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ZAP-70 expression in chronic lymphocytic leukemia: a new parameter for an old disease

Chronic lymphocytic leukemia (CLL) has a variable clinical course. Although the median survival of patients with this form of leukemia is around 10 years, in individual patients the prognosis is extremely variable, ranging from a very short to a normal lifespan.¹ Thus, some CLL patients will have an excellent prognosis and will never require treatment, whereas in others the prognosis is poor and prompt treatment is required. The clinical staging systems, independently developed by Rai *et al.*² and Binet *et al.*³ in the early 80's, based on easily obtainable biological and clinical variables, are the most useful prognostic parameters. These staging systems not only facilitate the treatment of patients according to individual prognosis, but also make it possible to conduct and to compare trials based on the risk of the disease. However, these systems are not accurate enough to identify subgroups of patients with progressive CLL and mechanisms causing cytopenias are not taken into consideration.

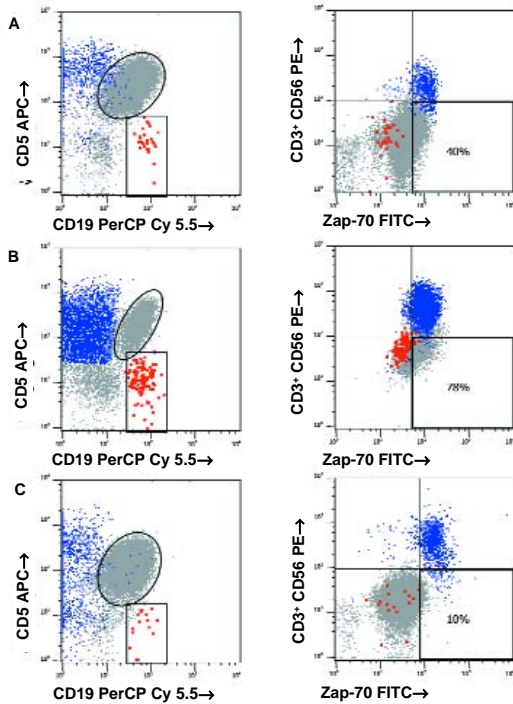


Figure 1. Flow cytometry of ZAP-70 in three CLL cases. T and NK cells (blue, showing high ZAP-70 expression), normal residual polyclonal B cells (red), and CLL cells (gray) were plotted. The left column is showing the expression of CD19 and CD5, with an oval gate selecting CLL cells. In the right column is represented the expression of ZAP-70 and CD3+CD56 antigens, with line markers selecting T and NK cells in the up-right quadrant. CLL cells in the low-right quadrant are ZAP-70 positive, and results are expressed as the percentage of CLL cells having as much ZAP-70 as T cells. Patients A and B (first and second row) express high percentage of ZAP-70 positive CLL cells, whereas patient C disclosed low ZAP-70 expression. Of note, residual B cells in all cases expressed low ZAP-70.

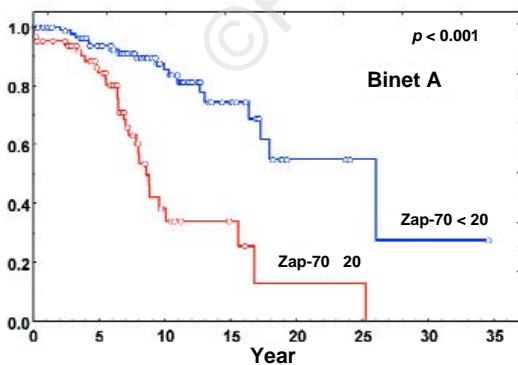


Figure 2. Survival curve of 155 patients CLL patients in Binet A stage according to the ZAP-70 expression. Cases with 20% or more ZAP-70 positive-cells had a median survival of 8 years, whereas it was of 25 years for those CLL cases with less than 20% positive-cells.

Since the introduction of staging systems, there has been a continuous effort to identify new prognostic factors in CLL. In 1999, two different groups reported an important breakthrough in CLL.^{4,5} These groups clearly showed that the mutational status of the somatic mutations of the variable region of the immunoglobulin genes (IgVH) correlates with different disease subsets: those patients with unmutated IgVH genes have a poorer prognosis than those displaying mutated IgVH genes.⁵⁻⁷ Of great interest, the prognostic value of the IgVH mutational status is independent of clinical stages.^{4,5}

Unfortunately, most laboratories are currently unable to isolate and characterize IgVH genes sequences and, even if the technique is available, it is quite costly and time consuming. For this reason, a surrogate for the IgVH mutational status would be extremely welcome. Damle et al first suggested a correlation between CD38 expression on the surface of neoplastic lymphocytes and IgVH mutational status. Nevertheless, the value of CD38 as a surrogate for the IgVH mutational is controversial.^{9,10} Moreover, Hamblin et al have reported that CD38 expression may vary during the course of the disease.¹¹ It is worth emphasizing, however, that CD38 expression has prognostic importance by itself.¹¹ Thus, Otaggio et al in this journal together with other authors demonstrated that CLL cases with increased CD38 expression exhibited more chromosome imbalances or p53 abnormalities.¹²⁻¹⁴

Investigations using DNA microarrays¹⁵ have shown that CLL cells exhibit a characteristic gene expression profile in which a small subset of genes, including ZAP-70, IM12860777, and C-type lectin, correlates with the mutational status of IgVH genes.^{15,16} ZAP-70, a member of the Syk/ZAP-70 protein kinase family, is normally expressed in T cells and NK cells, and plays a critical role in the initiation of T-cell signaling.^{17,18} We recently demonstrated¹⁹ that among patients with CLL, expression of ZAP-70, as detected by flow cytometric analysis (Figure 1), correlated with IgVH mutational status, disease progression and survival (Figure 2). Thus, ZAP-70 expression analysis is able to identify a subgroup of patients in Binet A stage with adverse prognosis. As opposite to CD38 expression, ZAP-70 expression seems to be stable over the time.¹⁹ More importantly, ZAP-70 expression can be studied by cytofluorometry,¹⁹ or RT-PCR²⁰ in purified B cells, which makes of this marker a more convenient parameter than IgVH mutations. For these reasons, ZAP-70 expression analysis should be included in the diagnosis workup of patients with CLL.¹⁹

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Current therapeutic options for subgroups of chronic lymphocytic leukemia. Planning risk-adapted treatment according to recognized prognostic factors

Chronic lymphocytic leukemia (CLL) is the most common of all adult leukemias and is not a homogeneous disorder.^{1,2} Although some have argued that this may not be a single entity³ it is probably one disease with different subgroups displaying different biological behavior patterns, manifesting as different clinical courses and varying responses to treatment.⁴ Most recently physicians have acquired more confidence in their approach and have dared to ask the once feared question: is CLL a curable disease?⁵ This change in approach is basically due to the fact that much has changed in our thinking about CLL in the last decade because of the knowledge and data which have accumulated regarding the biology, molecular genetics and prognostic factors, coupled with the development of novel drugs, new concepts of immunotherapy and the newer techniques for stem cell transplantation now available.⁵ All the latter have allowed us to entertain new ideas for therapy and the concepts of complete (CR) and molecular remission (MR) have now readily been incorporated into our new mode of thinking on how best to treat CLL. Concepts of possible clinical cure have been entertained and questions are asked such as whether very early disease in younger patients should be treated without necessarily waiting for the classical indications of progressive disease before treatment is given. In the light of all the above it is indeed difficult to outline rigid guidelines for what is best for CLL patients and many of these basic questions are the subjects of ongoing clinical trials.^{6,7} However it does seem that the correct questions are now being addressed and it is possible that in 5-10 years from now more answers will be available which may well alter the current concepts of therapy for many patients.

Importance of prognostic indicators for treatment selection

Before therapy can be discussed it has to be understood that the clinical presentation and course in CLL is far from uniform and disease progression and individual response to treatment are unpredictable, differing from patient to patient. Nowadays it seems evident that a proportion of patients have a long survival without major progression while an equal number (about one third) have more aggressive disease with progressive clin-