Lymphoproliferative Disorders

A comparison of the sensitivity of flow cytometry and bone marrow biopsy in the detection of minimal residual disease in chronic lymphocytic leukemia

We compared the sensitivity of bone marrow biopsy to blood flow cytometry in detecting minimal residual disease (MRD) in 29 patients with chronic lymphocytic leukemia (CLL) in clinical remission after treatment. These results demonstrate that flow cytometry is more sensitive than bone marrow biopsy in detecting MRD and in predicting relapse in CLL.

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Patients with chronic lymphocytic leukemia (CLL) have a variable course and a wide range of survival time from a few months to more than 25 years.[1] New therapeutic approaches for the treatment of CLL aim to induce phenotypic remission and molecular remission. Indeed minimal residual disease (MRD) assessment is becoming critical for treatment strategies and their monitoring in CLL.

Using flow cytometry and molecular biology techniques, we and others have previously shown that patients in complete remission with detectable MRD have an increased risk for early relapse, whereas those without detectable MRD experience prolonged remission.[2,3] The evaluation of response to treatment in CLL is based on the NCI criteria which include bone marrow biopsy.[4] Recently lymphoid infiltration detected in the biopsy after three or six courses of therapy was demonstrated not to correlate with time to progression or with overall survival.[5] Rawstron *et al.*, by using a very high sensitive flow cytometry technique, showed that patients with more than 0.01×10^9/L circulating CLL cells always had significant (> 5%) marrow disease, and blood monitoring could be used to time marrow assessment.[2] The main aim of our study was to compare the sensitivity of bone marrow biopsy and blood flow cytometry in detecting MRD and their respective predictive value for relapse.

Twenty-nine previously untreated patients with stage B (n=28) or C (n=1) CLL enrolled in a French trial were in complete clinical remission after treatment with six courses of oral fludarabine+cyclophosphamide[6] with a median follow up of 49 months (range 19-56). Two months after the treatment ended, blood flow cytometry and bone marrow biopsy with morphological study completed by immunohistochemistry were performed in all cases at the same time. Flow cytometry analysis was carried out to detect residual leukemic cells using a four-color method with a panel of antibodies including anti-CD19, anti-CD20, anti-CD5, and anti-CD79b antibodies. The result was expressed as a ratio ($CD19^+CD5^+CD79^{-/low}$ lymphocytes/total $CD19^+CD5^+$ lymphocytes) which is less than 0.2 in case of phenotypic remission with a sensitivity of 10^{-4} as previously described.[7] This ratio was preferred to absolute counts for two major reasons: (i) in most CLL patients the lymphocyte count is very low after treatment with fludarabine; (ii) in our hands, the B $CD5^+CD79b^{+low}$ subset in healthy donors varies from 0 to 0.06×10^9/L, values that are observed in some CLL cases with MRD^+ confirmed by polymerase chain reaction. Immunohisto-

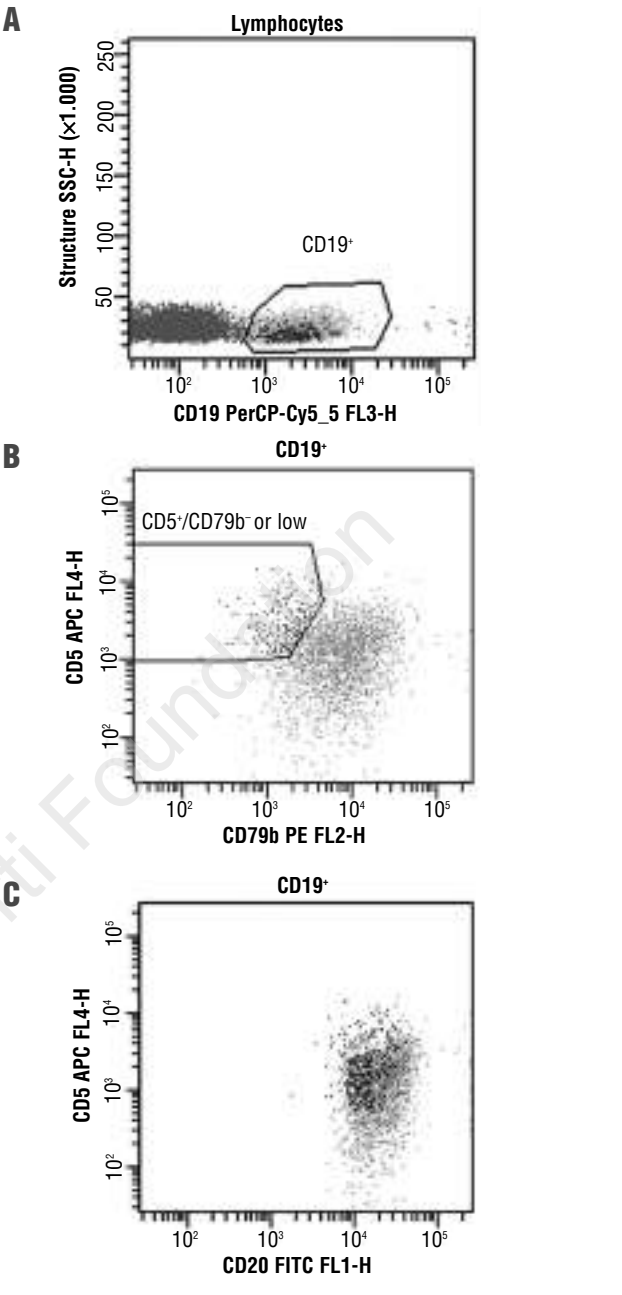

Figure 1. Example of a case with negative blood flow cytometry, an increase of polyclonal B $CD5^+$ lymphocytes and positive immunohistochemistry in the bone marrow. (A) gating on the $CD19^+$ lymphocytes; (B) the majority of B lymphocytes expressed both CD5 and CD79b molecules; (C) normal CD20 expression for all B lymphocytes (D) CD20 and (E) CD5 co-expression in a bone marrow lymphoid nodule demonstrated by immunohistochemistry.

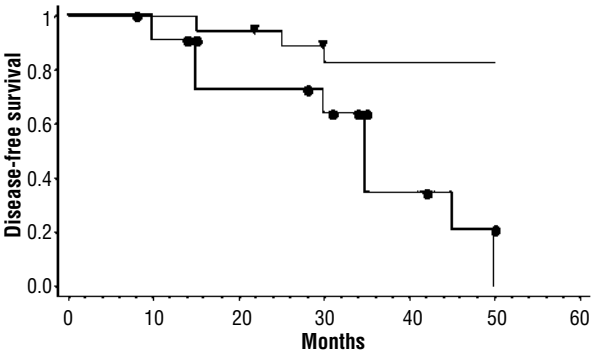

Figure 2. **Disease-free survival according to blood flow cytometry status. Patients achieving phenotypic remission (n=18) (▼) had longer responses than had patients with detectable MRD in the blood (n=11) (●); *p*=0.0041.**

chemistry was performed with anti-CD3, -CD5, and -CD20 antibodies. Bone marrow biopsy immunostaining was considered positive for MRD in the case of concurrent expression of CD20 and CD5 by the same cells assessed on consecutive sections.

The histological study identified six patients with persistent $CD20^+CD5^+$ lymphocyte marrow infiltrate and 23 patients with a normal bone marrow biopsy. In eight of these 23 cases with normal marrow biopsy, blood flow cytometry detected MRD. Regarding the six patients with positive biopsy, blood cytometry was positive in three cases, as expected, and negative in the other three patients who showed expansion of polyclonal B $CD5^+$ lymphocytes without any phenotypic characteristics of CLL cells (Figure 1).[7] When considering the patients according to their blood phenotype status at the time of the treatment evaluation, 18 were in phenotypic remission and only three of these relapsed 12, 22 and 30 months following completion of the treatment. In contrast, nine out of the 11 patients who were not in phenotypic remission relapsed 8, 14, 15, 28, 31, 34, 35, 42 and 50 months after the end of therapy (p=0.0041) (Figure 2). It should be noted that no correlation was demonstrated between histological status and clinical outcome since among the 23 patients with normal biopsy, ten relapsed at a median time to progression of 30 months (range 14-50) and two of the six patients with positive biopsy relapsed 8 and 42 months after the evaluation.

Our results are in agreement with data previously published by Weiss *et al*.[8] and by Moreton *et al*.[9] who showed that eradication of MRD as assessed by flow cytometry is associated with prolonged survival. On the other hand, Oudat *et al*. showed a significant correlation between time to progression and marrow infiltrate (<70% vs >70%) before therapy but no correlation was found after treatment.[5] Our study confirms the clinical utility of flow cytometry of peripheral blood lymphocytes for the detection of MRD and its ability to predict clinical outcome. In contrast, bone marrow biopsy appears to be less sensitive than blood cytometry in detecting MRD and in predicting relapse. Further studies on larger series are needed to consider whether blood flow cytometry can definitively replace bone marrow biopsy in the evaluation of treatment in CLL.

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Key words: bone marrow biopsy, flow cytometry, MRD, CLL.

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