The prognostic value of a proliferation-inducing ligand (APRIL) serum levels in treatment-naive patients with chronic lymphocytic leukemia

Abstract

**Background/aim:** A proliferation inducing ligand (APRIL) has been investigated as a prognostic marker in chronic lymphocytic leukemia (CLL) patients. However, there is no cut-off level for serum APRIL (sAPRIL) levels that predict time to treatment in CLL patients.

**Materials and methods:** Between May and December 2012, consecutive 94 CLL patients and 25 healthy controls were assessed. sAPRIL levels were measured by ELISA. Demographic data and the prognostic markers were obtained from the patients’ files. Treatment-naive patients were followed up to 6.5 years for any treatment need.

**Results:** Patients were divided into three groups: Treatment naive (n=47), chemotherapy receiving (n=25) and those who had received chemotherapy previously (n=22). No difference in median sAPRIL levels of patients who were receiving chemotherapy at the sampling time and the healthy controls indicates that sAPRIL levels might be influenced by treatment. For treatment naive patients, the best cut-off in predicting time to treatment was found at a sAPRIL level of 2.04 ng/ml, with 78% sensitivity and 63% specificity. Time to treatment was significantly earlier in APRIL high group (n=27) in APRIL low group (n=20) (p=0.010, log-rank test).

**Conclusion:** sAPRIL, a simple, promising blood test, which can be measured by ELISA, will seemingly attain a place in the wide range of prognostic markers in CLL. Prospective large-scale studies are required to validate and confirm the feasibility of the
proposed cut-off level of 2.04 ng/ml as a predictor of time to treatment in treatment naive CLL patients.

**Key words:** Chronic lymphocytic leukemia, treatment, survival, prognosis, chemotherapy

### 1. Introduction

Chronic lymphocytic leukemia (CLL) is a progressive malignant disease characterized by the accumulation of monoclonal lymphocytes in peripheral blood, bone marrow, and lymphoid tissues. Immunophenotypic analysis by flow cytometry reveals CD5, CD19, CD20 and CD23 expression on B cells [1]. Treatment is required in cases with active disease which is defined by the following conditions: B symptoms, progressive splenomegaly, hepatomegaly, lymphadenopathy or lymphocytosis, evidence of bone marrow failure that is not caused by autoimmune phenomena and organomegaly, andg autoimmune phenomena refractory to conventional therapy [2]. Unlike other types of leukemia, treatment is usually deferred until advanced stages of disease [2]. There are no curable treatment options except for allogeneic bone marrow transplantation. There are 2 clinical staging systems in CLL; the Rai [3] and the Binet systems [4]. Although they are easy to use and are widely used for determining prognosis, both staging systems fall short in identifying a portion of CLL patients who have early-stage disease but are at high risk for faster progression. Therefore, there is a considerable amount of research to stratify patients at risk of progression [5]. Several complementary parameters have been suggested to improve the prediction potential of the prognostic scoring systems, such as shortened lymphocyte doubling time (LDT), beta2-microglobulin (β2M), lactate dehydrogenase (LDH), thymidine kinase, CD49d levels,
CD38, and zeta-chain-associated protein kinase 70 (ZAP70) expression. Furthermore, cytogenetic abnormalities including deletions in the chromosomes 11, 13 and 17 or mutations in the immunoglobulin heavy chain gene variable region (IGHV) and TP53 and several mutated genes that are involved in DNA damage, Notch signaling, inflammatory pathways and cytokine signaling have been identified as prognostic markers [5, 6]. Since none of the aforementioned parameters can identify all patients at high risk and the routine clinical use of most of them is limited, there is an unmet need for a better marker of prognosis.

A proliferation-inducing ligand (APRIL) belongs to the family of tumor necrosis factor ligands and has been known to be associated with B cell proliferation and survival [7]. It plays different roles at various stages of B cell ontogeny. There are some observations that APRIL might play a role in the pathogenesis of CLL. First, CLL cells have been shown to express APRIL, which was held responsible for the resistance to apoptosis of CLL cells [7]. Second, nurse like cells that are a part of CLL microenvironment have been shown to express APRIL [8]. In addition to those, APRIL transgenic mice are prone to develop B-cell associated lymphoid tumors [9]. Moreover, serum APRIL (sAPRIL) levels have been found to be associated with a shorter treatment-free interval in newly diagnosed CLL patients [10, 11]. However, there are conflicting data regarding its prognostic role on survival [10, 12, 13]. Finally, there is no data whether sAPRIL levels are still a useful prognostic tool during the course of the disease and vary according to the treatment.

The aim of the present study was (1) to compare sAPRIL levels of CLL patients with those of age and gender matched healthy subjects, (2) to investigate the relationship between sAPRIL levels and other common prognostic factors, (3) to find out whether
sAPRIL levels influence by treatment, (4) to determine whether sAPRIL levels can predict time to treatment in the setting of a prospective observational study over a time span of up to 6.5 years and (4) to identify a cut-off level for prediction of time to treatment.

2. Materials and methods

Between May and December 2012, venous blood samples were drawn from 104 consecutive CLL patients and 25 age and sex-matched healthy controls. Treatment-naive patients have been followed up for 6.5 years for any treatment requirement and survival. CLL was diagnosed according to the National Cancer Institute Working Group criteria [14]. The study was supported by an unrestricted grant by Scientific Research Projects Coordination Unit of Istanbul University (project no: 19694), and was approved by the Ethics Committee of the Cerrahpasa Medical Faculty (43458/2011). Written informed consent was obtained from all the patients and healthy controls. This study was conducted in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Data at initial presentation of the patients were reviewed from the medical records for the following parameters: demographic features, the presence of organomegaly and peripheral lymphadenopathy, complete blood count, LDH and β2M levels, ZAP-70 positivity, the percentage of CD38+ cells in the flow cytometry, and time to treatment.

The cut-off levels for CD38+ positivity and β2M were 30% and 2 mg/L, respectively. The cut off of del17p performed by fluorescent in situ hybridization (FISH) in our laboratory was 10%.

Venous blood samples were collected in anticoagulant free tubes without venous stasis after 12 hours of overnight fasting and centrifuged immediately (3000g) for 10 min at
+4 °C. The serum was stored at -80 °C until the time of assay. sAPRIL levels were measured in duplicate aliquots, using a human enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s instructions (Bender MedSystems, Vienna, Austria). The coefficients of intra- and inter-assay variations were 4.1% (n=10) and 7.2% (n=10), respectively.

Statistical analysis was done using SPSS 17.0. Categorical variables were compared by Chi-square test. Continuous variables were compared by the Student’s t-test when data were parametric and by Mann-Whitney-U test when data were non-parametric. Spearman’s correlation test was used to assess the correlation between measures.

Summary receiver-operating characteristic (ROC) curve and log-rank test were used to calculate whether sAPRIL levels predicted time to treatment in treatment-naive CLL patients. Statistical significance was considered at the two-tailed 0.05 level.

**Results**

Of 104 patients, 10 were excluded from the study due to hemolysed blood samples (3 patients), Richter transformation at the time of recruitment (3 patients), and inadequacy of medical records (4 patients). Overall, samples from 94 CLL patients and 25 healthy donors were eligible for the final analysis.

Median sAPRIL levels of CLL patients were found to be significantly higher than those of 25 healthy donors [(2.63 ng/ml, IQR: 0.97-3.75) vs (1.29 ng/ml, IQR: 0.58-2.19) respectively; p=0.006] (Figure 1). At the sampling time, 47 patients were treatment-naive, 25 patients were actively receiving chemotherapy, and 22 patients had received chemotherapy previously and were treatment-free for ≥ 3 months. The median sAPRIL levels of 47 treatment-naive and 22 treated patients were significantly higher than that of the healthy controls [(2.78 ng/ml, IQR: 0.61-3.78) vs (1.29 ng/ml, IQR: 0.58-2.19);
p=0.028, (3.54 ng/ml, IQR: 2.23-6.51) vs (1.29 ng/ml, IQR: 0.58-2.19); p<0.001, respectively]. However, the median sAPRIL levels of 25 patients actively receiving chemotherapy and 25 healthy controls were not different [(1.56 ng/ml, IQR: 0.84-2.99) vs (1.29 ng/ml, IQR: 0.58-2.19); p=0.295) (Figure 1). Although we did not measure sAPRIL levels prior to and after chemotherapy in each patient, obtained data showing no difference in median sAPRIL levels of patients who were receiving chemotherapy and the controls made us think that sAPRIL levels might be influenced by treatment. We, then, extended the study to follow-up treatment-naive patients to delineate the prognostic role of sAPRIL in these patients.

Treatment-naive patients (group A)

There were 47 (M/F: 30/17) treatment-naive CLL patients. ZAP-70 results were available in 9 patients with 2 of them being positive. sAPRIL levels were found to be negatively correlated with haemoglobin levels (r=-0.298; p=0.037) and platelet counts (r=-0.321; p=0.025). There was no correlation with prognostic indicators such as age (r=0.069; p=0.64), Rai (r=0.151; p=0.31) and Binet stages (r=0.171; p=0.24), lymphocyte counts (r=0.039; p=0.79), β2M (r=0.121; p=0.18) and CD38 levels (r=0.037; p=0.85). The median sAPRIL levels were not different among the patients who had high or normal LDH, CD38 and β2M levels (data were not shown). Clonal abnormality was not evaluated due to low number of detected cases (5 patients).

The median follow-up times of the patients since diagnosis and serum sampling were 114 months (IQR: 90-138) and 78 months, respectively. Among the 47 patients, 23 received chemotherapy due to progressive disease. The median time from sampling to treatment in these 23 patients was 37 months (IQR: 17-47). Seven patients died during
the follow-up, and 5 of them received treatment due to progressive CLL and died due to refractory disease and infection after a median follow up of 1.5 years (range: 1-3) following treatment initiation. The remaining two treatment-naive patients died due to cardiovascular disease 3.5 and 4 years following the study entry, and at the time of death they still did not require therapy.

The ROC curve of sAPRIL levels in predicting time to treatment showed an area under the curve of 0.75 (Figure 2). The best cut-off in terms of prognostic effectiveness was found at a sAPRIL level of 2.04 ng/ml, with 78% sensitivity and 63% specificity. The patients were divided into 2 groups according to the cut-off level: sAPRIL high (n=27) and sAPRIL low group (n=20). Two groups were similar with respect to demographic data and prognostic factors (Table). In sAPRIL high group, 18 of the 27 patients received chemotherapy during follow-up whereas only 5 of the 20 patients required treatment in sAPRIL low group. Time to treatment from sampling (Figure 3a), and diagnosis (Figure 3b) were significantly earlier in sAPRIL high group than in sAPRIL low group (p=0.010, p=0.003, log-rank test, respectively). Among the 5 patients who had died due to refractory CLL and infection, 4 were in sAPRIL high group and 1 was in sAPRIL low group. One patient died in each group due to cardiovascular disease.

3. Discussion

Several studies have been conducted to predict outcome in patients with CLL since patients having early-stage disease have a variable clinical course. None of the prognostic factors mentioned above can stratify all patients at high risk for progression. Moreover, most of them are not available in routine clinical practice. It would be desirable to have a simple prognostic test. sAPRIL levels are easily measured by ELISA and have been investigated as a prognostic marker in CLL patients. However, previous
studies reported conflicting results with regard to the prognostic role of sAPRIL in terms of overall survival [12, 13, 10]. Additionally, its prognostic role has been mostly studied in newly diagnosed patients. In the present study, we included consecutive CLL patients. Although our patient population was heterogeneous regarding disease duration, sAPRIL levels again predicted time to treatment in treatment-naive CLL patients based on a ROC analysis defined threshold of 2.04 ng/ml. We also found that sAPRIL levels seem to be influenced by treatment.

Tecchio and colleagues [10] reported that high sAPRIL levels were associated with an earlier progression in low-risk CLL patients. Ferrer and colleagues [11] also supported this finding and showed that a combined analysis of B cell activating factor and APRIL levels may be more useful to predict disease progression in CLL patients. Our results confirmed those of the previous studies, showing that sAPRIL levels predict time to treatment in treatment naïve CLL patients. However, unlike the 2 previous retrospective studies, in which the serum samples were collected at the time of diagnosis, our study was cross-sectional and included samples obtained at the diagnosis or during the course of the disease. Despite this heterogeneity in the collection time points of the samples, sAPRIL remained to be a useful predictor of time to treatment in treatment-naïve CLL patients. This finding is remarkable as it indicates that sAPRIL levels may be used as prognostic factor independent of time of sample collection. Moreover, different from the previous studies, in our hands, ROC curve provided a cut-off level of 2.04 ng/mL, which clearly differentiated patients who would need treatment.

Serum [12] and plasma [13] APRIL levels were found to be associated with overall survival in 2 studies, but not in Tecchio’s study [10]. In the study by Planelles et al. [12], the patient group with high sAPRIL levels included more patients with advanced
stage disease, when compared to sAPRIL low group. This might be the reason for the conflicting result with regard to the prognostic potential of sAPRIL levels. On the other hand, the demographic and clinical differences among the sAPRIL high and sAPRIL low groups were not clearly represented in Bojarska’s study [13]. In our study, we could not evaluate the impact of sAPRIL levels on overall survival of CLL patients, because the follow-up period was relatively short, and we only lost 5 patients due to refractory disease during the follow-up. Though, 4 of these 5 patients were in sAPRIL high group. sAPRIL levels were detected to be increased in our CLL population compared to healthy controls paralleling the results of the previous reports. Moreover, we also demonstrated that sAPRIL levels in patients receiving chemotherapy were not different from those of the healthy controls. Thus, this finding indicates that sAPRIL levels were not useful for predicting the prognosis in patients on treatment. Chemotherapy apparently led to a decrease in sAPRIL levels. In addition to this, we could demonstrate a negative correlation between the sAPRIL levels and hemoglobin as well as platelet counts. This finding was considered as an indirect evidence for an association between the leukemic cell burden and sAPRIL levels.

Our study had some limitations. First of all, the correlation of sAPRIL levels with the cytogenetic abnormalities and VH mutation could not be investigated due to the low number of patients with sufficient cytogenetic data. However, sAPRIL high and sAPRIL low groups were similar with regard to other prognostic factors. Second of all, the impact of sAPRIL levels on overall survival could not be determined due to the inadequate follow-up time. And last but not least, time points of blood sampling were heterogeneous, some were drawn at diagnosis, others during the course of the disease prior or after a treatment episode. However, this limitation turned to be a strength of the
study demonstrating the prognostic role of sAPRIL regardless of the collection time of serum samples in treatment naive CLL patients. Finally, the low number of treatment naive patients, the cross-sectional design of the study and the relatively short duration of follow-up were the other limitations.

In conclusion, sAPRIL levels are higher in CLL patients than in healthy controls, a finding that is in line with the current literature. However, this only holds true for treatment naive or treatment free patients but not for those who are on chemotherapy. Furthermore, sAPRIL levels seem to be correlated with leukemic cell burden. sAPRIL, a simple, promising blood test, which can be measured by ELISA, will seemingly attain a place in the wide range of prognostic markers in CLL. Prospective large-scale randomised studies are required to validate and confirm the feasibility of the proposed cut-off level of 2.04 ng/ml as a predictor of time to treatment in treatment naive CLL patients.

Acknowledgement

We would like to thank Mrs Nazik Sari for her efforts in obtaining the blood samples used.

References


**Table.** The demographic and clinical characteristics of sAPRIL high and sAPRIL low group

<table>
<thead>
<tr>
<th></th>
<th>APRIL high group (n=27)</th>
<th>APRIL low group (n=20)</th>
<th>p-Value</th>
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<tbody>
<tr>
<td><strong>Mean ± SD age at</strong></td>
<td></td>
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<tr>
<td><strong>sampling time, years</strong></td>
<td>67±12.4</td>
<td>66.9±10.2</td>
<td>0.98</td>
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<tr>
<td><strong>Mean ± SD age at</strong></td>
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<tr>
<td><strong>diagnosis, years</strong></td>
<td>64.3±10.5</td>
<td>61±10.2</td>
<td>0.48</td>
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<tr>
<td><strong>Male, n (%)</strong></td>
<td>19 (70.4)</td>
<td>11 (55)</td>
<td>0.36</td>
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<tr>
<td><strong>Binet stage</strong></td>
<td></td>
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<tr>
<td>A</td>
<td>22</td>
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<tr>
<td>B</td>
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<tr>
<td>C</td>
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<td><strong>Modified Rai stage</strong></td>
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<tr>
<td>Low-risk</td>
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<td>Intermediate-risk</td>
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<tr>
<td>High-risk</td>
<td>2</td>
<td>2</td>
<td>0.75</td>
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<tr>
<td><strong>Median (IQR)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>lymphocyte, mm³</td>
<td>23.700 (11.100-56910)</td>
<td>18.755 (15.112-30300)</td>
<td>0.83</td>
</tr>
<tr>
<td>LDH, n (%)</td>
<td>25 (93)</td>
<td>17 (85)</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>Median (IQR)</strong></td>
<td></td>
<td></td>
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<tr>
<td>β2M (mg/L)</td>
<td>2125 (1680-2862)</td>
<td>1985 (1759-2637)</td>
<td>0.21</td>
</tr>
<tr>
<td>High CD38, n/N (%)</td>
<td>0/13</td>
<td>3/17 (18)</td>
<td>0.11</td>
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<tr>
<td>17p deletion, n/N (%)</td>
<td>4/15 (26)</td>
<td>1/9 (11)</td>
<td>0.36</td>
</tr>
<tr>
<td>Patients who required chemotherapy during the follow-up, n (%)</td>
<td>18 (67)</td>
<td>5 (25)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

APRIL: A proliferation-inducing ligand; β2M: beta2-microglobulin; LDH: Lactate dehydrogenase
Figure 1. Median sAPRIL levels of healthy controls, CLL patients, treatment-naive patients (group A), patients receiving chemotherapy (group B) and patients who were received chemotherapy previously (group C)
Figure 2. The ROC curve of sAPRIL in predicting time to treatment
Figure 3. Kaplan Meier graph shows an earlier time to treatment from sampling (3a) and diagnosis (3b)