APRIL but not BLyS serum levels are increased in chronic lymphocytic leukemia: prognostic relevance of APRIL for survival

APRIL (a proliferation-inducing ligand) and BLyS (B lymphocyte stimulator) expression have been reported in chronic lymphocytic leukemia (CLL) cells. We examined APRIL and BLyS serum levels in CLL patients and evaluated the prognostic significance of APRIL expression on survival.

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BLyS is a fundamental survival factor for transitional and mature B cells, whereas APRIL mainly affects B-1 cell activity, humoral responses and immunoglobulin class switching.[1] We recently reported that aging APRIL transgenic mice develop B-1 cell-associated tumors that are highly reminiscent of human CLL; our initial analysis showed increased levels of circulating APRIL in CLL patients (n=22) compared to healthy donors.[2] Two other reports described that BLyS and APRIL can act in an autocrine manner in CLL tumor cells, promoting cell survival.[3,4] In addition, APRIL produced by inflammatory cells that infiltrate tumor lesions appears to contribute to disease in B cell lymphoma.[5]

To further evaluate APRIL and BLyS implication in CLL, we performed a retrospective study of a cohort of 95 patients diagnosed with CLL according to NCI criteria.[6] After informed consent was obtained, peripheral blood samples were collected from 1991 to 2005, aliquoted and frozen. As a control, 32 sera from age- and sex-matched healthy donors were processed in the same manner. The sera tested were of patients at different stages (A, B and C), but mostly (80%) of untreated patients at either stage A or B. Circulating APRIL was measured by ELISA as described[2] and the mean APRIL serum level was 10.5 ng/ml in healthy donors (range=1.8-25.3 ng/mL; n=32) and 64.5 ng/mL in CLL patients (range=1.5-208.5 ng/mL; n=95; $p<0.0001$; Figure 1A); 75% of all CLL patients showed an increase in circulating APRIL protein compared to controls. We also observed significant differences in APRIL levels in CLL patients grouped by Binet's staging; the mean APRIL serum level was 53.5 ng/mL in stage A patients (n=57) and 79.9 ng/mL in B/C patients (n=37; $p=0.02$).

By contrast, the CLL patient sera tested by ELISA (R&D Systems) showed a moderate decrease in circulating BLyS levels compared to healthy controls (Figure 1B, $p<0.0001$). This was similar to recent observations by Haiat *et al.*[7] Another study revealed increased BLyS serum levels only in CLL patients with a familial history of B-cell lymphoproliferative malignancies.[8] In our study, information on patient familial history was not available. Whether the observed decrease of circulating BLyS levels is relevant for the development of CLL awaits further studies.

To evaluate the clinical relevance of circulating APRIL on CLL, we divided the patient cohort according to the median APRIL serum level. Patients were considered APRIL^{high} when serum APRIL concentration ≥56 ng/mL or APRIL^{low} when APRIL concentration was <56 ng/mL. A description of our CLL patient population and distribution according to the median APRIL levels is shown in Table 1. Fisher's exact test showed no significant association between APRIL levels and Binet's stage or VH mutational status when all patients where considered. Nevertheless,

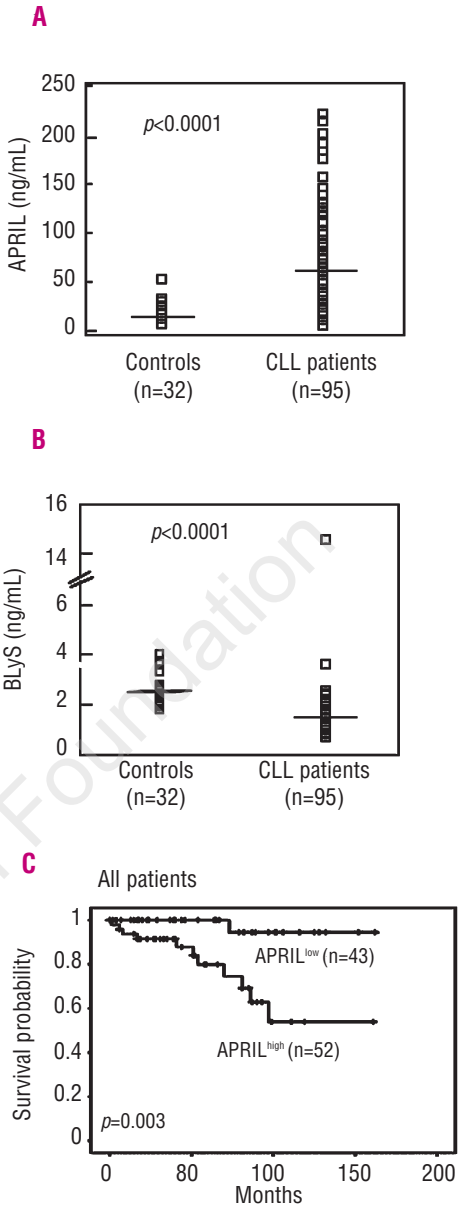

Figure 1. Serum APRIL (A) and BLyS (A) levels in CLL. The retrospective study included 95 patients diagnosed with typical CLL according to NCI criteria6 and 32 sera from age- and sex-matched healthy donors. Circulating APRIL and BLyS were measured by ELISA. Sera were tested three times each; solid lines represent the mean value for each group. (C) Kaplan-Meier curves of overall survival based on serum APRIL levels for all patients.

when analyzing the association of APRIL levels and VH mutational status in subgroups defined by Binet's stage, we found that B/C patients with unmutated VH genes were associated with higher serum APRIL levels than B/C patients with VH mutated genes ($p=0.04$).

We used Kaplan-Meier analysis to estimate the prognostic value of APRIL serum levels on overall survival (OS). APRIL^{high} patients had a significantly poorer prognosis than those classified as APRIL^{low} (survival probability of 53% and 94% respectively; $p=0.003$) (Figure 1C). Univariate Cox analysis confirmed that APRIL levels had a significant correlation with survival ($p=0.02$) and multivariate Cox analysis using the three variables; VH status,

Table 1. CLL patient distribution related to APRIL levels.

	All patients n (%)	*APRIL^{high} n (%)*	*APRIL^{low} n (%)*	*Fisher's exact test*
Patient number	95 (100%)	52 (55%)	43 (45%)	
Sex				
Male	68 (72%)	34 (50%)	34 (50%)	
Female	27 (28%)	18 (67%)	9 (33%)	
Age				
Median (range) years	64 (18-84)	65 (18-82)	62 (42-84)	
VH mutational status				ns
Mutated	53 (56%)	25 (47%)	28 (53%)	
Unmutated	33 (35%)	22 (67%)	11 (33%)	
Unknown	9 (9%)	5 (56%)	4 (44%)	
Binet's staging				ns
Binet A	57 (60%)	27 (47%)	30 (53%)	ns
	VH mutated	44 (77%)	22 (50%)	22 (50%)
	VH unmutated	9 (16%)	4 (44%)	5 (66%)
Binet B, C	37 (39%)	25 (68%)	12 (32%)	p=0.04
	VH mutated	9 (27%)	3 (33%)	6 (67%)
	VH unmutated	24 (65%)	18 (75%)	6 (25%)
Unknown	1 (1%)	1 (100%)		

ns: not significant.

Binet's stage and APRIL levels suggested two alternative models in which APRIL remained independent. In the first model, APRIL had additional prognostic value to VH mutational status (odds ratio, 2.35; 95% CI, 1.33 to 83.04; p=0.03) and in the second, to the Binet staging system (odds ratio, 2.15; 95% CI, 1.10 to 67.37; p=0.04). Due to the high correlation between Binet stages and VH mutation status relative to survival, these variables were not included simultaneously in the model to avoid a colinearity effect. To summarize, we show that serum APRIL levels are significantly elevated in CLL patients and correlate with survival. At present, we cannot conclude whether serum APRIL levels vary over time during disease development. To study the evolution of circulating APRIL in CLL, we have stated a prospective longitudinal study that will also allow evaluation of whether APRIL measurement is a useful indicator of CLL progression.

Lourdes Planelles, Sonia Castillo-Gutiérrez,° Jan Paul Medema,# Antonio Morales-Luque,° Hélène Merle-Béral,@+ Michael Hahne*+*

+These authors share senior authorship

**Institut de Génétique Moléculaire de Montpellier, UMR5535, Montpellier, France; °Department of Statistics and Operation Research, University of Jaén, Spain; #Laboratory for Experimental Oncology and Radiobiology, Amsterdam Medical Center, Amsterdam, The Netherlands; @Service d'Hématologie Biologique, Groupe Hospitalier La Pitié-Salpêtrière, Paris, France*

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Correspondence: Lourdes Planelles, PhD, Department of Immunology and Oncology, Centro Nacional de Biotecnologia/CSIC, Darwin 3, UAM Campus de Cantoblanco, 28049 Madrid, Spain. Phone: international +34.915854855. Fax: international +34.9.13720493. E-mail: lplanelles@cnb.uam.es

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