Bone Marrow Histologic Pattern—The Best Single Prognostic Parameter in Chronic Lymphocytic Leukemia: A Multivariate Survival Analysis of 329 Cases


In previous studies, the prognostic value of bone marrow (BM) histologic patterns in chronic lymphocytic leukemia (CLL) has been demonstrated. In order to investigate whether such a value is independent of other prognostic parameters, a multivariate survival analysis (Cox’s regression model) was undertaken in a series of 329 CLL patients in whom a BM had been performed. The following binary variables were included in the analysis: age (more than 60 years), lymphadenopathy (more than two areas involved), splenomegaly, hepatomegaly, absolute lymphocyte count (more than 30,000/μL), anemia (hemoglobin less than 10 g/dL), thrombocytopenia (less than 100,000 μL), and BM pattern (diffuse vs nondiffuse). Three variables entered the regression at significant level: BM pattern (P < .001), anemia (P < .001), and hepatomegaly (P = .03). The model was also tested by expressing the variables in a continuous way when possible. Again, BM pattern entered first in the regression (P < .001), followed by the hepatomegaly (P = .002), hemoglobin level (P = .02), and lymphadenopathy (P = .04). When both the binary and the continuous models were tested separately in 227 patients with BM as initial staging procedure and in 102 patients in whom this was performed later during the course of the disease, in all instances, BM pattern entered first in the regression at a highly significant level. BM histologic pattern appears to be a better single prognostic parameter than any one of the variables employed in current clinical staging systems. A combined clinicopathologic system incorporating the BM pattern, together with the usual clinical variables, is presented.

In CHRONIC LYMPHOCYTIC LEUKEMIA (CLL), a certain number of prognostic factors have been isolated. Some of them were grouped by Rai et al1 in a clinical staging system that has been widely accepted and verified.2 5 However, some attempts to improve this classification have been carried out.6-7 An International Workshop on CLL8 has recommended a modified system for CLL staging, which although not considered as a final one, provides advantages in planning treatment and in research because clinical stages are limited to three. In this staging system, patients are classified depending on the presence or absence of anemia and/or thrombocytopenia (group C) and the number of “lymphoid” areas enlarged (groups A and B). The International Workshop on CLL has particularly encouraged the investigation of methods to predict, within each of the A, B, and C groups, subsets of patients who develop a progressive or aggressive clinical course as compared to patients whose clinical course is benign or stable.

The prognostic value of bone marrow histologic patterns in CLL has been evaluated by some authors. In a study of 115 patients (including cases of lymphosarcoma cell leukemia), Gray et al19 showed that cases with diffuse bone marrow infiltration had a poor prognosis as compared with cases presenting with a nodular or mixed (nodular and diffuse) pattern. In a preliminary study, some of us described four different bone marrow patterns of involvement in CLL, namely, interstitial, nodular, mixed (interstitial and nodular), and diffuse.12 A significant correlation between these patterns and clinical stages according to Rai et al was found.13-15 A similar correlation has been found by Binet’s group for their anatomicoclinical staging system16 and by Carbone et al17 for the “active” and “indolent” forms of the disease. In 1981, three of us (C.R., E.M., and L.H.-N.) showed a significant difference of survival according to the bone marrow infiltration patterns.18

In order to investigate whether the prognostic value of the bone marrow histologic pattern is independent of other known prognostic parameters, the present multivariate survival analysis (Cox’s regression model) was undertaken in a series of 329 patients. This article is a detailed account of this study, in which the bone marrow histologic pattern emerged as a better single prognostic parameter than any one of the variables employed in current clinical staging systems.

MATERIALS AND METHODS

Patients and Diagnostic Criteria

Three hundred and twenty-nine patients (208 males and 121 females) from 17 Spanish institutions (Spanish Cooperative Group for CLL Study, Secretariat: Postgraduate School of Hematology “Farreras Valenti,” University of Barcelona, Spain.


Address reprint requests to Dr C. Rozman, Postgraduate School of Hematology “Farreras Valenti,” Hospital Clínico y Provincial, University of Barcelona, Spain.

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0006-4971/84/6403-0009$03.00/0

642 Blood. Vol 64, No 3 (September), 1984: pp 642–648
Marrow biopsy in prognosis of CLL

for CLL Study) were included. Mean age was 64.7 years (SD \pm 10). An interim report related to the clinical-pathologic correlations of a part (209 patients) of the present series has previously been referred to. In 227 patients (mean age 65.2 years, SD \pm 10.3), bone marrow biopsy was carried out as part of their initial staging procedure. In the remaining 102 cases (mean age 63.5 \pm 9.1 years), it was performed later during the course of the disease. The survival was calculated from the time at which the biopsy was done.

The diagnostic criteria for CLL were those usually recommended: (1) more than 15 x 10^9/L lymphocytes in peripheral blood; (2) bone marrow infiltration by lymphocytes of 50% or more; (3) less than 10% atypical lymphocytes in either the peripheral blood or the bone marrow. Lymphosarcoma cell leukemia, prolymphocytic leukemia, and leukemic reticulosis were excluded.

Most patients received no treatment or were treated with a single agent, either cyclophosphamide or chlorambucil. A few patients with an aggressive form of the disease received polychemotherapy (cyclophosphamide, vincristine, and prednisone).

Bone marrow biopsy specimens were reviewed independently by two observers, without any information concerning the clinical characteristics of the patients.

On the basis of previous experience, four different patterns (Figs 1 through 5) were recognized according to the following criteria:

1. Interstitial (Fig 2). Some degree of replacement of normal hemopoietic tissue by mature lymphocytes is observed, but with preservation of fat cells and bone marrow structure.

2. Nodular (Fig 3). Nodules made up of mature lymphocytes appear. These nodules are greater than normal lymphoid follicles and lack clear centers. There is no interstitial infiltration. Fat cells are preserved.

3. Mixed (Fig 4). A combination of interstitial and nodular patterns.

4. Diffuse (Fig 5). Diffuse lymphoid infiltration with massive replacement of normal hemopoietic tissue as well as fat cells.

For the present analysis, patterns 1, 2, and 3 were pooled as a nondiffuse group. So, in this analysis only two patterns were recognized: nondiffuse (201 patients) and diffuse (128 patients), respectively. The proportion of the diffuse pattern in whole series was 39%, in initial biopsies 37%, and in delayed biopsies 43%. As far as the correlation between BM patterns and the clinical stage according to the proposal of an International Workshop on CLL is concerned, the numbers of patients with nondiffuse diffuse bone pattern in different stages were as follows: stage A 126/16, stage B 55/38, stage C 20/74. In other words, the proportion of the diffuse pattern in stage A was 11.3%, in stage B 40.9%, and in stage C 78.7%; that is, there was some, but far from absolute, correlation of BM patterns with clinical stages.

Statistical Methods

Actuarial survival probability curves were plotted according to the method of Kaplan and Meier. Different curves were statistically compared by using the log-rank test. Chi square test for trend was computed as recommended by Peto et al.

A multiple regression model for censored survival data, developed by Cox, was employed in order to identify the most significant prognostic factors. A stepwise forward selection procedure was used that inserts variables in turn until the regression is satisfactory. The order of insertion is determined by using the maximum log likelihood value as a measure of the importance of variables not yet in the regression equation. At each step, the significance level is computed. As recommended by Kalbfleish and Prentice, ties in uncensored and censored observation times were avoided as far as possible by expressing such times in days instead of in months of observation.

In addition to the bone marrow biopsy pattern, we included those variables that are being used in the present clinical staging systems or have been suggested as useful in order to improve such systems. Thus, in addition to bone marrow pattern, lymphadenopathy, splenomegaly, hepatomegaly, anemia, and thrombocytopenia, we included age and lymphocytosis, which have proved to be useful in our previous work. The model was tested twice in each series of patients (whole series, initial biopsies, and delayed biopsies) (Table 1) by using a series of binary variables (model A), and by expressing
the variables, whenever possible, in a continuous way (model B). In
order to minimize the influence of extreme values, the logarithm of
some numerical values was used in the latter system. The cut-off
level of the parameters used in model A was chosen according to the
criteria used in the International Staging System (hemoglobin,
platelets) or the results of our previous work. In the case of
lymphocytosis, the cut-off levels of 50,000/μL and 40,000/μL have
been suggested as useful.10,11 In this series, both cut-off levels yielded
significant results, but the level of 30,000/μL was still better in
discriminating power. As the chief aim of the present work was to
ascertain whether the bone marrow pattern was better than the
remaining variables, the most discriminating cut-off level of lympho-
cytosis (30,000/μL) was employed.

RESULTS

The survival probability according to A, B, and C
groups is shown in Fig 6. As can be seen, there are
clear-cut differences in the life expectancy for these
groups of patients. However, the survival of group B is
quite similar to that of the whole series of patients. The
survival probability according to the bone marrow
histologic pattern is presented in Fig 7. There is a
striking difference between nondiffuse and diffuse
pattern (P < .001). The same statistical difference
(P < .001) was found when the survival probability
according to the BM pattern was studied separately in
both initial and delayed biopsy groups.

The results of the multivariate regression analysis
are presented in Tables 2 through 4. In all instances, by
using binary (model A) or continuous variables (model

Fig 3. Nodular pattern. Numerous lymphoid nodules can be
seen in marrow spaces (H & E; magnification 69×).

Fig 4. Mixed pattern. In addition to a nodular
formation, interstitial infiltration is present (H &
E; magnification 158×).

Fig 5. Diffuse pattern. There is a heavy lymphocytic
infiltration of all bone marrow spaces, with diffuse replace-
ment of fat cells (H & E; magnification 158×).
B), the bone marrow pattern was the first parameter to enter the regression model at a highly significant level.

In order to investigate whether the bone marrow pattern could be a useful variable for isolating subsets of patients within the established clinical stages, we analyzed the international stage B, whose survival probability is similar to that of the whole series. The survival probability of these patients was estimated according to the bone marrow pattern, that is to say, diffuse vs nondiffuse (Fig 8). The survival probability was much better in the stage B nondiffuse bone marrow pattern than in the stage B diffuse pattern \((P = .01)\). When the same analysis was performed in stage A and stage C patients, the difference did not reach statistical significance, but the same trend could be detected. Indeed, the observed/expected ratios (O/E) were as follows: stage A, nondiffuse O/E 0.90, diffuse O/E 1.80; stage C nondiffuse O/E 0.69, diffuse O/E 1.11.

In order to further explore the extent of independence of BM patterns from clinical staging, a converse analysis was performed, which examined the value of clinical staging while controlling for marrow pathology pattern. Thus, A, B, and C survival curves were analyzed separately in the nondiffuse and diffuse BM pattern group. The results were statistically significant—Nondiffuse: (A) O/E 0.57, (B) O/E 1.18, (C) O/E 3.64; \(\chi^2\) for heterogeneity 25.48 \((P < .001)\); \(\chi^2\) for trend 21.23 \((P < .001)\); diffuse: (A) O/E 0.29, (B) O/E 0.68, (C) O/E 1.46; \(\chi^2\) for heterogeneity 15.00 \((P < .001)\); \(\chi^2\) for trend 14.44 \((P < .001)\).

In order to investigate the efficacy of a combined clinicopathologic staging, we designed a provisional proposal based on the International Clinical Staging System \((A, B, C)\) and BM pattern \((\text{nondiffuse, diffuse})\), as follows: stage 1, A—nondiffuse; stage 2, A—diffuse + B—nondiffuse; stage 3, B—diffuse + C—nondiffuse; stage 4, C—diffuse. The corresponding survival curves are presented in Fig 9 and the log-rank analysis in Table 5. As can be seen, there is striking statistical significance, and the O/E ratios are either lower or higher than 1. In other words, all the stages designed in this way discriminate in respect to the whole population.

**DISCUSSION**

The histologic criteria for separating different patterns of bone marrow involvement in CLL have been demonstrated in our previous work.18 There are no conclusive data in the literature concerning the homogeneity of such patterns throughout the body. In a "geographic" bone marrow study, Block et al32 showed that the amount of lymphoid infiltration is progressively decreasing when estimated in biopsies taken from three different sites—posterior iliac crest, greater
trochanter, and proximal tibia—but this relationship is always constant. Moreover, in our experience based on the analysis of large bone marrow cores, the pattern of involvement tends to be homogeneous throughout the sample. On the other hand, studying sequential bone marrow biopsies, we have demonstrated that patients in stable clinical stages usually show an unchanged pattern of involvement.33

The prognostic significance of bone marrow histology in CLL has been the subject of several reports. In a study of 115 patients, including cases of lymphosarcoma cell leukemia, Gray et al32 showed that cases with diffuse bone marrow infiltration had a poor prognosis as compared to cases with nodular or mixed (nodular and diffuse) patterns. Although the bone marrow patterns in CLL have usually been described just as nodular (or focal) and diffuse, some authors have distinguished other types of bone marrow involvement in CLL. Thus, Rywlin34 recognized (1) an interstitial pattern in which the marrow architecture is preserved and lymphocytic infiltrates are found among the hemopoietic cells in-between the fat spaces; (2) a nodular pattern in which there are nodular lymphocytic infiltrates replacing the hemopoietic cell areas as well as fat cells, and (3) a diffuse pattern in which there is a total effacement of marrow architecture and substitution by lymphocytes of normal hemopoietic and fat cells. In addition, according to Rywlin,34 there is a mixed pattern formed by the combination of nodular and diffuse infiltration types. Although in some cases of otherwise diffuse bone marrow involvement, nodular or confluent structures can be seen among the lymphocytic infiltrations, this particular type of mixed pattern (nodular plus diffuse) was classified by us as diffuse, as in these cases, a great tumoral load is usually present. It is important to note that the mixed type described by Rywlin34 and also recognized by Gray et al32 is not the same mixed type that we use in our study (nodular plus interstitial).

Recently, Bartl et al,35 assessing the bone marrow structure in patients with CLL, have described two major types of bone marrow involvement: (1) diffuse (nonnodular patterns) that could be subclassified in interstitial and packed marrow types, and (2) nodular patterns. In this work a certain degree of correlation has also been found between the bone marrow pattern and the clinical stages. It is not clear from the description of these authors whether the interstitial type described by them is the same interstitial type recognized by Rywlin34 and also measured by Gray et al32.
The prognostic significance of bone marrow biopsy has been clearly demonstrated in the previous work of some of us.18 Thus, patients with interstitial and nodular bone marrow involvement had a longer survival than those with mixed and diffuse ones. Because the overall follow-up of the present series is still rather short, no definitive conclusions can be drawn in respect to the eventual differences in the survival probability among patients with interstitial, nodular, and mixed bone marrow patterns. The different prognostic of patients with nondiffuse patterns in comparison to those with diffuse bone marrow involvement is clear.

A certain degree of correlation between BM patterns and clinical stages has previously been demonstrated, although it is far from being absolute.13,18,19 In the present study, it is also evident that the proportion of patients with the diffuse pattern increases progressively from low-risk to high-risk clinical stages. But again, a certain degree of independence between BM patterns and clinical stages has clearly emerged. So, patients in clinical stage B could be subdivided into two different prognostic subsets on the basis of their BM pattern. Conversely, each of two BM pattern subgroups (diffuse and nondiffuse), could be separated according to the clinical stages A, B, and C.

Though developed on an empirical basis, the Rai staging system1 has represented major progress in the prognostic of CLL, permitting the isolation of a “high-risk” group (stages III and IV), a “low-risk” group (stages 0 and I), and an “intermediate” group (stage II) of patients. However, a critical review demonstrates certain limitations of this system, namely, there are too many prognostic categories to be useful for therapeutic trials, static and progressive forms of the disease are not separated, and a considerable number of patients belong to stage II, showing a survival expectancy equal to the whole population of CLL.

The new staging system derived from a simultaneous multivariate survival analysis by Binet et al (1981)37 represents further progress. In the work performed by these authors, the prognostic value of the number of involved lymphatic areas has been recognized. This staging system is reproducible, and it has been confirmed in large series of patients and endorsed by the International Workshop on CLL.10 The need to isolate different subsets of patients within each prognostic group has been emphasized, as there can be patients among them with a more aggressive course and others with quiescent forms of the disease that probably do not need therapy.

From our present study, it is clearly evident that the histologic bone marrow pattern is an important prognostic factor in CLL. The high statistical significance of BM pattern, both in initial and delayed biopsy groups, suggests the prognostic value of this parameter not only at the diagnosis, but also during the course of the disease. In addition, at least in present series, this parameter is better than any one of the remaining variables employed in current clinical staging systems1,10,37 and their modifications.7,9,30 The use of this parameter clearly permits the isolation of subsets in some groups of patients; for example, in stage B, which usually exhibits a survival similar to the whole series of patients. Taking into account the great prognostic value of BM pattern when compared to other single parameters and the partial independence of the BM pattern from clinical stages, we devised an integrated clinicopathologic staging system (Fig 9 and Table 5). In addition to displaying a strong discriminating capacity, this system appears to have the advantage of being able to discriminate all stages with respect to the whole population. In our opinion, it would be interesting to have the value of this system tested by other groups and by the International Workshop on CLL.

ACKNOWLEDGMENT

We express our gratitude to the Department of Biomathematics of the University of Texas Cancer Center for kindly supplying us with the computer program of Cox's multiple regression analysis. We are also grateful to Begoña Ramirez for her secretarial work.
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Bone marrow histologic pattern--the best single prognostic parameter in chronic lymphocytic leukemia: a multivariate survival analysis of 329 cases

C Rozman, EMontserrat, JM Rodriguez-Fernandez, R Ayats, T Vallespi, R Parody, A Rios, D Prados, M Morey and F Gomis