (648±330 and 305±85 ng/ml) for patients and controls, respectively (Fig. 1).

When serum levels of sVCAM-1were compared in different stages of the disease (according to the modified Rai classification system) (Fig. 2), there was a highly significant difference ($p = 0.02$) between the low risk group (stage 0) and the intermediate risk group (stage I and II). Their mean serum levels were (710±280 and 1090±580 ng/ml for the low risk and the intermediate risk groups, respectively). Significant difference ($p = 0.003$) was found also between the Intermediate risk group and the high risk group (stages III and IV). Their mean serum levels were (1090±580 and 1430±495 ng/ml for the intermediate risk and the high risk groups, respectively). Regarding sICAM-1, a highly significant difference ($p = 0.001$) was found between the low risk group and the intermediate risk group (330± 158 and 560±192ng/ml respectively). However, an insignificant difference ($p = 0.34$) was found between the Intermediate risk group (560±192 ng/ml) and the high-risk group (615±210 ng/ml).

An insignificant correlation was found between sICAM-1 levels and any of the prognostic markers of the disease (hemoglobin concentration, total leukocyte count, platelets count, lymphocyte count, serum LDH and serum β_2-m). The same insignificant correlation was found between sVCAM-1 levels and either hemoglobin concentration, total leukocyte count, platelet count or serum LDH. On the other hand, a highly significant correlation was found between sVCAM-1 levels and both lymphocyte count ($r = 0.622$, $p < 0.01$) and serum β_2-microglobulin ($r = 0.712$, $p < 0.01$).

The median duration of clinical follow up was 21.9 months (ranging from 7 months to 30 months). Due to the significant correlation between sVCAM-1 and prognostic markers of the disease, it had been chosen to be measured serially in the patients (every 3 months) for a

total of 1 year. A total of 18 patients were treated by chemotherapy with weekly follow up by a complete blood picture. According to the modified Rai classification system, 10 patients were stage I and II (treated because of disease complications i.e. painful lymphadenopathy, obstructive lymphadenopathy or disfigurement) and 8 patients were stage III and IV. The remaining 9 patients were kept under strict follow up with serial estimations of sVCAM-1 every 3 months. In the treated group, during the follow up period, 12 patients showed progressive decline of the sVCAM-1, 10 remained alive for the whole duration of follow up and 2 patients died because of unrelated causes. On the other hand, the remaining 6 patients showed, more or less, stationary levels of sVCAM-1. Of these 6 patients, one died from intercurrent disease (myocardial infarction), one died from cerebral hemorrhage and 4 died from massive infection. In the untreated group of patients (n=9), 5 patients with the highest levels of sVCAM-1 (1215±352 ng/ml) progressed rapidly to more advanced stages of the disease and developed complications. On the other hand, the remaining 4 patients who had the lowest levels of sVCAM-1 (677±147 ng/ml) during the follow up period did not show progression and did not require treatment.

Table (1): Clinical and hematological characteristics of both patients and controls.

	Patients	Controls
Number	27	15
Sex (Male / Female)	18/9	11/4
Age range (years)	39-72	35-65
Lymph node enlargement	23	
Hepatomegaly and/or splenomegaly	17	
Rai staging:		
Stage 0	3	
Stage I	8	
Stage II	8	
Stage III	5	
Stage IV	3	
Hb concentration (g/dl)	10.5±4.6	13.5±5.4
TLC ($x10^6$/l)	49.7±27	8.0±2.3
Platelets count ($x10^6$/l)	236.1±99	466.0±200
Lymphocytes in PB ($x10^6$/l)	39.3±7.0	5.5±1.1
Lymphocytes in BM (%)	17.3±2.4	Not done

Hb = Hemoglobin. PB = Peripheral blood.
TLC = Total leukocyte count. BM = Bone marrow.

Table (2): Cases of B-CLL with their immunophenotypes.

Patients No.	CD5%	CD19%	CD20%	CD23%	SIgM	Lightchain
1	81.7	91.0	0.4	90.6	–	–
2	74.5	73.9	39.5	61.9	63.9+	λ
3	87.3	80.2	65.3	74.7	47.0+	K
4	84.1	61.1	64.8	66.4	59.4+	K
5	82.7	80.0	14.5	78.1	–	–
6	96.5	95.3	0.5	90.2	71.3+	K
7	80.9	87.1	9.3	46.1	–	–
8	74.1	80.7	0.4	66.9	60.0+	K
9	80.6	67.1	61.8	60.1	41.0+	–
10	88.5	80.6	65.3	87.4	–	–
11	91.4	91.0	65.2	90.3	79.4++	K
12	79.6	71.4	45.4	67.6	63.6+	K
13	81.7	91.8	0.6	90.3	14.0	–
14	95.3	92.0	13.6	90.7	30.1++	λ
15	80.6	61.1	63.8	60.9	–	–
16	77.1	79.5	52.1	66.1	13.7	–
17	97.5	90.3	13.5	90.7	76.0+	K
18	93.8	90.2	8.0	92.0	67.2+	K
19	80.6	71.7	71.1	70.3	63.3+	K
20	74.5	88.0	0.4	69.0	–	–
21	81.7	91.8	0.7	90.6	14.4	–
22	79.5	77.3	49.1	66.7	63.7+	λ
23	95.3	90.2	13.5	92.0	30.0+	κ
24	90.0	91.0	65.1	90.3	–	–
25	88.5	80.8	78.4	78.4	–	–
26	87.5	47.0	8.1	40.2	–	–
27	84.7	85.9	0.1	83.6	–	–

sIgM = Surface immunoglobulin M, κ = Kappa light chain, λ = Lambda light chain, % = Percent of positive lymphocytes.

Table (3): Correlation between sVCAM-1, sICAM-1 and other prognostic markers studied in the patients.

Parameter	sVCAM-1		sICAM-1	
	r	*p* value	*r*	*p* value
Hb	0.178	> 0.05	0.201	> 0.05
TLC	0.063	> 0.05	0.193	> 0.05
Platelets	0.139	> 0.05	0.145	> 0.05
Lymphocyte	0.622	< 0.01	0.218	> 0.05
LDH	0.137	> 0.05	0.172	> 0.05
β_2-m	0.712	< 0.01	0.190	> 0.05

Hb = Hemoglobin. LDH = Lactate dehydrogenase.
TLC = Total leukocyte count. β_2-m = β_2 microglobulin.

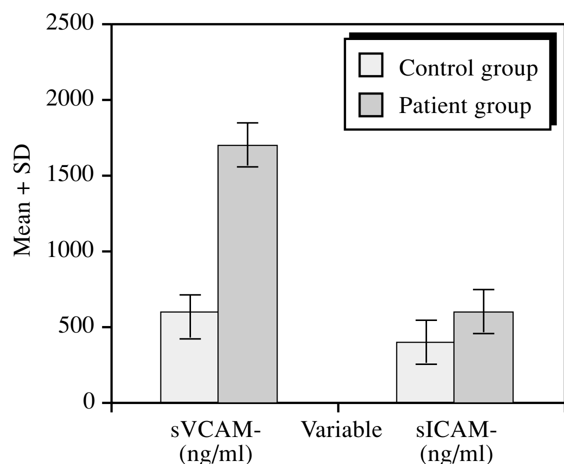

Fig. (1): Statistical analysis of the sVCAM-1 and sICAM-1 levels among patients and controls.

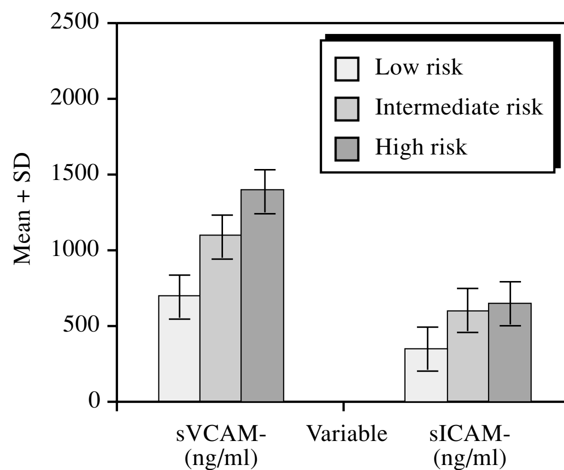

Fig. (2): Statistical comparison of serum sVCAM-1 and sICAM-1 levels among different clinical stages of the disease according to modified Rai staging.

DISCUSSION

Chronic lymphocytic leukemia (CLL) is a markedly heterogenous disease with regard to its prognosis and clinical course. Several staging systems have been proposed [37] that identify three major prognostic subgroups and guide the treatment decision. There is general agreement that only patients with advanced Rai stages require chemotherapy [38]. Patients with early stages of CLL, i.e. Rai stages 0 to II, do not usually receive treatment until progression. However, approximately 30% to 40% of patients with early CLL show a short progression-free survival (PFS) and might benefit from early and/or intensified treatment.

Considerable progress has been made over the past 20 years in identifying new prognostic parameters in CLL. In addition to advanced Rai stages, at least four factors generally seem to predict poor prognosis in CLL: diffuse BM infiltration, blood lymphocyte counts greater than 50,000/µl, a lymphocyte doubling time less than or equal to 12 months and multiple or complex abnormalities of karyotype [4,7]. However, it should be emphasized that some of these four prognostic factors were tested in a limited number of studies or showed independent prognostic values in small subgroups of patients.

The value of serum parameters as β_2-m, serum LDH and serum thymidine kinase (TK) may add prognostic information to the current staging systems [1,32]. The versatility and ease of serum tests which yield quantitative rather than qualitative results are important advantages, in comparison to the laborious classic cytogenetics or investigator-based evaluation of BM histology.

In the present study, elevation of serum levels of sVCAM-1in all B-CLL patients was found, with highly significant difference between patients and controls. Moreover, when sVCAM-1 levels were compared in different stages of the disease, according to the Rai staging system, there was significant difference between the levels in the low risk group and in the intermediate risk group. The same significant difference was found between the intermediate risk group and the high-risk group. Serum VCAM-1 levels were found to be correlated with disease activity as indicated by lymphocyte count and serum β_2-

m. These findings agree with the results of Christiansen et al. [11] who showed the same results except that in their study no significant correlation was found between sVCAM-1 levels and lymphocyte count.

In our study, sICAM-1 was significantly increased in all 27 B-CLL studied patients when compared to controls, as reported also by Wang et al. [37] and Mustjoki et al. [24]. Significant difference was noted between the levels of sI-CAM-1 in the low risk group and in the intermediate risk group. However, no significant difference was found between the intermediate risk group and the high-risk group. Our results are in agreement with those of Musolino et al. [23].

Christiansen et al. [10] and Molica et al. [22] reported in their studies that sICAM-1 level was correlated with tumor burden and its pre-treatment levels were elevated in all patients and were correlated with clinical stage, lymphocyte doubling time, thymidine kinase, LDH and β_2-m. However, in our study no such correlation was found between sICAM-1 and any of the laboratory prognostic markers of the disease. Our results are in agreement with those of Beksac et al. [3] who were not able to find any correlation between sICAM-1 levels and clinical or laboratory parameters of the patients.

Beksac et al. [3] reported that sICAM-1 could not be used in their work for monitoring therapy in B-CLL patients. Mustjoki et al. [24] also stated that although increased sICAM-1levels in leukemia patients had an important role in disease progression yet, it correlated only weakly with leukocyte count while no correlation was found between its level and response to therapy. Hjalmar et al. [16] added that surface antigen expression of CD54 (ICAM-1) on B and T cells, but not the soluble form, can give prognostic information about the disease.

The functional consequence of dysregulated levels of soluble adhesion molecules remains unclear, apart from disrupted cell-cell signaling [36] and the intriguing suggestion of sVCAM-1 as a promoter per se of angiogenesis [26]. However, elevated serum levels of soluble adhesion molecules do not necessarily reflect the cellular events. The interplay between cellularity expressed adhesion molecules and the soluble counterparts needs elucidation [8]. The cellular origin of high sVCAM-1 levels in B-CLL is presently unknown. A possible source for sV-CAM-1 in CLL could be the malignant cells themselves, but since all stages of CLL have elevated levels of sVCAM-1 it seems unlikely that a simple proteolytic cleavage is the origin of sVCAM-1 in all subgroups. Other possible inducers for sVCAM-1 could be cytokines such as IFN-δ, TNF-α and interleukins [2,23].

The strongest correlation that was found in our work between sVCAM-1 and clinical stage of the disease, lymphocyte count and β_2-m, i.e. tumor load, made it of better predictive value than sICAM-1that did not correlate with any of the serum markers of disease activity. That is why follow up of the patients was restricted to serial measurements of sVCAM-1 only. Significant lowering of sVCAM-1 occurred with response to treatment and indicated better survival. Ten out of 12 patients with progressive decline of sVCAM-1 remained alive during the period of the study and only 2 died. Meanwhile, 6 patients with more or less stationary levels of sVCAM-1 died during the course of follow up. Regarding patients who did not receive therapy during the follow up period, the 5 patients with the highest levels of sVCAM-1 progressed rapidly to more advanced stages of the disease and developed complications. On the other hand, 4 out of 9 patients with the lowest levels of sV-CAM-1 during the follow up period did not show progression and did not require treatment. This means that the study has to be done on larger scale and for a longer follow-up period to decide whether we can use the sVCAM-1 as a follow-up marker and as a guide to select more aggressive therapy for the high risk group of patients who have stationary or progressive increase in the level of sVCAM-1.

From the previously mentioned data, we can conclude that sVCAM-1 is a more sensitive prognostic marker than sICAM-1 as it significantly correlates with tumor burden and with serum markers that are known for their prognostic importance such as β_2-m. Moreover, sVCAM-1 measurement is easy, reproducible and accurate and can be used as a laboratory tool for deciding a treatment or no treatment policy for patients in early stages of the disease. This needs to be confirmed in prospective studies on larger numbers of patients.

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