Serum Levels of Soluble Intercellular Adhesion Molecule 1 Are Increased in Chronic B-Lymphocytic Leukemia and Correlate With Clinical Stage and Prognostic Markers

By Ilse Christiansen, Cecilia Gidlöf, AnnaCarin Wallgren, Bengt Simonsson, and Thomas H. Töterman

The serum levels of soluble intercellular adhesion molecule 1 (ICAM-1) were significantly elevated (P < .001) in patients with chronic B-lymphocytic leukemia (B-CLL, n = 113) compared with healthy controls (n = 31). sICAM-1 levels in B-CLL were positively correlated to the tumor mass as reflected by the modified Rai and the Binet staging systems, lymphocyte counts, and isolated spleno/hepatomegaly. During disease progression or regression on cytoreductive therapy, the circulating sICAM-1 levels changed accordingly. sICAM-1 was also correlated to a kinetic parameter such as the lymphocyte doubling time. Furthermore, the serum sICAM-1 levels were inversely correlated to hemoglobin levels in patients with early clinical stage, and this may turn out to be of prognostic value. sICAM-1 was compared with other serum markers to reflect disease activity in B-CLL, ie, soluble CD23, thymidine kinase, lactate dehydrogenase (LDH), and β2-microglobulin. sICAM-1 was equally well or better correlated to clinical stage and lymphocyte doubling time. In univariate regression analysis, all serum markers but LDH correlated with survival, and in multivariate analysis, sICAM-1 was the only marker approaching significance for additional prognostic information when included after clinical stage and lymphocyte doubling time. Based on the present observations, it appears that prospective studies repeatedly monitoring serum sICAM-1 in B-CLL are justified.

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MATERIALS AND METHODS

Patients and Control Subjects

We studied 113 B-CLL patients (75 men and 38 women, mean age, 69; range, 44 to 88), all fulfilling the diagnostic criteria of the International Workshop on CLL (IWCLL). Serum samples were collected at varying intervals after diagnosis (12 to 460 months) and stored at −70°C until use. Seventy-one patients were untreated, five patients were splenectomized only (91, 76, and 4 months before study), and 15 patients had received chemotherapy, but were off treatment (3 to 240 months). Two of the latter patients had been splenectomized (39 and 48 months earlier). Twenty-two patients were on chemotherapy (nine chlorambucil-prednisolone, five COP, two CHOP, two prednisolone, one cyclosporine-A, one teniposide, and one prednimustine). Three of these cases had been splenectomized (60, 12, and 2 months before study). At the time of serum sampling, all 113 patients were restaged according to the systems of Rai et al and Binet et al. The LDT’s were calculated retrospectively in the 76 untreated patients all having observation times in excess of 1 year. All 22 patients undergoing chemotherapy had LDT’s <1 year before treatment, and were classified accordingly. In the 15 cases receiving intermittent chemotherapy, LDT’s were estimated a minimum of 3 months after treatment. At the time of serum sampling, analysis of routine hematologic variables including determination of serum LDH (n = 95) and serum TK (n = 74) were performed. Repeated sampling of sera was done in 10 patients with disease progression (n = 8) or regression (n = 2).
Serum samples from 31 healthy elderly blood donors (mean, 51 years; range, 40 to 60 years) were analyzed for comparison.

**Assays**

**sICAM-1.** The sICAM-1 levels in serum were determined by enzyme immunoassay (EIA) kits purchased from British Biotechnology Products Ltd (Oxon, UK). The assay is based on two monoclonal antibodies (MoAbs) directed against different epitopes on the sICAM-1 molecule. The capture MoAb is bound to solid phase and the detection MoAb is conjugated to horseradish peroxidase (HRP). 3, 3, 5, 5-tetramethylbenzidine (TMB) is used as substrate. The colored product was quantified photometrically using a spectrophotometer (Titertek Multiskan MCC340, Solna, Sweden) set at 450 nm with a correction wavelength of 620 nm. Data were evaluated by DeltaSoft 3.3 MCC software for the Macintosh (both from Apple Computers, Cupertino, CA). The sensitivity of the assay was 0.35 ng/mL, the interassay coefficient of variance (CV) was less than 7.4% and the intra-assay CV varied between 3.3% and 4.8%. Serum samples from 10 patients were tested fresh and after freeze thawing. The sICAM-1 levels were unaltered.

**β₂-microglobulin.** Soluble β₂m levels in serum were determined by EIA kits (T Cell Diagnostics, Inc, Cambridge, UK) based on a competitive assay in which HRP-labeled β₂m competes with cold serum ligand for binding to solid phase antiserum. After incubation with TMB, the colored product was measured using the spectrophotometer set at 450 nm. The sensitivity of the assay was 0.25 μg/mL, the intra-assay CV was less than 6% and the interassay CV was less than 13%.

**sCD23.** sCD23 EIA kits (The Binding Site, Birmingham, UK) were based on three antibodies. The capture MoAb is bound to solid phase, and detection involves sheep-antihuman sCD23 followed by HRP-conjugated antisheep IgG. TMB is used as substrate and the colored product is measured by spectrophotometry. The intra-assay precision CV was 4.2% and the interassay CV was 18.9%.

**Thymidine kinase.** TK is a routine analysis at our institution. In a radioenzymatic technique (Prolifigen assay; Sangtec Medical, Bromma, Sweden), TK converts [³²P] deoxythymidine into [³²P] deoxythymidine monophosphate, and the reaction product, but not the unconverted substrate, is absorbed to a granulate. The radioactivity of the granulate is then measured after several washings. The mean serum TK level in healthy adults was 2.4 ± 1.3 U/L. TK values above 5 U/L (mean + 2 SD) were considered elevated.

**Lactate dehydrogenase.** LDH is a routine analysis at our institution. LDH (EC 1.1.1.27) was analyzed on a Hitachi 717 spectrophotometer (Boehringer Mannheim Diagnostica, Titzing, Germany) using an LDH reagent from the same manufacturer (Catalogue No. 191353). In short, LDH catalyzes pyruvate to lactate + nicotinamide adenine dinucleotide (NAD) in the presence of reduced NAD (NADH) + H⁺. Kinetic measurement of NAD at 340 nm proportionally reflects the concentration of LDH. The serum levels in healthy subjects ranged from 3.8 to 6.7 μkat/L.

Statistical Methods

Data are presented as mean ± SD. P values less than .05 were considered significant. Analysis of variance was used to compare serum sICAM-1 levels with the clinical classification systems of Rai and Binet. The paired Student’s t-test was used to compare serum sICAM-1 levels to lymphocyte counts, LDT, and Hb levels. Spearman’s correlation coefficient was used to determine the association between sICAM-1 and other serum markers, and the association between lymphocyte count and serum markers. The Cox proportional hazard regression model was used in univariate and multivariate analyses.

**RESULTS**

**Serum sICAM-1 Is Elevated in B-CLL**

All 113 B-CLL patients and 31 control subjects had detectable serum sICAM-1 (Fig 1). Circulating sICAM-1 levels were significantly elevated (P = .0009) in the patient group (533 ± 293 ng/mL, mean ± SD) compared with the control group (329 ± 132 ng/mL), although values were widely scattered in B-CLL. The sICAM-1 levels in patients representing the lowest Rai stage 0 (395 ± 169 ng/mL) were not different from controls (P = .06). However, when comparing Binet stage A patients (412 ± 205 ng/mL) with controls, a significant difference was found (P = .03) (see below).

**sICAM-1 in Relation to Clinical Staging Systems**

When applying the modified Rai classification system (Fig 2), B-CLL patients representing high-risk stages had significantly higher (P < .0001) serum levels of sICAM-1 (711 ± 336 ng/mL) compared with intermediate-risk (443 ± 217)
and low-risk (380 ± 185) patients. There was a trend toward higher sICAM-1 levels in the intermediate-risk versus low-risk group, but the difference was not statistically significant. Using the Binet staging system, we likewise found that the sICAM-1 levels in stage C (739 ± 335 ng/mL) were significantly elevated ($P < .0001$) compared with stages B and A (481 ± 245 and 448 ± 233, respectively). Again, no difference was found between stages A and B (data not shown).

Among B-CLL patients in Binet stage A, a survival advantage has been reported for patients with Hb values greater than 120 g/L compared with cases having Hb values 120 g/L or less. Therefore, we compared circulating sICAM-1 levels with Hb levels in 48 stage-A patients. Patients with Hb values ≥120 g/L had significantly higher ($P = .001$) serum sICAM-1 levels compared with patients having higher Hb values (583 ± 241 v 366 ± 163 ng/mL) (Fig 3).

**sICAM-1 Versus Lymphocyte Doubling Time, Lymphocyte Count, and Organomegaly**

Patients having an LDT less than 1 year had significantly higher levels of serum sICAM-1 compared with cases having longer LDT’s (640 ± 331 v 434 ± 213 ng/mL, $P = .01$) (Fig 4A).

Neither the modified Rai nor the Binet classification accounts for hepatomegaly and/or splenomegaly as single factors. Because this might be relevant when considering adhesion molecules and their soluble counterparts, we studied the relationship between splenomegaly/hepatomegaly and sICAM-1 (n = 39, of which only 4 had hepatomegaly, excluding splenectomized cases). Patients with organomegaly had significantly ($P = .0006$) higher levels of sICAM-1 (707 ± 317 v 430 ± 240 ng/mL), as shown in Fig 4B.

A correlation existed between the lymphocyte counts and serum levels of sICAM-1 ($r = .32, P = .0009$) but data were widely distributed (not shown). Excluding patients on therapy or off treatment with cytostatics, the same pattern was seen (not shown).

**Serial Determinations of sICAM-1**

In 10 B-CLL patients, sera were obtained prospectively at 2 to 8 occasions and analyzed for sICAM-1. According to the original Rai classification, 8 of 10 patients exhibited a change in clinical stage (7 progressions and 1 regression on therapy). As shown in Fig 5, a switch in Rai stage was consistently paralleled by a corresponding change in serum sICAM-1 level in all individuals studied. The same results were registered using the Binet (9/10 changes in stage) and
SICAM-1 is elevated in B-CLL

Fig 4. (A) Serum SICAM-1 levels are correlated with the lymphocyte doubling time (LDT; \( P = .01 \)). (B) Patients with splenomegaly and/or hepatomegaly have higher serum levels of SICAM-1 than patients without organomegaly \( (P = .0006) \). Horizontal bars indicate mean values. (n=53) (n=60)

Fig 5. Serial analysis of serum SICAM-1 in eight patients changing in Rai stage (seven progressing and one regressing on therapy).

SICAM-1 compared with other serum markers

Among serum markers claimed to be associated with clinical stage and disease activity in B-CLL, we chose to consider sCD23, TK, \( \beta_m \), and LDH and compared these with SICAM-1 (Tables 1 through 3). The correlation between each individual serum marker and the corrected Rai classification is shown in Table 1. SICAM-1 was equal or superior to sCD23, TK, and LDH. No significant correlation between \( \beta_m \) and corrected Rai stage was registered. Table 2 shows the correlation between each individual serum marker and the LDT. With the exception of LDH, the other markers sCD23, TK, and \( \beta_m \) were correlated to LDT, with marginal significance for \( \beta_m \). Table 3 shows the cross-correlations between serum levels of the five markers and the lymphocyte count. SICAM-1 was most strongly correlated to TK and sCD23, followed by \( \beta_m \) and the lymphocyte count. No correlation was seen with LDH.

SICAM-1 and prognosis

Table 4 shows the prognostic value (survival) of SICAM-1 and the four other serum markers in univariate and multivariate Cox regression analysis. A significant \( (P < .002) \) survival advantage was registered in patients with lower serum SICAM-1 levels. However, no cutoff value was found. With the exception of LDH, the other markers sCD23, TK, and \( \beta_m \) also showed significant correlation to survival. Mul-
Table 1. Rai Staging System Correlated With Different Serum Markers

<table>
<thead>
<tr>
<th>Serum Marker</th>
<th>Rai Stage</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>I + II</td>
</tr>
<tr>
<td>sICAM-1 (ng/mL)</td>
<td>380 ± 185 (n = 22)</td>
<td>443 ± 217 (n = 62)</td>
</tr>
<tr>
<td>sCD 23 (µg/mL)</td>
<td>339 ± 283 (n = 22)</td>
<td>724 ± 689 (n = 61)</td>
</tr>
<tr>
<td>Δm (µg/mL)</td>
<td>5.23 ± 9.24 (n = 22)</td>
<td>4.92 ± 6.45 (n = 61)</td>
</tr>
<tr>
<td>TK (U/L)</td>
<td>2.60 ± 0.99 (n = 13)</td>
<td>7.50 ± 5.84 (n = 43)</td>
</tr>
<tr>
<td>LDH (µkat/L)</td>
<td>6.04 ± 1.39 (n = 20)</td>
<td>6.34 ± 1.63 (n = 54)</td>
</tr>
</tbody>
</table>

Abbreviation: NS, not significant.

* Mean ± standard deviation.

Table 2. Lymphocyte Doubling Time Correlated With Serum Markers

<table>
<thead>
<tr>
<th>Serum Marker</th>
<th>Lymphocyte Doubling Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1 yr (n = 48)</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>575 ± 297*</td>
</tr>
<tr>
<td>sCD 23</td>
<td>1,216 ± 1,158</td>
</tr>
<tr>
<td>Δm</td>
<td>732 ± 793 (n = 47)</td>
</tr>
<tr>
<td>TK</td>
<td>17 ± 23 (n = 34)</td>
</tr>
<tr>
<td>LDH</td>
<td>7.8 ± 3.9 (n = 40)</td>
</tr>
</tbody>
</table>

Abbreviation: NS, not significant.

* Mean ± SD.

tivariate analysis was performed with two, three, or four prognostic factors against each serum marker. The results were identical, and therefore, only the analyses testing the corrected Rai staging plus LDT versus each serum marker are shown (Table 4). The highest P value was obtained for sICAM-1, although this did not reach significance (P = .08) in the present patient material.

DISCUSSION

The most commonly used methods for predicting prognosis in B-CLL are clinical staging systems that estimate the static tumor mass, whereas additional information is obtained by studying the kinetics of leukemic cell expansion, ie, lymphocyte doubling time, the lymphocyte and marrow morphology, and cytogentic aberrations. The prediction of prognosis is particularly difficult in patients representing early clinical stages and serial monitoring of different serum markers and activated lymphocyte phenotypes reflecting disease activity have been proposed.

The present investigation is, to our knowledge, the first to show that serum levels of 82-kD sICAM-1 are elevated in B-CLL and correlate with survival in univariate analysis. However, elevated serum levels of sICAM-1 have recently been described in patients with other hematopoietic tumors such as HD and childhood ALL, and also in malignant melanoma. In HD patients, serum sICAM-1 levels were related to tumor mass, B symptoms, circulating interleukin-2 (IL-2) receptor (CD25) levels, and disease-free survival. In melanoma, increased sICAM-1 serum levels and tumor cell ICAM-I expression were typical for metastatic disease. In the case of HD, it was shown by histochemistry that the malignant cells overexpress ICAM-1, and that HD cell lines release sICAM-1 when exposed to various cytokines.

In the present study, the serum sICAM-1 levels in B-CLL patients were positively correlated to the tumor mass as reflected by the modified Rai and Binet stagings, lymphocyte counts and isolated splenomegaly/hepatomegaly. In patients repeatedly monitored during disease progression or regression on cytoreductive therapy, the circulating sICAM-1 levels changed accordingly. sICAM-1 was also correlated to a kinetic parameter represented by the lymphocyte doubling time. This indicates that sICAM-1 may not simply reflect the static tumor mass in B-CLL. Further, the serum sICAM-1 levels were inversely correlated to Hb levels in patients with early clinical stage, and this may turn out to have prognostic significance. We also compared sICAM-1 with other serum markers said to reflect disease activity in B-CLL, ie, sCD 23, TK, LDH, and Δm. sICAM-1 was equally well correlated with survival, and in multivariate analysis, sICAM-1 was the only marker approaching significance (P = .08) for prognostic information when included after clinical stage and lymphocyte doubling time. Based on all the present observa-

Table 3. Correlation of sICAM-1 With Other Serum Markers and Lymphocyte Count

<table>
<thead>
<tr>
<th>Serum Marker</th>
<th>sICAM-1</th>
<th>sCD 23</th>
<th>Δm</th>
<th>TK</th>
<th>LDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>sICAM-1</td>
<td>0.32* (P = .0009)</td>
<td>—</td>
<td>0.47 (P &lt; .0001)</td>
<td>0.45 (P &lt; .0001)</td>
<td>0.52 (P &lt; .0001)</td>
</tr>
<tr>
<td>sCD 23</td>
<td>0.41 (P = .0001)</td>
<td>0.47 (P &lt; .0001)</td>
<td>—</td>
<td>0.42 (P &lt; .0001)</td>
<td>0.47 (P &lt; .0001)</td>
</tr>
<tr>
<td>Δm</td>
<td>0.06 (NS)</td>
<td>0.45 (P &lt; .0001)</td>
<td>0.42 (P &lt; .0001)</td>
<td>—</td>
<td>0.46 (P = .0001)</td>
</tr>
<tr>
<td>TK</td>
<td>0.21 (NS)</td>
<td>0.52 (P &lt; .0001)</td>
<td>0.47 (P &lt; .0001)</td>
<td>0.45 (P = .0001)</td>
<td>—</td>
</tr>
<tr>
<td>LDH</td>
<td>0.06 (NS)</td>
<td>0.18 (NS)</td>
<td>0.24 (P = .02)</td>
<td>0.12 (NS)</td>
<td>0.50 (P &lt; .0001)</td>
</tr>
</tbody>
</table>

Abbreviation: NS, not significant.

* Spearman's correlation coefficient.
SICAM-1 IS ELEVATED IN B-CLL

tions, we think that a prospective study repeatedly monitoring serum SICAM-1 in B-CLL is justified.

The cellular origin of SICAM-1 in B-CLL is presently unknown. Soluble ICAM-1 is thought to be released from the cell surface through proteolytic cleavage.51,52 Some 40% of B-CLL patients were reported to have surface ICAM-1+ leukemic cells in the blood.43 However, in our experience the surface expression of ICAM-1 on circulating B-CLL cells is low and poorly correlated with serum SICAM-1 levels (unpublished observations). Further, circulating B-CLL cells are arrested in the G0 phase of the cell cycle,53 and the proliferative tumor cell compartment is likely represented by the bone marrow, lymph nodes, and spleen. Several inflammatory mediators including IL-1, tumor necrosis factor α (TNFα), interferon γ, and IL-4 enhance cell surface expression of ICAM-1,44-48 and B-CLL cells have been reported to release at least TNFα, IL-1, and possibly IL-2.49-51 Furthermore, T-cells and NK-cells in these patients are phenotypically activated.38 Thus, circulating SICAM-1 in B-CLL may originate from either tumor cells, regulatory cell types, or both, and this is presently under investigation.

ACKNOWLEDGMENT

We are grateful to Dr. J. Bring and A. Taube at the Department of Statistics, Uppsala University, for their expert help with the statistical analysis.

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Table 4. Prognostic Value of sCAM-1 and Other Serum Markers Tested in the Cox Proportional Hazard Regression Model

<table>
<thead>
<tr>
<th>Marker</th>
<th>Univariate Analysis (P value)</th>
<th>Multivariate Analysis (P value)</th>
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</thead>
<tbody>
<tr>
<td>sCAM-1</td>
<td>.002</td>
<td>.08</td>
</tr>
<tr>
<td>sCD23</td>
<td>&lt;.001</td>
<td>.23</td>
</tr>
<tr>
<td>β2m</td>
<td>.006</td>
<td>.30</td>
</tr>
<tr>
<td>TK</td>
<td>.002</td>
<td>.27</td>
</tr>
<tr>
<td>LDH</td>
<td>.068</td>
<td>.95</td>
</tr>
</tbody>
</table>

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Serum levels of soluble intercellular adhesion molecule 1 are increased in chronic B-lymphocytic leukemia and correlate with clinical stage and prognostic markers [see comments]

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