Prognostic Importance of Serum Soluble CD23 Level in Chronic Lymphocytic Leukemia

By Marika Sarfati, Sylvie Chevret, Claude Chastang, Guy Biron, Pierre Strycxkman, Guy Delespessere, Jacques-Louis Binet, Helene Merle-Beral, and Dominique Bron

Prognosis of B-cell chronic lymphocytic leukemia (CLL) is based on clinical staging whose limitation is the failure to assess whether the disease will progress or remain stable in early stage (Binet A, or Rai 0, I, II) patients. We previously reported that soluble CD23 (sCD23), a protein derived from the B-cell membrane CD23 Ag, is selectively elevated in the serum of CLL patients. This prospective study assessed the predictive value of serum sCD23 level measured at study entry on the overall survival of all CLL patients and on disease progression of stage Binet A patients. Prognostic value of repeated measurements of sCD23 over time in stage A patients was also analyzed. One hundred fifty-three CLL patients were prospectively followed with a median follow-up of 78 months. Eight clinical or biological parameters were collected from the date of the first sCD23 measurement. At study entry, by Cox model, Binet staging ($P = .0001$) and serum sCD23 level ($P = .03$) appeared as prognostic factors for survival. Patients with sCD23 level above median value (>$574 \text{ U/mL}$) had a significantly worse prognosis than those with lower values (median survival of $53 \pm 109$ months, $P = .0001$). During follow-up, sCD23 doubling time increased by 3.2 the risk of death ($P = .001$). Among stage A patients ($n = 100$), sCD23 determination at study entry was the sole variable predictive of disease progression, patients with sCD23 level above $574 \text{ U/mL}$ had a median time progression of 42 months versus 88 months for those with lower levels ($P = .0001$). Stage A patients who doubled their sCD23 level exhibited a 15-fold increased risk of progression ($P = .0001$) and, in addition, the sCD23 increase preceded by 48 months disease progression. We conclude that in CLL patients, serum sCD23 level provides significant additional prognostic information in terms of overall survival. Most interestingly, among early stage patients, sCD23 determination at diagnosis and during the course of the disease may help to the early identification of patients who will rapidly progress to upper stages.

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THE CD23 ANTIGEN (the low-affinity receptor for IgE) is a type II membrane glycoprotein which is cleaved into soluble fragments (soluble CD23, sCD23) of various sizes displaying pleiotropic biological activities. The CD23 gene has been cloned and encodes two CD23 isoforms (type A and B) that only differ by six amino-acids in their intracytoplasmic domain. Normal B cells exclusively express CD23 type A, whereas interleukin-4 (IL-4) and IL-13 are potent inducers of CD23 type B. Membrane CD23 is involved in the IgE-dependent enhancement of specific immune response in vitro and in vivo. However, most of the functions ascribed to sCD23 are IgE-independent, including (1) prevention of germinal center B cells from their entry into apoptosis; (2) proliferation of myeloid precursor cells; and, more recently, (3) costimulation of interferon-γ (IFN-γ) production by T cells and triggering of monokine release by monocytes.

Chronic lymphocytic leukemia (CLL) is characterized by the accumulation of monoclonal CD5+ B cells coexpressing sIgM, sIgD, and CD23 Ags, that are arrested at the G0/G1 stage of the cell cycle. CLL represents 30% of reported leukemia cases in Western Europe and North America. Our previous studies have shown that the CD23 protein and gene expression are abnormally regulated in B-CLL cells. We have recently established a link between CD23 expression and normal or leukemic B-cell proliferation in that selective increase of CD23 (mainly type B) in B-CLL lymphocytes provokes their entry into the S phase of the cell cycle. Finally, we have reported that CLL sera contain 3- to 500-fold more sCD23 than control sera or sera from patients with other B lymphoproliferative disorders. Recently, Reinish et al have shown that sCD23 reliably reflects disease activity in CLL without establishing the prognostic significance of this biological parameter.

Clinical staging (Binet or Rai) are widely accepted to stratify CLL patients into low-, intermediate-, and high-risk categories that exhibit approximately 9, 5, and 2 years mean survival, respectively. Additional prognostic factors include pattern of bone marrow (BM) infiltration, chromosome abnormalities, and lymphocyte doubling time.

In the present multicenter prospective study, we followed 153 CLL patients to assess the predictive value of serum sCD23 level recorded at study entry on their overall survival and most particularly on freedom from progression of stage Binet A patients. We also studied the prognostic significance of doubling time of sCD23 level in stage A patients.

MATERIALS AND METHODS

Prospective data were obtained on 153 patients with B-CLL between 1983 and November 1, 1994, the reference date used for the present analysis. CLL was defined as $>5,000/\mu L$ lymphocytes bearing both CD5 and CD20 antigens in peripheral blood (PB) and BM infiltration greater than 40%. There were 71 patients from Brussels, Belgium, 46 from Montreal, Canada, and 36 from Paris, France. Their age ranged from 29 to 84 (median: 60) at the time of diagnosis; 92 were male and 61 were female. The main characteristics of the patients at study entry, which is the time of first sCD23 measurement.

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Table 1. Main Characteristics of the 153 CLL Patients at the Time of Inclusion, According to Binet Stage

<table>
<thead>
<tr>
<th>Variables</th>
<th>Stage A (n = 100)</th>
<th>Stage B (n = 26)</th>
<th>Stage C (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph nodes</td>
<td>32 (32%)</td>
<td>26 (100%)</td>
<td>22 (81%)</td>
</tr>
<tr>
<td>Spleen involvement</td>
<td>8 (8%)</td>
<td>17 (65%)</td>
<td>21 (78%)</td>
</tr>
<tr>
<td>Liver involvement</td>
<td>2 (2%)</td>
<td>13 (50%)</td>
<td>10 (37%)</td>
</tr>
<tr>
<td>Anemia</td>
<td>—</td>
<td>—</td>
<td>15 (66%)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>—</td>
<td>—</td>
<td>21 (78%)</td>
</tr>
<tr>
<td>RAI's classification</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>57 (57%)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>I</td>
<td>35 (36%)</td>
<td>9 (38%)</td>
<td>—</td>
</tr>
<tr>
<td>II</td>
<td>8 (8%)</td>
<td>15 (59%)</td>
<td>—</td>
</tr>
<tr>
<td>III</td>
<td>—</td>
<td>1 (4%)</td>
<td>4 (15%)</td>
</tr>
<tr>
<td>IV</td>
<td>—</td>
<td>1 (4%)</td>
<td>22 (81%)</td>
</tr>
<tr>
<td>sCD23 level (U/mL)</td>
<td>674 ± 849</td>
<td>1,694 ± 1,303</td>
<td>1,784 ± 2,201</td>
</tr>
</tbody>
</table>

are shown in Table 1. According to Binet classification system, 100 patients were stage A (mean sCD23 level: 674 ± 849), 26 patients stage B (mean sCD23 level: 1,694 ± 1,303), and 27 stage C (mean sCD23 level: 1,784 ± 2,201) with statistically significant differences for sCD23 distribution according to clinical staging (nonparametric Wilcoxon test;  \( P < .0001 \)). A total of 96 patients (63%), including 68 stage A, 14 stage B, and 14 stage C were free of treatment at study entry. The median follow-up time of the study was 78 months, and 3 patients were lost to follow-up and 49 patients have died. Sampling for sCD23 measurement was performed at diagnosis in 49 patients (65% stage A, 17% stage B, 17% stage C), and the median time from diagnosis to study entry was 30 months in 104 patients (65% stage A, 16% stage B, 16% stage C). Repeated measurements of sCD23 over time (at least 3 samples per patient) were requested for the longitudinal study. The 1,121 sera collected between 1983 and 1994 were aliquoted and kept frozen at −20°C; the serum sCD23 level was measured at once in all the samples at a single center by a sandwich radio-immunoassay using two anti-CD23 monoclonal antibodies (MoAbs) as previously described. The sensitivity of the assay was 2 U/mL (equivalent to 200 pg/mL) and the mean value of sCD23 in control donors was IO U/mL (1 ng/mL). Coefficient variation of the assay was less than 10%.

For statistical analysis, failure time data (overall survival and disease progression in stage A patients) were collected from the date of study entry were estimated by the Kaplan and Meier method.
The weakness of the clinical staging is the failure to predict whether the disease will progress or remain stable in stage A patients. Therefore, we assessed whether sCD23 measurement could be useful to identify a subgroup of patients with a high probability of disease progression and death. Disease progression was defined as the appearance of upper stages according to Binet classification. Thirty-four of 100 stage A patients progressed during the observation period; 12 of these 34 patients had died. The 1-year worsening was estimated at 12% (95% confidence interval, 5% to 18%). The results of univariate prognostic factor analysis in Table 3 indicate that 3 out of 5 variables tested at study entry were associated with disease progression, namely sCD23 level ($P = .0001$), Rai stage ($P = .0002$), and involvement of lymph nodes ($P = .0003$). As shown in Fig 2, patients with sCD23 level at study entry higher than 574 U/mL had a median progression time of 42 months compared to 88 months for the patients with sCD23 level below 574 U/mL ($P = .0001$). Of note, 29% of patients who progressed were treated at study entry compared to 33% in the group of patients with a nonprogressive disease. Most interestingly, when these three variables were introduced into a multivariate Cox model, sCD23 level remained the sole predictive factor of disease progression in stage A patients ($P = .0001$); patients with sCD23 higher than 574 U/mL demonstrated a relative risk of clinical worsening at 5.8 (95% confidence interval, 2.7% to 12.3%) compared to the patients with sCD23 level lower than 574 U/mL ($P = .0001$).

### Table 3. Stage A Progression: Univariate Prognostic Analysis Based on Baseline Information

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Patients (n = 100)</th>
<th>Progression (n = 34)</th>
<th>3-yr Event Rate (24%)</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAI stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>57</td>
<td>10</td>
<td>12%</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>35</td>
<td>21</td>
<td>43%</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>8</td>
<td>3</td>
<td>27%</td>
<td>.0002</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>67</td>
<td>15</td>
<td>17%</td>
<td>.0003</td>
</tr>
<tr>
<td>Yes</td>
<td>32</td>
<td>19</td>
<td>39%</td>
<td></td>
</tr>
<tr>
<td>Involvement of the spleen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>92</td>
<td>30</td>
<td>24%</td>
<td>.66</td>
</tr>
<tr>
<td>Yes</td>
<td>8</td>
<td>4</td>
<td>14%</td>
<td></td>
</tr>
<tr>
<td>Involvement of the liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>98</td>
<td>32</td>
<td>25%</td>
<td>.37</td>
</tr>
<tr>
<td>Yes</td>
<td>2</td>
<td>2</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>sCD23 (U/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤574*</td>
<td>60</td>
<td>11</td>
<td>11%</td>
<td>.0001</td>
</tr>
<tr>
<td>&gt;574</td>
<td>40</td>
<td>23</td>
<td>46%</td>
<td></td>
</tr>
</tbody>
</table>

*Median value on the entire sample (n = 153).

Fig 1. Overall survival according to sCD23 level and Binet clinical stage. (A) Overall survival was calculated from the date of first sCD23 measurement (sCD23 level at study entry). 574 U/mL of sCD23 corresponds to the median value of the entire group (n = 153) ($P < .0001$). (B) Overall survival according to Binet classification ($P < .0001$).

Fig 2. Freedom from progression for 100 Binet stage A patients according to sCD23 level at study entry. Freedom from progression was calculated according to sCD23 level at study entry as in Fig 1. 574 U/mL of sCD23 corresponds to the median value of the entire group (n = 153) ($P < .0001$).
of the liver \( (P = .0001) \) or of the lymph nodes \( (P = .0001) \), hemoglobin level \( (P = .0001) \), platelet count \( (P = .0001) \), and sCD23 \( (P = .0003) \). Sixty patients developed a twofold increase in sCD23 level during the follow-up period, 47 stage A, 10 stage B, and 3 stage C with 11\% (95\% confidence interval 6\% to 17\%) estimated doubling rate at 1 year. The doubling time of sCD23 level increased by 3.2 the risk of death \( (P = .001) \), when adjusting for both Binet stage and baseline sCD23 level. When incorporated simultaneously into a Cox model, only three variables were retained by the stepwise procedure as adding to each other prognostic information, namely Binet stage \( (P = .0001) \) and involvement of the spleen \( (P = .003) \) and of the liver \( (P = .04) \).

Most interestingly, in stage A patients, the monitoring of sCD23 level was strongly associated with disease progression in both univariate and multivariate analysis \( (P = .0001) \). At any time during the course of the disease, patients who displayed an elevation of sCD23 level above 574 U/mL augmented their relative risk of progression by 10 compared with patients who did not experience such an increase \( (P = .0001) \). Moreover, when adjusting on sCD23 measurement at study entry, patients who doubled their sCD23 level (even without reaching median value) increased by 15 their relative risk of disease progression \( (P = .0001) \). Finally, doubling of sCD23 level not only augmented the risk of developing aggressive disease but significantly preceded by a median time estimated at 48 months the progression to upper stages.

**DISCUSSION**

CLL is the most frequent type of leukemia in Western countries.\(^7\) Some patients have a survival very similar to the general population whereas others have a rapidly fatal outcome. Prognostic value of clinical staging proposed by Rai and Binet are widely validated,\(^13,14\) but predicting the prognosis of a given patient is still a challenge. More particularly, in early stage of the disease (Binet A or Rai 0-I), some patients will rapidly progress to upper stages requiring early treatment and it is of major importance for clinicians to distinguish between indolent or aggressive CLL.\(^7\)\(^7\)

We have previously reported that soluble CD23 is a specific marker of B-CLL and that serum sCD23 level is correlated with clinical stage.\(^11\) These observations have recently been confirmed by others who reported a significant correlation between serum sCD23 level, CLL disease activity and tumor load.\(^12,24\) Other soluble receptors have been studied and some relationship with disease activity has been reported for sCD25\(^24\) and tumor necrosis factor (TNF) receptors.\(^25\) However, these serologic markers are not selective for CLL disease and have no prognostic value for the patients.

Therefore, we initiated a prospective survey in 153 CLL patients from three different institutions with the aim to assess the prognostic value of serum sCD23 level measured at study entry or during follow-up in terms of overall survival and to investigate whether this biologic parameter could help to delineate a group of patients with poor prognosis among Binet stage A patients.

Using as a cut-off point the sCD23 level corresponding to the median value of the entire group (574 U/mL), results clearly show that patients with sCD23 level above the median value have a significantly shorter survival time than those with lower values. In multivariate analysis taking into account Rai and Binet stages, lymph node, spleen or liver involvement, hemoglobin, platelets, and sCD23 determinations, only Binet staging \( (P = .0001) \) and serum sCD23 level \( (P = .03) \) appear as independent prognostic factors for survival, indicating that sCD23 determination provides prognostic information additional to clinical staging.

Of interest, the expression of membrane CD23 on CD5\(^+\) B cells from CLL patients has been previously related to survival by Geisler et al.\(^20\) who reported a favorable outcome for patients with high membrane CD23 expression compared with cases with low CD23 expression. An inverse relationship between membrane CD23 expression and disease activity was not confirmed by other recent studies.\(^12,27\) These controversial data may be explained by the difficulty in assessing quantitatively surface CD23 expression by flow cytometry due to the instability of the CD23 molecule, which is rapidly cleaved from the cell surface into soluble CD23.\(^1\) In contrast, the measurement of soluble CD23 has been shown to be a highly reproducible and quantitative method.\(^11,12,24\)

Several investigators have reported prognostic factors that add discriminant power to clinical stages; so far, three of these parameters have been well studied and correlated with survival: (1) the lymphocyte doubling time (LDT),\(^19,20\) (2) the pattern of marrow infiltration,\(^14,17\) and (3) the karyotype abnormalities.\(^18\)

Although LDT requires at least a 12-month follow-up before identifying a low-risk group of patients with a stable lymphocyte count, a follow-up of a few months is often accepted and safe in this disease. However, in the present study, using time-dependent covariate analysis, the increase in lymphocyte count was not significantly predictive of disease progression \( (P = .34) \). Interestingly, Reinisch et al.\(^12\) reported a significant reciprocal relationship between sCD23 and LDT in 24 patients examined.

The pattern of marrow infiltration requires a BM biopsy, which is not performed as a routine procedure at the time of the diagnosis but is strongly recommended by the International Workshop on CLL (IW CLL) as well as by the National Cancer Institute working group on CLL.\(^26\) Finally, karyotype abnormalities are only detected in 50\% of patients with CLL and some patients with a normal karyotype may display a poor prognosis. Of note, BM biopsy and/or cytogenetic analysis were not performed in the CLL patients of our study group.

The second aim of this study is of major importance for clinicians: we prospectively followed 100 patients with stage Binet A to investigate whether sCD23 level could discriminate between smouldering and progressive CLL.

A multivariate analysis of stage A CLL patients shows that sCD23 determination at study entry is the sole variable predictive of disease progression with a relative risk of clinical worsening at 5.8 for patients with sCD23 value above 574 U/mL. The median time to progress to upper stages is significantly shorter for patients with higher sCD23 values compared with sCD23 levels below 574 U/mL (42 v 88 months).

The French Cooperative Group on CLL\(^29\) described a sub-
group of stage A CLL patients with smouldering disease characterized by a normal hemoglobin level and a rather low lymphocyte count (<30,000/µL). The life expectancy of this group of patients is not different from that of a sex- and age-matched healthy population. In our stage A group, among the 34 patients who progressed, only 10 fulfilled the criteria of nonsmouldering CLL, (lymphocyte count > 30,000/µL) showing thus the limited predictive value of this sub-classification. However, according to Montserrat and Rozman, identification of smouldering versus nonsmoulding CLL patients should be mainly based on the pattern of BM infiltration and the lymphocyte doubling time.

Finally, in a longitudinal study, we analyzed the doubling time of sCD23 level at study entry and its prognostic significance for the overall survival of the entire group and for disease progression among stage A patients. The results indicate that doubling of sCD23 level increases by 3.2 the risk of death of the entire population. Most interestingly, in stage A patients, increase of sCD23 level during follow-up not only significantly precedes by 48 months their progression to upper stages but also significantly increases by 15 their relative risk of disease progression.

We conclude that serum sCD23 is a unique tumor marker of B-CLL disease (which may be rapidly determined) that proved to be a reliable parameter for prognostic evaluation. Soluble CD23 determination is a prognostic factor that provides additional information to clinical staging not only at diagnosis but also during the course of the disease. Moreover, longitudinal monitoring of sCD23 levels may help to assess the relative risk of death and the response to therapy.

Finally, and most importantly, in early stage A patients, serum sCD23 level measured at diagnosis or during follow-up may be useful to delineate a population at high risk for disease progression who so far remained untreated and therefore might benefit earlier from novel therapeutic approaches such as purine nucleoside analogues or even BM transplantation for younger patients.

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REFERENCES

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