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# CLINICAL SIGNIFICANCE OF sIL2R, sCD23, sICAM-1, IL6 AND sCD14 SERUM LEVELS IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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**ABSTRACT**

***Background.*** **The aim of the study was to establish the role exerted by some soluble factors in B-CLL disease mechanisms.**

***Materials and Methods.*** **Serum levels of sIL2R, sCD23, sICAM-1, IL6 and sCD14 were detected in 47 B-CLL patients. Thirty-seven out of the 47 cases were in advanced/progressive stage, while the remaining 10 patients were defined as smouldering B-CLL. Twenty normal controls provided the reference values. Serum samples of 24 out 37 advanced/progressive cases were measured before and six months after the start of chemotherapy.**

***Results.*** **The advanced/progressive patients showed significantly higher levels of sIL2R, sICAM-1 and sCD23 as compared to normal subjects. Furthermore, sIL2R, sICAM-1 and IL6 values were significantly higher in advanced/progressive B-CLL than in smouldering B-CLL patients. A statistically significant difference was found between smouldering B-CLL and controls for sCD14 only. sIL2R and sICAM-1 levels directly correlated with total tumor mass (TTM) score, sCD23 with both TTM score and lymphocytosis, and sCD14 with IgG serum values. sIL2R and sCD23 levels lowered significantly after chemotherapy, but only sCD23 and TTM variations after chemotherapy were closely correlated.**

***Conclusions.*** **sCD23 may be considered the only indicator of tumor mass, while the other soluble factors can be released through different mechanisms. In particular, sICAM-1 seems to correlate with the ability of the tumor to spread, while the sCD14 increase could indicate a role for this soluble factor in preventing infections in B-CLL patients.**

*Key words: B-CLL, cytokines, soluble factors, tumor mass, response*



B-cell chronic lymphocytic leukemia (B-CLL) is a lymphoproliferative disorder involving B monoclonal lymphocytes whose origin and disease progression have been widely investigated.[1,2]

Soluble factors, whether derived from the tumor (autocrine) or from the surrounding host cells (paracrine), were postulated to play an important role in the growth mechanism of the neoplastic clone.[1] In this respect, the soluble interleukin 2 receptor (sIL2R) and soluble CD23 (sCD23), which are cleavage products of CD25 (IL2R) and low affinity IgE receptor (CD23) respectively, have been detected in large amounts in sera from B-CLL patients.[3,4] IL6, a cytokine which promotes B-cell lineage proliferation and differentiation, has been supposed to act as an autocrine growth factor in B-CLL.[5] Intercellular adhesion molecules (ICAM), which are involved in cellular interactions, have been implicated in tumor progression and diffusion.[6,7] A soluble form of ICAM-1, sICAM-1, has been discovered[8] and is expressed at high levels in the serum of patients with various solid tumors[9,10] as well as in inflammatory conditions.[11] Finally, CD14, a molecule strongly expressed on monocyte cell sur-

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faces[12] and on the neoplastic cells of some B-CLL patients,[13] is also detectable in serum as a soluble form.[14] sCD14 is involved in the interaction between bacterial endotoxins and the monocyte/macrophage system.[15]

Serum levels of sIL2R, sCD23, sICAM-1, IL6 in B-CLL and their correlation with the tumor burden have been previously reported.[3,4,16,17] The aims of the present study were i) to assess the level of these factors with the addition of sCD14 in different phases of the disease, namely in advanced/progressive and in smouldering cases; ii) to correlate these findings with the tumor burden as expressed by different staging systems, as well as with the main clinical and hematological B-CLL features, and iii) to evaluate the impact of response to therapy on these serum factor levels.

## *Materials and Methods*

### *Patients*

Serum samples were obtained after informed consent from 47 B-CLL patients. Thirty-seven cases, 18 males and 19 females, mean age 69.8±9.6 years (range 47-88 yrs), were classified as advanced/progressive B-CLL by a total tumor mass (TTM) score > 9[18] and/or bone marrow failure (anemia or thrombocytopenia or both) and/or doubling time (DT)[16] < 12 months. In particular, the TTM score is the sum of the square root of peripheral lymphocytosis plus the diameter in cm. of the largest palpable lymph node plus the extent of spleen palpable below the costal margin; for example, a patient with $16\times10^9$/L peripheral lymphocytes, a lymph node 3 cm diameter and spleen palpable 2 cm below the costal margin has a TTM score of 9 (4+3+2). According to the Rai staging,[19] 12 were in stage 0-I, 12 in stage II and 13 in stage III-IV. With regard to the Binet classification,[20] the distribution was: 12 stage A, 11 stage B and 14 stage C. TTM score ranged from 3.4 to 27.0 (mean 11.8), with 28 cases exhibiting a TTM score > 9. DT, calculated in 24 patients (in 13 cases chemotherapy was started at the first observation), was <12 months in 16 cases. Bone biopsy, accepted by 22 patients, disclosed a non diffuse pattern of lymphoid infiltration in 16 cases.

All 37 advanced/progressive patients received chemotherapy. Thirty cases were treated with a high-dose chlorambucil schedule,[21] administered continuously until major response or grade 3 toxicity was reached, followed by maintenance therapy with the same drug given twice a week; the remaining 7 cases received six cycles of Binet's modified CHOP scheme,[22] administered every 28 days. In 24 patients, serum samples were obtained before and 6 months after the start of therapy without intermediate determinations. In the remaining 13 patients soluble factor levels were assessed only before the start of therapy.

We considered a major clinico-hematological response to be the achievement of a TTM score < 2.3, which is the B-CLL diagnostic threshold, in the absence of bone marrow failure.[18] For the purposes of the present study, we defined as *responders* only those patients who obtained a major response, and as *non responders* the remaining cases. TTM variation before and after chemotherapy was also evaluated as an additional indicator of sensitivity to therapy and response. The short median follow-up time of this series prevented any analysis of the impact of soluble factors on patient survival.

At the end of 6 months of therapy, 22 out of 24 patients studied before and after therapy displayed a reduction in TTM score, with 12 cases defined as responders because a major response was obtained.

Ten additional B-CLL patients, 4 males and 6 females with a mean age of 66.8 years steadily in clinical stage A/0 for more than 4 years, and fulfilling the criteria for smouldering B-CLL,[23] entered the study.

Finally, 20 normal controls provided reference values for comparison with these two B-CLL groups.

### *Detection of soluble factors*

Sera were separated, aliquoted and stored at –80°C until assayed. sIL2R, IL6 (Eurogenetics, Turin, Italy), sICAM-1, sCD23 (Cellfree, Cambridge, MA, USA) and sCD14 (IBL, Hamburg, Germany) serum levels were determined using

an ELISA immunoassay according to the manufacturer instructions. Sera belonging to treated cases before and after treatment were assayed with the same kit for all determinations.

*Statistical analysis*

The SAS/STAT software package (release 6.06, SAS Institute Inc., 1993) was used for all statistical calculations. The Wilcoxon rank sum test was employed to compare serum level results between controls and either smouldering or progressive B-CLL, and between responding and non responding patients. Pearson's correlation coefficient was used to determine the association between serum data and clinico-biological parameters and to relate TTM score variations and differences in serum factor levels before and after therapy. The comparison of soluble factor values before and after chemotherapy was performed with the paired Student's t-test.

## *Results*

Serum soluble factor levels in advanced/progressive B-CLL, smouldering B-CLL and normal subjects are reported in Table 1. Compared to smouldering cases, patients with advanced/progressive disease had significantly higher sIL2R, sICAM-1, IL6, but not sCD14 serum levels. sCD23 was not analyzed in smouldering B-CLL. Compared to normal subjects sIL2R, sICAM-1, sCD23, sCD14, but not IL6, levels were found to be significantly higher in advanced/progressive B-CLL. No difference between smouldering B-CLL and controls was observed in any soluble factor but sCD14.

Table 2 shows the statistically significant correlations between the main hematological parameters and soluble factor levels in advanced/progressive B-CLL, as evaluated by Pearson's coefficient. The hematological parameters taken into account were TTM score, IgG serum level, lymphocytosis, Rai and Binet stages, DT and bone biopsy pattern. Concerning correlations with clinical parameters, sIL2R and sICAM-1 were directly related to TTM score, sCD23 both to TTM score and the lymphocyte count, and sCD14 only to IgG levels; Rai and Binet stages, DT and bone marrow pattern were not correlated with any cytokine level. When each soluble factor was compared with the remaining ones, a significant direct relationship was found between sIL2R and IL6, and sICAM-1 and sCD23.

Serum factor levels before and after chemotherapy are reported in Table 3, irrespectively of the grade of response. A statistically significant reduction was observed in sIL2R and sCD23 serum levels; IL6 showed a trend toward decreasing and sCD14 and sICAM-1 displayed a slight increase after chemotherapy. Considering the grade of response, no significant difference in serum factor levels before and after chemotherapy was noted between major responders and non-responders (data not shown).

Finally, considering TTM score variation before and after chemotherapy as a continuous variable indicating sensitivity to therapy, only sCD23 differences had a close relationship with TTM changes (Figure 1): p=0.02 with Pearson's correlation coefficient test.



Table 1. Serum levels of sIL2R, IL6, sICAM-1, sCD23 and sCD14 in B-CLL in two disease states and in normal controls. Data are reported as mean±SE values. Statistical analysis was performed with the Wilcoxon rank sum test.

| *Disease status* | *No. of cases* | *sIL2R U/mL* | *p* | *IL6 pg/mL* | *p* | *sICAM-1 µg/mL* | *p* | *sCD23 U/mL* | *p* | *sCD14 µg/mL* | *p* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Progressive | 37 | 456.6±82.6 | 0.002 | 18.8±13.4 | 0.02 | 467.3±26.8 | 0.0005 | 3425±456 | — | 10,094±1,120 | ns |
| Smoldering | 10 | 114.3±48.3 | ns | 1.77±0.57 | ns | 307.8±17.6 | ns | not done | — | 11,311±1,725 | 0.03 |
| Normals | 20 | 88.7±32.6 | | 3.0±0.69 | | 273.0±21.4 | | 154.8±33.7 | | 6,695±639 | |
| Normal vs. progressive | | | 0.001 | | ns | | 0.0002 | | 0.0001 | | 0.04 |

| Variables | serum IgG | lymphocytosis | TTM | sIL2R | IL6 | sICAM-1 | sCD23 |
|---|---|---|---|---|---|---|---|
| sIL2R | | | $R = 0.395$<br>$P = 0.022$ | | $R = 0.506$<br>$P = 0.004$ | | |
| IL6 | | | | $R = 0.506$<br>$P = 0.004$ | | | |
| sICAM-1 | | | $R = 0.508$<br>$P = 0.002$ | | | | $R = 0.656$<br>$P = 0.003$ |
| sCD23 | | $R = 0.532$<br>$P = 0.018$ | $R = 0.560$<br>$P = 0.012$ | | | $R = 0.656$<br>$P = 0.003$ | |
| sCD14 | $R = 0.446$<br>$P = 0.022$ | | | | | | |

Table 2. Correlation analysis between each serum soluble factor and the remaining factors and the main clinico-hematological parameters in B-CLL. Statistical analysis was performed with Pearson's test.

| | No. of cases | sIL2R | sCD23 | IL6 | sCD14 | sICAM-1 |
|---|---|---|---|---|---|---|
| Before | 24 | 370.1± 74.9 | 3,532± 406 | 24.0± 17.6 | 9,718± 1,169 | 454.3± 32.2 |
| After | 24 | 174.6± 25.7 | 1,410± 352 | 9.6 ± 3.3 | 10,722± 1.49 | 528.2± 61.5 |
| $P =$ | | 0.02 | 0.002 | NS | NS | NS |

Table 3. Serum levels of soluble factors before and after chemotherapy in 24 previously untreated B-CLL patients (mean values±SE). Data were analyzed by the paired Student's t-test.



## *Discussion*

The results of the present study indicate that patients with advanced/progressive B-CLL display significantly higher levels of all the serum factors tested, except IL6, than normal controls. On the contrary, in smouldering B-CLL sIL2R, sICAM-1 and IL6 values are substantially in the normal range. Only sCD14 levels appear to be higher in this latter group than in normal individuals. The present results give support to the importance of the role exerted by these factors in the pathogenesis of the disease and its progression.[2]

sCD23, a cleavage product of the low affinity IgE receptor (FcR II) constitutively expressed on B-CLL cells, derives directly from leukemic cells.[4] The direct correlation between sCD23 serum level and both the lymphocyte count and the TTM score, together with its significant reduction after chemotherapy, gives additional evidence of the origin of this soluble factor from leukemic cells, as already suggested by other authors.[24,25] Moreover, taking into account TTM score differences before and after chemotherapy as a parameter of response, we could demonstrate that sCD23 levels paralleled TTM score variations, thus confirming that this factor is produced by the leukemic population and can be considered a true indicator of the tumor mass. It has also been shown that that CD23 plays a role in the maintenance and progression of B-CLL.[26]

The high sIL2R levels observed in patients with solid tumors have been explained as the expression of i) increased cellular immunity against the tumor; ii) general activation of the immune system; iii) release from the neoplastic cells. In B-CLL, early reports demonstrated that malignant cells release large amounts of sIL2R.[3,27] Our study indirectly confirms these data by the significant correlation between sIL2R levels and TTM score and the reduction in the values of this cytokine after chemotherapy. Conversely, no correlation was found either with the grade of response or with the TTM differences before and after chemotherapy, probably because other mechanisms, such as T cell re-expansion and/or activation, could affect sIL2R levels.

The observations about IL6 production in B-CLL are controversial. Some authors did not detect IL6 mRNA in neoplastic B cells,[28] while others found IL6 gene expression.[5] These apparently discordant data could be explained by postulating that only a subgroup of B-CLL patients is able to produce IL6. This hypothesis is supported by the great variability in IL6 serum values observed in our patients; a general trend towards an increase in advanced/progressive B-

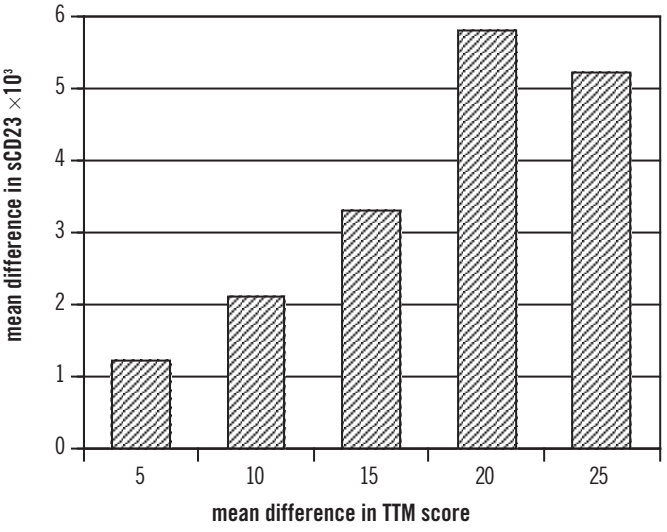


Figure 1. Relationship between TTM score and sCD23 differences (mean values) before and after therapy. The reduction of the TTM score parallels the decrease in sCD23 values (p=0.0268 by Pearson's correlation coefficient).

CLL was found with respect to normal subjects and smouldering B-CLL.

ICAM-1, together with its counter receptor LFA-1, plays a key role in the homing and migration processes of tumor cells,[7] as well as in cell-cell interactions of the immune response.[29] sICAM-1 has been found in large amounts in several neoplastic diseases,[9,10] especially in the metastatic phase. Similarly to previous experiences,[16,30] in this study we demonstrated that sICAM-1 levels are higher in advanced/progressive B-CLL than in normal controls and smouldering B-CLL. Besides, we have found a direct correlation of sICAM-1 concentrations and TTM score, possibly indicating that the size of the neoplastic burden is related to the tumor's spreading capacity. On the other hand, a tendency of post-therapy sICAM-1 levels to increase was observed. Since sICAM-1 levels are not reduced after therapy, it can be hypothesized that non neoplastic cellular compartments could be involved in sICAM-1 release. It is known that T and NK cells, whose functioning is regulated by ICAM-LFA1 interactions, are phenotypically activated in B-CLL.[31] Taken together, these observations support the hypothesis that reduction of the neoplastic mass is followed by restoration of the immune system.

The CD14 molecule is expressed on the monocyte-macrophage surface and operates as a receptor for lipopolysaccharide (LPS) bound to LPS-binding protein in mediating LPS-induced tumor necrosis factor (TNF) production.[32] This glycoprotein is also present in variable amounts on the surface of B-CLL cells able to secrete IL1.[13] The soluble form of CD14, whose role is still debated, was discovered some years ago.[14] In our study, significantly higher sCD14 values were detected both in advanced/progressive and smouldering B-CLL than in controls, and were directly correlated with serum IgG levels. Interpretation of this last correlation is, so far, difficult. It has been reported that sCD14 functions as an endogenous down-regulator of TNF overproduction, possibly preventing septic shock.[15] If the protective role of sCD14 is confirmed, our data could support the hypothesis that sCD14, together with IgG serum levels, exerts a role in the defense against infections in B-CLL.

In conclusion, the present observations confirm that i) sCD23 and sIL2R correlate positively with the tumor mass, but only sCD23 parallels the behavior of the neoplastic mass; therefore it can be considered the sole indicator of the neoplastic cell burden; ii) the high sICAM-1 levels detected in advanced/progressive B-CLL patients seem to correlate with the spreading ability of the neoplastic clone; iii) the increase of sCD14 concentration in B-CLL patients and its significative correlation with IgG serum values

could indicate that this soluble factor exerts a protective role against infections in B-CLL.

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