Prognostic Significance of Immunoglobulin Phenotype in B Cell Chronic Lymphocytic Leukemia


Seventy-six consecutive untreated patients with B cell chronic lymphocytic leukemia (B-CLL) and classified according to Binet’s staging system were studied at the clinical presentation. Several immunologic parameters (number of total and T circulating lymphocytes and their surface membrane immunoglobulin [SmIg] phenotypes and levels of serum Ig) were evaluated with the aim of identifying a biologic marker of prognostic relevance. In this series of persons, Binet staging confirmed its usefulness as a prognostic index (P < .001). With regard to SmIg, they were μ-type in 41 cases (53.9%), μ-type plus δ-type in 29 cases (38.2%), α-type in one case, and not detectable in five cases. No correlations were found between clinical stage and immunoglobulin phenotype, although all but one patient in stage C showed μ-type SmIg alone. On analyzing the survival curves of our patients according to different SmIg phenotypes, we found that patients with only μ-type SmIg had a poorer prognosis (P < .05) than those with μ-type plus δ-type; this difference was even more significant (P < .01) in patients in stage A, whereas there were no statistical differences in those in stages B and C. Because the appearance of surface heavy chain of δ-type could be an expression of cell maturation, these results suggest that in B-CLL the presence of phenotypically more mature leukemic cells may correlate with better clinical prognosis, particularly in the early phase of the disease.

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B CELL chronic lymphocytic leukemia (B-CLL) is a lymphoproliferative disease that varies widely in its clinical course, ranging from indolent forms with many years’ survival to aggressive forms with survival of only a few months. The need for clearly established prognostic factors is therefore imperative for the choice of correct therapeutic strategies. Many attempts have been made to identify such prognostic factors; morphologic, cytometric, and kinetic characteristics of leukemic cells have been claimed to be of prognostic relevance.1-5 In this respect, the most important and clinically useful results have been reported by Rai6 and Binet,7 who demonstrated a close correlation between the prognosis of CLL patients and some easily determinable clinico-hematologic parameters. More recently, Baccarani et al8 and Rozman et al9 identified subsets of patients in Rai’s or Binet’s staging systems with different prognosis according to circulating lymphocyte count. However, these staging systems do not take into account strictly biologic parameters, such as degree of cell maturation and differentiation, which have been shown to correlate with aggressiveness of the disease10,11 both in solid tumors and in hematologic malignancies. It has been claimed for many years that different surface membrane immunoglobulin (SmIg) phenotypes are closely related to different steps in normal B lymphocyte maturation12; this model of B lymphocyte ontogeny has been supported by recent advances in molecular biology.13 Clonal expansion of B cell tumors could start at different maturation levels, thus giving rise to populations bearing different SmIg phenotypes and possibly different “intrinsic malignancy.”

We reviewed 76 persons with B-CLL classified according to Binet’s staging to investigate the prognostic significance of a series of immunologic parameters, including particularly SmIg phenotypes, with the aim of identifying a biologic marker of prognostic relevance and possibly subsets of patients with different prognosis in the different stages.

MATERIALS AND METHODS

Patients

Seventy-six consecutive untreated persons with B-CLL who were admitted to our institution were studied. Diagnosis was made on the basis of peripheral lymphocytosis (lymphs > 10 x 10^9/L) accompanied by immunologic demonstration of clonal proliferation of lymphocytes (SmIg with the same light chain or mouse rosette receptor present in more than 50% of circulating lymphocytes). In doubtful cases, bone marrow infiltrate of >50% lymphocytes was required. The cases were classified according to Binet’s staging. The following parameters at diagnosis were also considered to ascertain their prognostic significance: sex, age (≥60 years), total and T lymphocyte counts, reduced levels of one or more serum immunoglobulin (Slg) classes, and types of Slg heavy and light chains.

Treatment Criteria

Treatment was usually the same. Asymptomatic patients with a small tumor burden did not receive any therapy. In the other cases, the first treatment was always chlorambucil and small doses of corticosteroids. Other treatments, including multiple drug combinations, were reserved for unresponsive cases.

Immunologic Studies

Peripheral blood drawn into heparin (Liquemin-Roche) was diluted twice with phosphate-buffered saline (PBS) and layered on a
The degree of monocyte contamination was always <5%.

**E rosettes.** Fresh sheep erythrocytes (SRBC) in Alsever’s solution (ISM, Milan) were washed three times in PBS. To obtain E rosettes, 100 µL of 4 x 10⁷/mL SRBC solution was placed in a 12 x 75-mm glass tube with 100 µL of a 4 x 10⁹/mL lymphocyte suspension. This was centrifuged at 200 g for four minutes and incubated overnight.

**Mouse rosettes.** The test was performed at the time of diagnosis in 58 patients and later in 18. Fresh mouse blood was collected into citrated saline and washed three times. One hundred microliters of packed mouse red blood cells (MRBC) was resuspended in 10 mL PBS. Fifty microliters fetal calf serum (Eurobio) and 250 µL of MRBC suspension were added to 250 µL of 4 x 10⁹/mL lymphocyte suspension. After centrifugation at 200 g for two minutes and incubation at 4 °C for one hour, the cells were resuspended by gently rolling the tubes. The percentage of cells forming E or M rosettes was determined by examining 200 cells.

**Surface membrane immunoglobulins.** SmIg were examined by direct immunofluorescence. The method included the incubation of the lymphocytes in RPMI 1640 at 37 °C for one hour to remove all traces of absorbed serum IgG. One hundred microliters of 10 x 10⁶/mL lymphocyte suspension was incubated with an appropriate dilution of a commercial preparation (Behringwerke, Marburg-Lahn, Germany) of fluoresceinated rabbit antihuman immunoglobulins, α, γ, δ, µ, κ, and λ chains at 4 °C for one hour. After washing with PBS with 2% of bovine albumin (BSA, Sigma Chemical Co, St Louis), the cells were resuspended in 50% buffered glycerol on a glass slide. At least 200 cells were examined at a Leitz (Wetzlar, FRG) Dialux 20 EB microscope by the same person. In order to remove aggregated material, all antisera were routinely ultracentrifuged and absorbed by liver acetone powder (Sigma) before use. Controls were included in all experiments.

**Serum immunoglobulins.** SmIg levels were determined by the nephelometric method (Beckman Immunochemistry Analyzer, Brea, Calif).

**Statistical Analysis**

Survival curves were computed according to Kaplan and Meier2,3; the differences among these were tested by the logrank test.4 Correlations between immunologic pictures and patients’ characteristics at diagnosis were investigated with analysis of variance and χ² test.

SURV-C program (M.C. Pike, S.V. Howard, P.G. Smith, J. Casagrande, personal communication, 1976) and SPSS package5 supplemented on Univac 1100/80 of CILEA were used for the above analysis.

**RESULTS**

The characteristics of the 76 patients are summarized in Table 1. Twenty-three of them have now died and two were lost to follow-up. The minimum follow-up was 26 months (median 43 months). Figure 1 shows the survival curves according to Binet’s stages; the difference between the three curves was highly significant (P < .001). Also, the number of peripheral blood lymphocytes (≤20, >20, ≤50, >50 x 10⁹/L) was significantly related with a prognosis (P < .05). We found no significant differences in survival relating to sex, age (≥60 years), reduction of one or more SmIg classes, or absolute number of T lymphocytes. Table 2 shows the distribution of patients according to SmIg class on the leukemic lymphocytes and the correlation with the stage; in almost all cases, the membrane immunofluorescence was weak, as already reported by various authors.21-23 Of the heavy chain, only µ were present in 41 cases (53.9%), µ were associated with δ in 29 cases (38.2%), and in one case the SmIg were α-type. In five cases (6.6%), SmIg were undetectable, but (confirming the diagnosis of B-CLL) there was a high percentage of mouse rosetting lymphocytes (>60%). No significant correlation was found between SmIg type and clinical stage; it should be noted that of the seven stage C patients, six had only µ-type and one had α-type.

Figure 3 shows the survival curves according to the type of SmIg heavy chain. The patients with lymphocytes bearing only µ-type SmIg had less favorable prognosis than those with other types of heavy chains (P < .05). The five cases in which SmIg were not detectable seemed to have an intermediate pattern. There was no correlation between SmIg type and circulating lymphocyte levels or patient’s age. No differences in survival were shown when the SmIg light chains were considered (κ/λ ratio: 3.36).

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**Table 1. Characteristics of CLL Patients According to Binet Staging**

<table>
<thead>
<tr>
<th>Binet Stage</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>39</td>
<td>30</td>
<td>7</td>
<td>76</td>
</tr>
<tr>
<td>Age (years), mean (range)</td>
<td>63.7 (45-85)</td>
<td>62.1 (38-83)</td>
<td>70.6 (54-81)</td>
<td>63.7 (38-85)</td>
</tr>
<tr>
<td>No. of patients aged &gt;60 years</td>
<td>26</td>
<td>20</td>
<td>6</td>
<td>52</td>
</tr>
<tr>
<td>Male/female</td>
<td>21/18</td>
<td>19/11</td>
<td>5/2</td>
<td>45/31</td>
</tr>
<tr>
<td>Lymphocyte count (x 10⁹/L), mean (range)</td>
<td>22.0 (10-84)</td>
<td>43.1 (10-270)</td>
<td>103.3 (18-203)</td>
<td>37.8 (10-270)</td>
</tr>
<tr>
<td>Alive/dead</td>
<td>34/5</td>
<td>18/12</td>
<td>1/6</td>
<td>51/23*</td>
</tr>
<tr>
<td>Median observation period (months)</td>
<td>52</td>
<td>43</td>
<td>25</td>
<td>43</td>
</tr>
</tbody>
</table>

*Two lost to follow-up.
Table 2. SmIg Phenotype According to Binet Staging

<table>
<thead>
<tr>
<th>Binet Stage</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SmIg μ</td>
<td>18</td>
<td>17</td>
<td>6</td>
<td>41</td>
</tr>
<tr>
<td>SmIg μ + δ</td>
<td>18</td>
<td>11</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>SmIg α</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SmIg not detectable</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 3. Characteristics of Patients in Binet's Stage A According to SmIg Phenotype

<table>
<thead>
<tr>
<th>Heavy Chain Type</th>
<th>μ</th>
<th>μ + δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Age &gt; 60 years</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Lymphocytes &gt; 50 x 10^9/L</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Age ≥ 60 years and lymphocytes ≥ 50 x 10^9/L</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Age ≤ 60 years and lymphocytes ≥ 50 x 10^9/L</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Rai's stages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>I</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Dead</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

We also studied survival according to SmIg within each stage. Although in stage A the difference between μ and μ + δ was still more significant (P < .01), no difference was found in the other two stages, which have a worse prognosis (Fig 2). As can be seen in Table 3, the stage A subgroups with different phenotype and prognosis were comparable for Rai stages, age, and circulating lymphocyte counts.

**DISCUSSION**

Various clinical staging systems have been proposed for B-CLL, and their prognostic reliability has been demonstrated.

Also in our series Binet's staging system appeared to have statistical prognostic relevance, with an extremely benign trend for patients in stage A. Baccarani et al and Rozman et al recently identified subsets of patients in the early Rai or Binet stages with different prognosis according to peripheral lymphocytosis.

In order to investigate if immunologic parameters also have prognostic value, we evaluated a series of 76 patients as a whole and subdivided according to Binet's staging system. In fact, the possibility of identifying subgroups at greater risk in the stages with favorable prognosis could have precise therapeutic applications. Because it has been demonstrated that aggressiveness of a tumor may correlate with its degree of cellular differentiation, we sought correlations between the SmIg type that was present on the leukemic lymphocytes (as an indication of the degree of maturation) and prognosis. In fact, it is known that in the ontogeny of normal B lymphocytes, μ-type SmIgs are the first to appear, and that only in subsequent maturation stages do other SmIgs become associated with or substituted by first the δ-type and later the γ-, α-, and ε-types.

No significant correlation was found between SmIg type and clinical stage in our series, but only one of the patients in stage C had lymphocytes with SmIg, indicating greater maturity of the leukemic cell (α-type).

Many attempts have been made to identify correlations between clinical stage and membrane immunoglobulin phenotype. Foa et al found no such correlation. Recently, Van Scoy-Mosh et al and Ligler et al...
al noted that the leukemic lymphocytes with γ-type SmIg correlated with disease in the initial Rai stages. Despite the relatively high frequency of this immunoglobulin phenotype in these two studies, the actual number of γ-type cases was rather small. No patient in our series expressed this phenotype.

Because clinical stages reflect a transient picture in a developing disease, but immunologic findings on the lymphocyte membrane are constant, the fact that no correlations could be found between them is not necessarily relevant. Moreover, with regard to stage C, the usually relatively small numbers of patients in this stage (as in our series) make statistical analysis difficult.

Correlations have also been investigated between immunologic phenotype and phase of the disease (indolent or active). Foa et al and more recently Baldini et al found no statistically relevant correlation, although the former observed a greater frequency of patients with μ-type SmIg in the active forms than in the indolent forms. Also in this case, however, the parameters considered to distinguish between indolent and active forms did not take the actual evolution of the disease into account.

Therefore, we considered the survival according to degree of cell maturity, and the difference between the survival curves of the patients with μ-type SmIg only compared with those with μ + δ or α was statistically significant. The disease in the patients with no detectable SmIg appeared to have an intermediate pattern, which could be due, at least in some cases, to the absence of the SmIgs (a sign of greater cell immaturity), but to their presence at a density at which they are undetectable with our methods.

Considering survival in the individual stages according to SmIg type, no difference was found for stages B and C, whereas in stage A the difference was highly significant (P < .01), so much so that all the patients with SmIg μ + δ are still alive. These differences in prognosis, both for the whole series and for the group of stage A patients, were not due to imbalance in the distribution of patients according to parameters that were found per se to have prognostic relevance.

Our findings do not agree with those of Hamblin and Hough, who identified a subset of CLL patients with less aggressive disease bearing only μ-type SmIgs on their leukemic cells. The results of this study, which reported a high percentage of patients with undetectable SmIgs and which analyzed prognostic factors singly, are difficult to compare with those of other more recent studies using clinical staging systems that take several variables into account simultaneously.

In a recent study on the survival of patients with non-Hodgkin’s lymphomas in relation to cell surface marker phenotype, Rudders et al found that survival was significantly longer in the patients with μ + δ-type SmIgs than in those with μ-type SmIgs alone; this difference was even more significant in a homogeneous subgroup of patients with small cleaved follicular cell lymphoma.

Our study also indicates that in CLL, a more favorable prognosis may be associated with the presence on the leukemic lymphocytes of SmIgs other than the μ-type alone. This seemed to be particularly true of the patients in stage A and suggests that, whereas in the stages in which the disease is already advanced, the immunologic prognostic factors are probably overtaken by the clinical prognostic factors. In stage A, when the prognosis is more favorable, the demonstration of a phenotype associated with greater cell immaturity correlates with less favorable prognosis. This finding, if confirmed, could identify a subset of B-CLL patients in stage A with a worse prognosis for whom treatment could be indicated.

REFERENCES

Prognostic significance of immunoglobulin phenotype in B cell chronic lymphocytic leukemia

L Baldini, R Mozzana, A Corteletzzi, A Neri, F Radaelli, B Cesana, AT Maiolo and EE Polli