Advances in Therapy® | *Volume 24 No. 1*
January/February 2007

Expression of Soluble CD27 and Interleukins-8 and -10 in B-Cell Chronic Lymphocytic Leukemia: Correlation With Disease Stage and Prognosis

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ABSTRACT

Investigators in this study explored levels of soluble CD27 (sCD27), interleukin (IL)-8, and IL-10 in B-cell chronic lymphocytic leukemia (B-CLL), and the correlation of these levels with disease stage and prognosis. Plasma IL-8, IL-10, and sCD27 levels were assessed with enzyme-linked immunosorbent assay tests in 22 healthy donors and 70 patients with B-CLL (49 men and 21 women). Mean patient age was 61.57 y (range, 44–75 y). Mean healthy donor age was 62.09 y (range, 40–72 y). In the study group, mean values were as follows: plasma IL-8, 284.758 pg/mL (0–1000 pg/mL); plasma IL-10, 26.152 pg/mL (0–100 pg/mL); sCD27, 731.357 U/mL (139.9–1000 U/mL); white blood cell count, 59.9 × 10^9/L (0.8–250.0 × 10^9/L); hemoglobin count, 11.2 g/dL (5.0–16.2 g/dL); platelet count, 162.5 × 10^9/L (29.8–317 × 10^9/L); B_2 microglobulin (B_2M) 3350.2 mg/L (274.7–7499.9 mg/L); CD38, 19.5%; and lactate dehydrogenase (count, 497.5 U/L (263.0–1507 U/L). Patients represented all Rai stages, with 22.9% at stage 0, 11.4% at stage I, 11.4% at stage II, 41.4% at stage III, and 12.9% at stage IV. Plasma levels of IL-8, IL-10, and sCD27 were correlated between study and control groups; significantly higher IL-8 (*P*=.001) and sCD27 (*P*=.000) levels were found, but the IL-10 level was not significant (*P*=.139). Plasma IL-10 (*P*=.01) and sCD27 (*P*=.008) were positively correlated with Rai stage, but IL-8 was not (*P*=.146). Levels of sCD27 were significantly correlated with values for B_2M (*P*=.000), hemoglobin (*P*=.028), lactate dehydrogenase (*P*=.001), CD19 (*P*=.03), and IL-10 (*P*=.000). IL-8 was significantly correlated with white blood cell (*P*=.000) count, and CD38 (*P*=.001) and CD5 (*P*=.006) levels. IL-10 was

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significantly correlated with B_2M (*P*=.017), CD19 (*P*=.000), platelet (*P*=.002), and CD27 (*P*=.000). In survival distributions for CD27, IL-8 and IL-10 were found to have more significant relationships for all parameters (*P*=.0000). In conclusion, the authors suggest that sCD27, IL-8, and IL-10 are more significant prognostic factors for B-CLL when compared with others, and these values should correlate with new prognostic factors (eg, zeta-associated protein-70, mutated/unmutated immunoglobulin variable heavy chain).

Keywords: B cell; B-CLL; CD27; IL-8; IL-10

INTRODUCTION

Chronic lymphocytic leukemia (CLL), a malignancy of well-differentiated monoclonal B cells, is the most common adult form of leukemia in the United States, accounting for roughly 25% of patients.[1] Reliable prognostic factors are important because they may be useful in counseling patients regarding treatment, in assessing the likelihood of response to treatment, and in gaining insight into the biology or pathophysiology of the disease. Prognostic factors have been identified in CLL, namely, that poor prognosis is associated with advanced stage[2]; increased levels of serum B_2 microglobulin (B_2M),[3,4] soluble CD23 (sCD23)[4,5] and CD27 (sCD27),[6,7] and lactate dehydrogenase (LDH)[8]; cytogenetic abnormalities[9,10]; leukemia cell expression of CD38[11,12] and the nonmutated immunoglobulin variable heavy chain gene[11,13]; and diffuse pattern of involvement of bone marrow[14] and zeta-associated protein-70.[15]

Interleukin-8 (IL-8) is a 72 to 77 amino acid, 6 to 8 kDa inflammatory chemokine that is produced by hematopoietic cells.[16] B cells of patients with CLL have been found to constitutively express IL-8; in addition, increased levels of IL-8 compared with values in normal control subjects have been noted in the serum of patients with CLL,[17] and CLL B cells express IL-8 receptor mRNA and bind radioisotope-labeled IL-8, indicating expression of surface receptors for IL-8.[18] Additionally, exogenous IL-8 can upregulate IL-8 mRNA expression in cultured CLL B cells, suggesting an autocrine mechanism of leukemia cell stimulation[18]; it follows that IL-8 may correlate with factors known to be prognostic in B-CLL.

Interleukin-10 (IL-10) is a pleiotropic cytokine that is produced by type 2 helper cells.[19] IL-10 has a potent stimulating effect on B cells, inducing proliferation and differentiation. It is interesting to note that in cell lines derived from B-cell lymphomas, IL-10 has been found to serve as an autocrine growth factor.[20-22] Serum IL-10 levels have been found to be important prognostic factors for B-cell lymphoma and, when assays that detect both human and viral IL-10 were used, for non-Hodgkin's lymphomas.[23-27]

The disulfide-linked 55-kDa homodimer CD27 is a member of the tumor necrosis factor receptor family.[28-30] CD27 is expressed on the cell surfaces of leukocytes, including germinal center B cells, some peripheral blood B cells, activated T cells, and natural killer cells. Early work revealed distinct subsets of CD27+ and CD27– CD4+ T cells.[31]

Although the earliest research into the function and signal transduction of CD27 was carried out with primary isolates of T cells, more recent work has focused on primary isolates of B cells. CD27 interaction with its ligand, CD70, results in increased

cell proliferation and immunoglobulin release in primary B-cell isolates from normal individuals.[32] Specifically, CD27/CD70 interaction induces increased immunoglobulin (Ig)G and IgM production by peripheral B cells.[33]

Additionally, CD27 has recently been suggested as a marker for human memory B cells.[34,35] CD27 expression of variable intensity was detected on almost all malignant B cells at a broad range of developmental stages.[6] The mechanism of CD27 expression by malignant B cells, however, is not yet known. Hence, it is necessary to investigate the regulation of CD27 expression and the functional role of this molecule in the course of B-cell differentiation.

In the present study, investigators explored plasma levels of IL-8, IL-10, and sCD27 in patients with B-CLL and determined their correlation with disease stage and other prognostic factors.

MATERIALS AND METHODS

After informed consent was provided, plasma was collected from 70 patients with CLL and 22 healthy donors who were seen at the Cukurova University Medical Oncology Department. Plasma IL-8 concentration was determined with the use of human IL-8 enzyme-linked immunosorbent assay (ELISA), plasma IL-10 with human IL-10 ELISA, and CD27 with human soluble CD27 ELISA. Concurrently, physical examination and routine clinical laboratory tests were performed, including complete blood count and LDH and B_2M levels; flow cytometry was performed to determine the proportions of cells that were positive for CD38, CD19, and CD5 for diagnosis of CLL; in addition, Rai stage was identified.

Descriptive statistics were calculated with the Statistical Package for the Social Sciences (SPSS) for Windows, version 10 (SPSS Inc., Chicago, Ill, USA), for the following: IL-8, IL-10, sCD27, and covariates such as age and sex, Rai stage, CD38, CD19, CD5, hemoglobin (Hb), platelet (PLT) count, white blood cell (WBC) count, LDH, and B_2M. The Wilcoxon rank sum test and the Kruskal-Wallis rank sum test were used to compare median levels of IL-8, IL-10, and sCD27 at various Rai stages. Further, Spearman's rank correlation coefficients were calculated for IL-8, IL-10, sCD27, and all continuous covariates. Survival was calculated by means of Kaplan-Meier analysis. Covariates that were statistically significant (significance level, .05) in the univariate analyses were used in the multivariate models.

RESULTS

Levels of plasma IL-8, IL-10, and sCD27 were determined in 22 healthy donors (12 men and 10 women) and 70 patients with B-CLL (49 men and 21 women) (Table 1). Mean age was 61.57 y (range, 44–75 y) for patients and 62.09 y (range, 40–72 y) for healthy donors. In the study group, mean values were as follows: plasma IL-8, 284.758 pg/mL (0–1000 pg/mL); plasma IL-10, 26.152 pg/mL (0–100 pg/mL); sCD27, 731.357 U/mL (139.9–1000 U/mL); WBC count, 59.9×10^9/L ($0.8–250.0 \times 10^9$/L); Hb count, 11.2 g/dL (5.0–16.2 g/dL); PLT count, 162.5×10^9/L ($29.8–317 \times 10^9$/L); B_2M, 3350.2 mg/L (274.7–7499.9 mg/L); and LDH count, 497.5 U/L (263.0–1507 U/L).

Table 1. Patient Characteristics

Characteristic	Value (range)
Male/female, n	49/21
Rai stage, n	
0	16
I	8
II	8
III	29
IV	9
Mean value, range	
Age, y	61.57 (44–75)
WBC count, × 10^9/L	59.9 (0.8–250.0)
Hb, g/dL	11.2 (5.0–16.2)
PLT count, × 10^9/L	162.5 (29.8–317)
B_2M, mg/L	3350.2 (274.7–7499.9)
IL-8, pg/mL	284.758 (0–1000)
IL-10, pg/mL	26.152 (0–100)
sCD27, U/mL	731.357 (139.9–1000)
LDH, U/L	497.5 (263.0–1507)

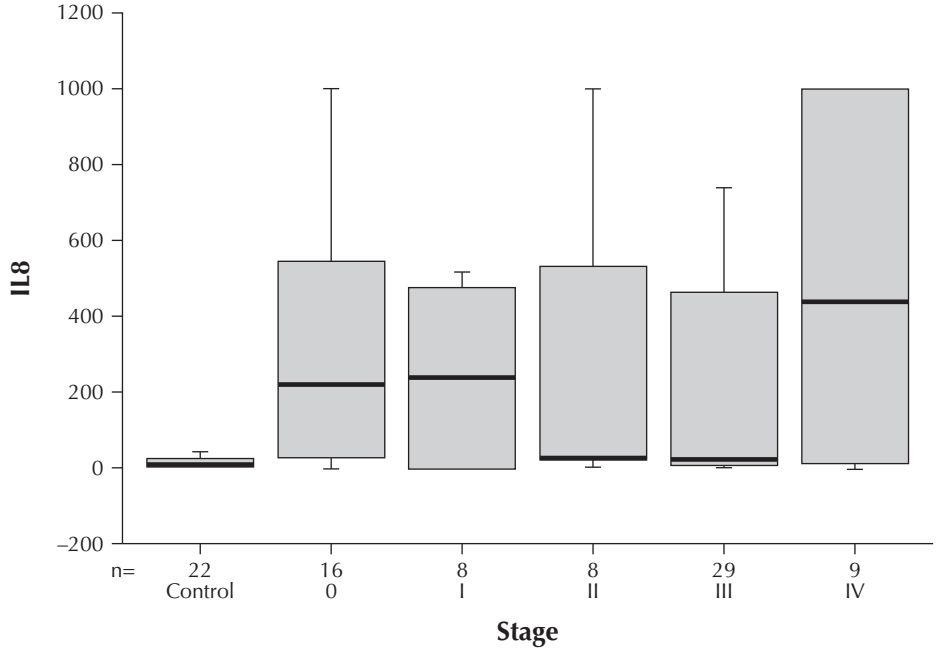
Patients at all Rai stages were represented, with 22.9% at stage 0, 11.4% at stage I, 11.4% at stage II, 41.4% at stage III, and 12.9% at stage IV. A correlation was noted between study and control groups for IL-8, IL-10, and sCD27 values. This correlation was strong for IL-8 (*P*=.001) and sCD27 (*P*=.000), but not for IL-10 (*P*=.139) (Table 2). Patients at high Rai stage had significantly higher plasma IL-8 levels; mean plasma IL-8 level for patients at Rai stage IV was 539.911 pg/mL, and values of 318.639, 243.524, 272.585, and 201.613 pg/mL were attained for Rai stages 0, I, II, and III, respectively (Fig 1). Patients at low Rai stage had lower plasma IL-10 levels; the mean plasma IL-10 level for patients at Rai stage 0 was 6.597 pg/mL, and values were 25.613, 20.42, 37.568, and 29.708 pg/mL for Rai stages I, II, III, and IV, respectively (Fig 2). In addition, plasma levels of sCD27 were higher at high Rai stage and lower at low Rai stage than 542.056 U/L for stage 0, and 632.513, 694.738, 804.71, and 951.944 U/L for stages I, II, III, and IV, respectively (Fig 3). Although plasma IL-10 (*P*=.01) and sCD27 (*P*=.000) were positively correlated with Rai stage, IL-8 was not so correlated (*P*=.146). Additional correlations were noted between sCD27, IL-8, and IL-10, and levels of B_2M, WBC, Hb, PLT, and LDH, as well as among these 3 factors.

After correlations were discerned, investigators identified positive correlations for sCD27 with B_2M (*P*=.000), Hb (*P*=.028), LDH (*P*=.001), CD19 (*P*=.03), and IL-10 (*P*=.000). With Spearman's rank correlation, IL-8 was correlated positively with CD38 (*P*=.001), CD5 (*P*=.006), and WBC (*P*=.000) (Fig 4). Also with Spearman's rank correlation, IL-10 was found to be correlated with B_2M (*P*=.017), CD19 (*P*=.000), PLT (*P*=.002), and CD27 (*P*=.000). In survival distributions for CD27, IL-8, and IL-10, more significant relationships were observed for all parameters (*P*=.0000).

Table 2. Correlations of IL-8, IL-10, and sCD27 Between Study Groups

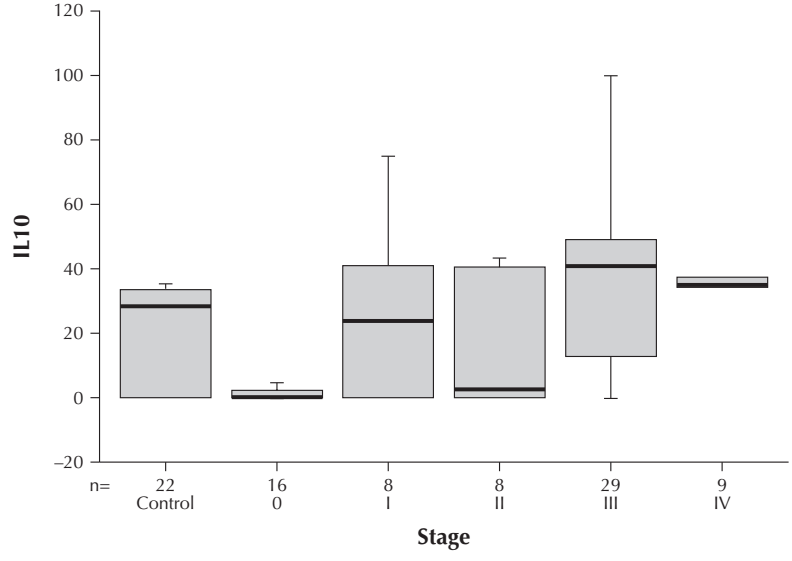
Tested Factor	Study Group, Mean Value	Control Group, Mean Value
IL-8, pg/mL	284.758	20.9 (*P*=.001)
IL-10, pg/mL	26.152	17.6 (*P*=.139)
sCD27, U/L	731.357	264.3 (*P*=.000)

Fig 1. Plasma levels of patients were significantly higher than those of healthy donors (*P*=.001).



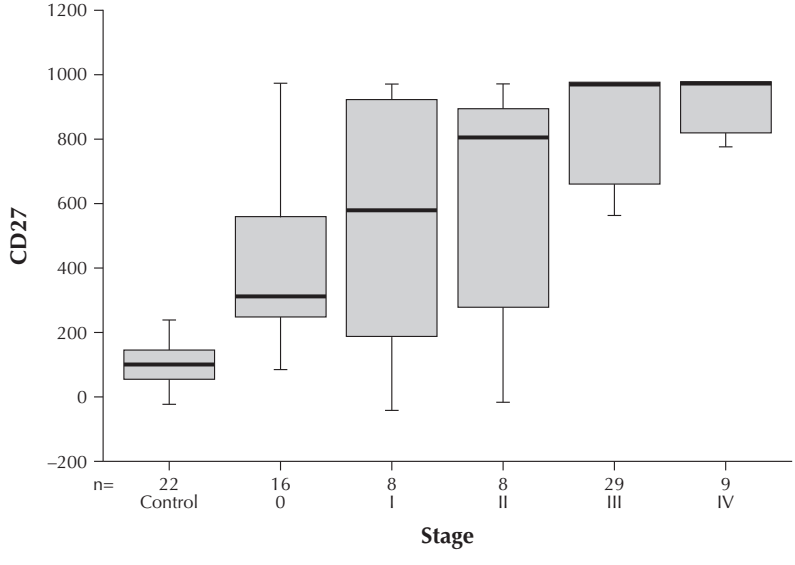
Patients with high Rai stage had significantly higher plasma IL-8 levels; mean plasma level of patients for Rai stage IV was 539.911 pg/mL; values for Rai stage 0, I, II, and III were 318.639, 243.524, 272.585, and 201.613 pg/mL, respectively.

Fig 2. No significance was found for differences between levels of plasma IL-10 in study and control groups (*P*=.139).



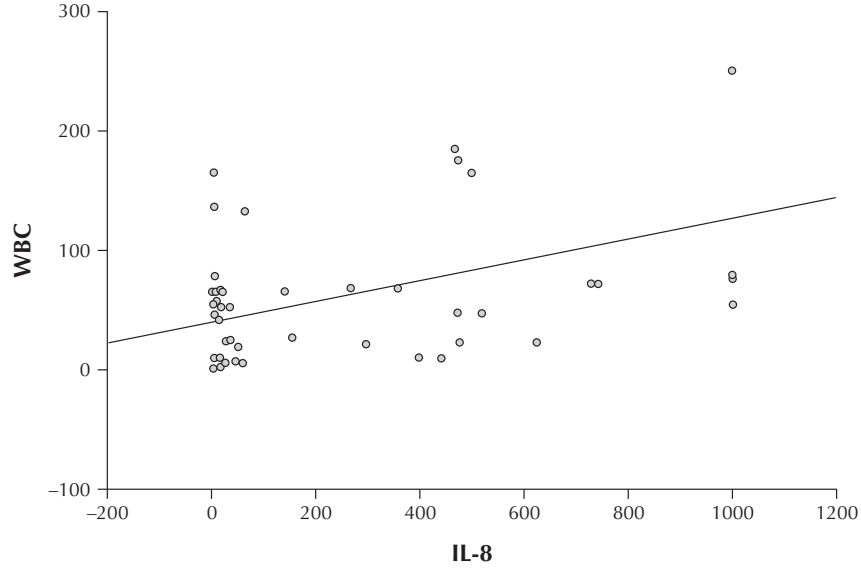
Mean plasma IL-10 level of patients for Rai stage 0 was 6.597 pg/mL; values for stages I, II, III, and IV were 25.613, 20.42, 37.568, and 29.708 pg/mL, respectively.

Fig 3. Plasma levels of sCD27 were significantly higher in the study group than in the control group (*P*=.000).



Plasma levels of sCD27 were 542.056, 632.513, 694.738, 804.71, and 951.944 U/L for stages 0, I, II, III, and IV, respectively.

Fig 4. Level of IL-8 was correlated significantly with WBC count (*P*=.001).



DISCUSSION

An understanding of the biology and pathophysiology of CLL is enhanced by identification and validation of factors that are prognostic for response to treatment and disease-free and overall survival. Conceptually, CLL is a disease of accumulation. As such, a pool of proliferating malignant B cells in bone marrow or other lymphoid organs such as the spleen gives rise to mature monoclonal cells. These malignant B cells lack normal cellular mechanisms and machinery that are responsible for a normal cell life cycle, resulting in cellular senescence and apoptosis.[36] Resistance to apoptosis results in the accumulation of long-lived leukemia cells.

Investigators in the present study found that plasma IL-8 levels were higher than those in the healthy control group, and that their relationships with WBC, CD38, and CD5 were significant. In vitro, IL-8 has been shown to be a constitutively expressed autocrine growth factor for CLL.[17] Furthermore, stimulation of CLL B cells with IL-8 induces increased expression of IL-8 and the antiapoptotic protein, bcl-2, suggesting an enhanced resistance to apoptosis.[18] As such, IL-8 may play a role in leukemia cell motility and migration through lymphoid tissue. Given that IL-8 may function in vivo as an autocrine growth factor for leukemia cells, it follows that levels may correlate with factors known to be prognostic in CLL.

Molica et al[4] evaluated serum IL-8 levels in 58 chemotherapy-naive patients and found that 26% had increased serum levels of IL-8 when compared with normal control subjects; no significant correlation was noted with stage, bone marrow pattern, lymphocytosis, lymphocyte-doubling time, serum B_2M, or sCD23 or sCD27 levels, as were found for CD27 and B_2M.[4] Furthermore, for Binet stage A patients, progres-

sion of disease was more likely if serum IL-8 concentration was above the median level, suggesting that this value may be prognostically useful in early-stage disease.[4] Although Molica et al[4] found no correlation between serum IL-8 levels and prognostic factors such as clinical stage, bone marrow involvement, B_2M, sCD23, and sCD27 in the 58 patients studied, they did demonstrate a likelihood for patients with early-stage disease to progress to a more advanced stage compared with patients below the median plasma IL-8 level. Data show that in 70 patients who were studied, increased plasma IL-8 levels in patients with CLL were not correlated with other validated prognostic factors such as Rai stage and B_2M level, but IL-8 was correlated with CD38, which was known to be a prognostic factor for CLL. At higher Rai stages, however, IL-8 levels were elevated in individual analyses performed at each stage; this may be related to local concentrations in the leukemia cell microenvironment. Furthermore, elevated plasma IL-8 levels were associated with increased WBC count and CD5 levels.

It is known that a high WBC count is the sole manifestation of early disease stages; together with CD5, this value may reflect the tumor burden at these stages. The data presented here may suggest that IL-8 and CD38 levels combined can be used to delineate groups of patients with a very bad or very good prognosis, thus providing the potential for stratification of patients with CLL to select better therapeutic approaches.

It has been reported that B-CLL lymphocytes express IL-10 mRNA,[37] as well as its receptor.[38] IL-10 mRNA expression in B-CLL has been found to correlate inversely with progression of the disease because it has been associated with stable disease.[39] However, data on in vivo production of IL-10 during the natural history of the disease have been equivocal. Investigators in the present study reported significantly higher levels of IL-10 between stages. Egle et al[37] reported that levels of IL-10 were significantly greater in patients at Rai stages III and IV than those at stages 0 to II; these results have been confirmed by another group.[40] In contrast, in a previous report, median serum level IL-10 values were found not to differ between healthy controls and patients with B-CLL, although some of those in the latter group exhibited elevated levels that had no clinical relevance.[41]

The findings of this study show that high serum IL-10 levels correlated with unfavorable prognostic features of the disease, such as Rai stage and elevated levels of B_2M, PLT, and CD27. The source of IL-10 in CLL appears to be polyclonal B cells rather than leukemic cells.[42] A study of the role of IL-10 in CLL has suggested that this molecule inhibits proliferation.[43] With regard to survival, some investigators[44] suggest that IL-10 prevents apoptotic death of CLL cells; others[45] suggest that IL-10 promotes the death of these cells.

IL-10 may prevent programmed cell death of normal human germinal center B cells and Epstein-Barr virus blasts,[46,47] but contrasting data have reported the effects of IL-10 on the survival of neoplastic B-CLL cells. Indeed, this cytokine has been shown to both inhibit and induce apoptosis of B-CLL lymphocytes. Fluckiger et al[45] found that IL-10 was capable of inhibiting spontaneous thymidine incorporation in a proportion of B-CLL samples and of inducing B-CLL cells to die from apoptosis with a concomitant decrease in bcl-2 protein levels. In contrast, other authors have suggested that IL-10 might act as an autocrine growth factor for B-CLL cells because in their studies, B-CLL lymphocytes spontaneously released IL-10 in culture, and

this cytokine enhanced the survival of B-CLL cells in a dose-dependent fashion by inhibiting the process of apoptotic cell death.[44] Recently, in an extensive study on IL-10 receptor expression by B-CLL cells, Jurlander and colleagues[38] found that IL-10 could prolong survival of B-CLL cells, with a pattern of STAT (signal transducer and activator of transcription) protein phosphorylation identical to the pattern of IL-10 receptor activation observed in normal B cells. Investigators have also reported that the activation pathway that leads to IL-10–mediated B-CLL cell survival was similar to that induced through the receptors for interferon-α and interferon-γ—cytokines known to inhibit apoptosis in B-CLL cells.[48,49] The potential heterogeneity of IL-10–induced effects on B-CLL cells was further underlined by a recent article in which IL-10 was found to be capable of increasing in vitro apoptotic B-CLL cell numbers in stage 0 patients, but not in stage I and II patients.[50]

As was previously discussed, CD27 has been suggested recently as a marker for human memory B cells.[34,35] Investigators in the present study found higher levels of CD27 expression on B-CLL cells in correlation with healthy patients (P=.000); correlation with other prognostic factors such as B_2M (P=.000), Hb (P=.028), and LDH (P=.001) was also statistically significant. The findings reported here parallel those of Molica et al,[7] which showed that changes in sCD27 level correlated with clinical stage, B_2M, and LDH. In another clinical study, a strong correlation between sCD27 levels in the serum and tumor load was found, indicating that sCD27 can be used as a disease marker in patients with acute and chronic B-cell malignancies.[6]

The mechanism of CD27 expression by malignant B cells is not yet known. Additional research is needed to investigate the regulation of CD27 expression and the functional role of this molecule in the course of B-cell differentiation, along with its relationship to IL-10.

In summary, CD27 expression shows higher serum levels in patients with B-CLL than in healthy donors. It is significantly correlated with all Rai stages and may serve as a reliable marker for patients with B-CLL. IL-8 for high Rai stage has significantly higher plasma levels, which makes IL-8 a reliable tumor burden marker for patients at late Rai stage; the opposite is true for IL-10. Together with CD27, IL-10 has prognostic value for patients with CLL in accordance with Rai stages. As a result, it is suggested that CD27 and IL-10 are good reliable tumor markers for B-CLL, and that after their role in disease progression is better understood, specific treatment modalities involving anti-CD27 and anti–IL-10 may be added to standard chemotherapy regimens.

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