

CD69 expression in B-cell chronic lymphocytic leukemia: a new prognostic marker?

Ninety-two patients suffering from immunologically typical (CD5⁺CD23⁺) B-cell chronic lymphocytic leukemia (B-CLL) were tested for the expression of CD69, an antigen that is precociously expressed on normal stimulated T lymphocytes and B cells. Forty-eight (52%) patients displayed CD69 antigen on cell surface and the expression of such a molecule was found to be related to higher peripheral blood lymphocytosis, more advanced clinical stage, diffuse pattern of bone marrow infiltration, and trisomy 12. By contrast, del13q14 was more frequently detected in CD69-negative group. Finally, CD69 expression had a significantly negative impact on survival of patients. These data suggest that CD69 could be a promising new immunological prognostic marker for B-CLL.

A highly variable clinical course of the disease characterizes B-cell chronic lymphocytic leukemia (B-CLL).¹ In fact, some patients do not require any treatment for many years and have a long-standing disease, while others may die within a few months from diagnosis because of B-CLL itself or disease-related complications.

In an attempt to identify subgroups of B-CLL patients with peculiar features predicting for the clinical behaviour of the disease, several clinical and laboratory parameters have been tested so far.² In particular, a number of immunophenotypic markers has been proposed as having prognostic relevance, such as the intensity of CD20 expression,³ and, more recently, the expression of CD38.^{4,5}

CD69 identifies a type II integral membrane protein with a single transmembrane domain belonging to the C-type lectin family of surface receptors.^{6,7} Initially described as an antigen expressed early in the activation of lymphoid cells, CD69 was retained restricted to activated lymphocytes. As a matter of the fact, resting peripheral blood lymphoid cells do not express CD69. However, the stimulation of the T-cell receptor/CD3 complex in T-cells induce a quickly expression of CD69.⁸ In addition, CD69 expression is inducible by immature thymocytes, B-cells (through crosslinking of surface immunoglobulin), natural killer cells, monocytes, neutrophils and eosinophils. Despite a specific ligand has not yet been identified, CD69 generates intracellular signals with various cellular responses.⁹

We analyzed CD69 (clone L78, Becton Dickinson Immunocytometry Systems, San José, CA, USA, BDIS) expression on neoplastic cells by means of flow cytometry (FACSCalibur, BDIS) in peripheral blood samples from 92 immunologically typical (CD5⁺CD23⁺) B-CLL untreated patients. An additional panel of fluorescein (FITC) and phycoerythrin (PE) directly-conjugated monoclonal antibodies including CD19 (Leu-12), CD20 (Leu-16), CD22 (Leu-14), CD23 (Leu-20), CD5 (Leu-1), CD38 (Leu-17), CD69 (L78), FMC7, κ/λ light chains, all purchased from BDIS, and CD79b (CB3-1, Immunotech, Marseille, France), was used. Finally, the number of CD20 and CD22 molecules/per cell, evaluated as antibody binding capacity (ABC), was detected by means of QuantiBRITE technology (BDIS), as described elsewhere.¹⁰

Forty-eight (52%) patients expressed CD69 in more than 30% of CD19-positive cells. In Table 1 the clinico-biological features of B-CLL patients according to the expression of CD69 are reported. As shown, no differences by age, gender, typical or atypical morphology (FAB criteria), expression of FMC7, CD79b and CD38, as well as density of CD20, CD22 and surface membrane immunoglobulins were observed between the two groups of patients. However, peripheral blood lymphocytosis, B and C Binet stages, and diffuse pattern of bone marrow infiltration as well, were found to be closely associated with CD69 expression. In addition, in the CD69-positive group trisomy 12 (a cytogenetic

Table 1. Clinico-biological features of B-CLL patients according to the expression of CD69 molecules.

Features	CD69 expression		p
	Negative	Positive	
Age	62.6±10.4	63.1±11.8	ns ^o
Sex (M/F)	34/10	27/21	ns*
Morphology			
Typical	37	38	ns ^o
Atypical	7	10	ns ^o
Clinical stage (Binet)			
A	20	37	0.0095*
B	11	5	
C	11	5	
Peripheral blood lymphocytosis (/ μ L)	44,383±75,581	16,460±9,613	0.0015 ^o
Pattern of bone marrow infiltration [§]			0.0006*
Nodular/interstitial	12	21	
Diffuse	7	3	
Surface Ig density			
Low	43	46	ns*
High	1	2	
FMC7 expression			
Positive	35	35	ns*
Negative	9	13	
CD79b expression			
Positive	38	35	ns*
Negative	6	13	
CD38 expression			
Positive	31	34	ns*
Negative	13	14	
CD20 ABC values	10,022±9,137	12,231±11,214	ns ^o
CD22 ABC values	7,639±7,001	6,232±4,210	ns ^o
Cytogenetics [¶]			
Trisomy 12	0	9	0.03*
Del 13q14	7	1	

Data are expressed as number of cases displaying or not CD69 molecule with the exception of peripheral blood lymphocytosis, CD20 and CD22 ABC values, reported as mean \pm standard deviations. ^oMann-Whitney test; *Chi-square test; [§]evaluated only in 43 cases; [¶]evaluated only in 27 cases.

marker of poor prognosis) was significantly more represented, while del 13q14 (usually correlated with a better prognosis) was detected more frequently in the CD69-negative group. Ten patients (5 in the CD69-positive and 5 in the CD69-negative group) did not carry any of these abnormalities. As a result, median overall survival of CD69-positive B-CLL patients was 98 months, while it is still not reached at 150 months in the CD69-negative B-CLL patients (Figure 1). Finally, multivariate analysis (Cox model) confirmed the independent positive prognostic weight of CD69 expression at diagnosis in B-CLL (p 0.015) (*data not shown*). Thus in our hands, CD69 was found to be expressed on neoplastic B-cells of more than half of B-CLL patients. Since such an expression correlated with worse clinico-biological findings as well as a shorter survival in comparison to CD69-negative forms, CD69 could be considered as a new promising immunological prognostic parameter in B-CLL. However, what is

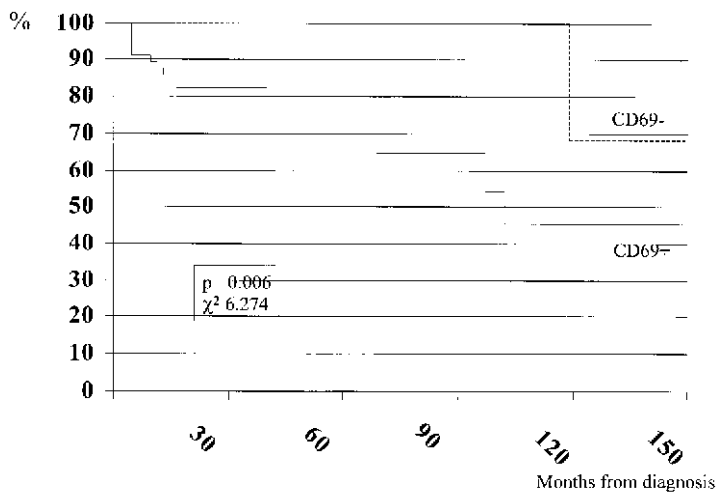


Figure 1. Actuarial survival based on CD69 expression in B-CLL (Kaplan-Meier and Pearson's correlation test).

the exact role of CD69 in the pathogenesis and in the clinical behaviour of B-CLL remains to be better established, needing further investigations.

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