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PROGNOSTIC VALUE OF BONE MARROW HISTOLOGY IN CHRONIC LYMPHOCYTIC LEUKEMIA. A STUDY OF 335 UNTREATED CASES FROM A SINGLE INSTITUTION

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ABSTRACT

Background and Methods. **A significant correlation between bone marrow (BM) histology, survival and disease progression (DP) probability has been observed by several authors in chronic lymphocytic leukemia (CLL). The prognostic value of BM histologic patterns on survival and disease progression probability was investigated in 335 B-CLL patients.**

Results. **Actuarial survival probability estimated by univariate analysis proved to be significantly influenced by stage ($p<0.0001$), BM histology ($p=0.01$), and by the following parameters: anemia ($p=0.0005$), splenomegaly ($p<0.001$), CLL-related symptoms ($p<0.01$), thrombocytopenia ($p<0.01$), number of involved nodal areas ($p=0.01$) and peripheral lymphocyte count over 30×10^9/L ($p<0.05$). In this series we did not detect a discriminating prognostic effect for BM histology within the individual stages. Cox multivariate regression analysis failed to demonstrate a significant value for BM histology, while stage and anemia emerged as the best prognostic variables. Actuarial DP free probability in 294 untreated patients with A and B stages was significantly related to stage ($p<0.00001$) and to BM pattern ($p=0.01$).**

Conclusions. **Despite the clear correlation between the D pattern of BM involvement and advanced and early progressive disease, we were unable to demonstrate an independent prognostic value for BM histology. These findings suggest that stage emerged as the best predictive factor of survival probability in our B-CLL patients.**

Key words: CLL, bone marrow histology, prognosis

Current staging systems for chronic lymphocytic leukemia (CLL) that include a certain number of clinical variables have been widely accepted as defining life expectancy and optimal therapeutic management of the disease.[1-3]

The prognostic importance of stage has recently been confirmed in a large series of patients by the MRC CLL trial,[4] as well as by many others in the past.

Nevertheless, it is still unclear why within the low risk stages some patients develop unpredictably more aggressive disease while others show an indolent course. This observation lead to investigation of the prognostic value of other clinical factors not included in the well-known staging criteria.

In the last decade, the histologic bone marrow (BM) pattern of infiltration in CLL and its prognostic significance have been the subject of several studies, and it is generally agreed that patients with a diffuse pattern of BM infiltration are characterized by advanced stage and poor prognosis.[5-12]

Recently, the Spanish Cooperative Group for CLL demonstrated that the BM histologic pattern has emerged as the best independent prognostic parameter;[13] moreover, Pangalis et al.

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have shown that BM histology may be of predictive value in disease progression.[14,15]

The aim of our study was to evaluate the prognostic role of BM histologic pattern on survival and disease progression probability in a large series of B-CLL patients observed at our Department.

Patients and Methods

Patients

Three hundred and thirty-five untreated patients (219 males and 116 females) with a diagnosis of CLL were included in this retrospective study. All patients were observed at our Department; the median follow-up was 34 months and median age was 60 years (range 31-81 yrs).

Criteria for B-CLL diagnosis are reported elsewhere.[16] Briefly, the minimum required for B-CLL diagnosis included sustained peripheral blood lymphocytosis ($\geq 5\times10^9$/L) characterized by morphologically mature small lymphocytes (prolymphocytes $\leq$ 10%) with B immunophenotype, CD5 antigen expression and low clonal expression of Sm Ig. All patients were classified according to the three different staging systems proposed by Rai,[1] Binet[3] and Mandelli.[17] Continuous chlorambucil (CHL) and prednisone (PDN) were administered as first line therapy to stage C patients; stage A and B patients were not treated unless their disease progressed. Disease progression was defined as a stage progression or observation of one or more of the following clinical signs: significant and progressive peripheral lymphocytosis with lymphocyte doubling time less than 12 months, increasing nodal size, severe splenomegaly and/or hepatomegaly. For the purpose of this analysis, DP was evaluated at the start of therapy.

Bone marrow biopsy analysis

BM biopsy was performed in all patients at the time of diagnosis. BM biopsy specimens were reviewed by two pathologists and classified according to the four different patterns described by the Spanish group: nodular, interstitial, mixed and diffuse.[7,9] In the present study only two histologic patterns were considered: diffuse (D) and non diffuse (ND); the nodular, the interstitial and the mixed patterns were included in ND pattern.

Statistical methods

Actuarial survival probability curves were calculated according to the method of Kaplan and Meier.[18]

Different curves were statistically compared by using the log-rank test.[19] In order to evaluate the relative significance of prognostic factors, the multiple regression model of Cox was applied.[20] The model was tested by using a series of binary variables. The same cut-off levels for parameters employed by Rozman[13] were applied in this study.

The sets of variables analyzed were: sex (male vs. female); age ($\leq$ 60 years vs $>$ 60 years); one or more CLL-related systemic symptoms[21] (present vs absent); number of involved lymph node areas ($<$ 3 vs $\geq$ 3); hemoglobin value ($\geq$ 10 g/dL vs $<$ 10 g/dL); platelet count ($\geq 100\times10^9$/L vs $< 100\times10^9$/L); peripheral lymphocyte count ($\leq 30\times10^9$/L vs $> 30\times10^9$/L); splenomegaly (present vs absent); hepatomegaly (present vs absent); bone marrow histology (ND vs D). In addition to these variables, stage according to the Binet (A vs B vs C) and the Rai classifications (0+I vs II vs III+IV) was tested.

Results

Distribution of patients according to BM histology

The ND pattern of BM involvement was observed in 251 patients (75%) and the D pattern in 84 (25%). A comparison between the initial clinical variables and the two types of BM pattern showed that patients with D histology had a significantly higher incidence of the following parameters: three or more nodal areas involved ($p<0.05$); splenomegaly ($p=0.005$); thrombocytopenia ($p<0.05$) and peripheral blood lymphocytosis $>30\times10^9$/L ($p<0.01$)(Table 1). When the distribution of the two types of BM histology was analyzed according to stages, a significantly higher proportion of patients

Table 1. Initial clinical parameters according to BM histology.

Clinical parameters	*BM pattern*		*p value*
	non diffuse %	*diffuse %*	
Sex M	66	63	NS
Sex F	34	37	
Age > 60 years	47	56	NS
CLL-related symptoms	6	12	NS
No. of involved nodal areas > 3	8	21	<0.01
Splenomegaly	16	32	<0.01
Hepatomegaly	14	13	NS
Anemia (Hb < 10 g/dL)	1	4	NS
Thrombocytopenia (Plts < 100×10^9/L)	4	11	<0.05
PB lymphocytosis (Lymph > 30×10^9/L)	21	37	<0.01

Table 2. Distribution of patients according to clinical stage and BM histology.

Clinical stage (no. of patients)	*BM pattern*		*p value*
	non diffuse %	*diffuse %*	
RAI classification			
0+I (139)	47	24	
II (161)	45	57	< 0.0001
III+IV (35)	8	19	
BINET classification			
A (257)	82	62	
B (50)	12	24	< 0.0001
C (28)	6	14	
MANDELLI classification			
I (183)	61	34	
II (116)	32	43	< 0.0001
III + IV (36)	7	23	

with the D pattern appeared to be allocated among the advanced stages in all three of the clinical staging systems considered (Table 2).

Survival analysis

Thirty-eight patients (11%) died; the main causes of death were directly related to CLL progression (31 pts). Other causes of death were cancer in three patients (bladder, lung and melanoma), myelomonocytic leukemia in one patient, hepatic failure in two and cardiovascular complications in another.

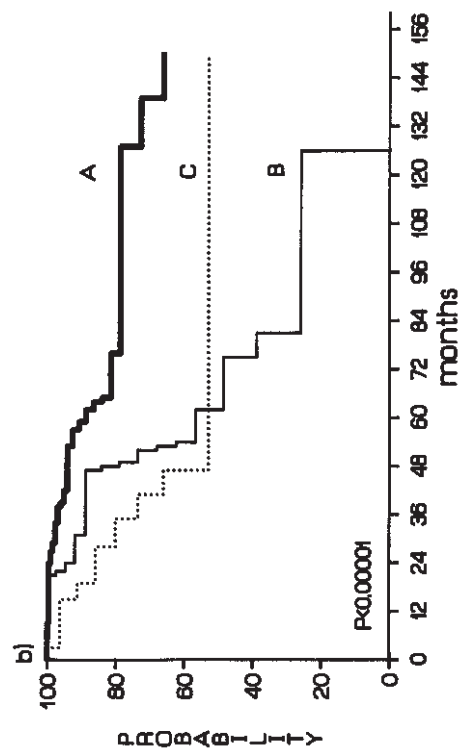
In the univariate analysis the survival probability was significantly influenced by the following clinical features: disease related symptoms ($p<0.01$), number of enlarged lymph node areas ($p=0.01$), splenomegaly ($p<0.001$), anemia ($p=0.0005$), thrombocytopenia ($p<0.01$) and absolute lymphocyte count ($p<0.05$). In this series, sex, age, hepatomegaly did not show any clear prognostic effect.

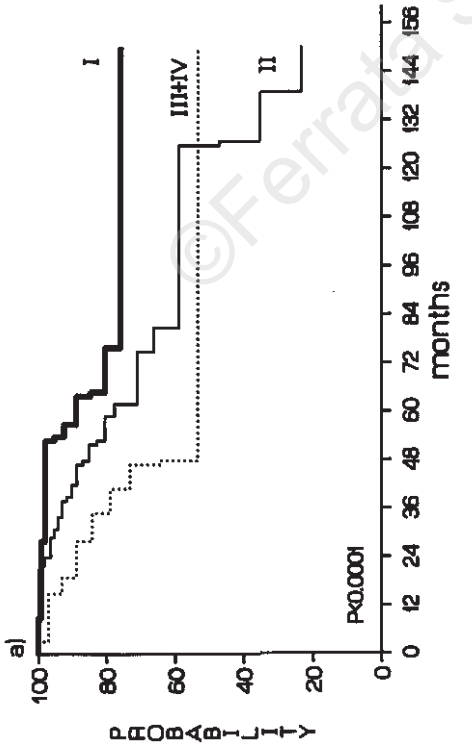
The prognostic importance of stage on survival probability was confirmed in every clinical staging system tested: Rai ($p<0.0001$); Binet ($p<0.00001$) and Mandelli ($p<0.00001$) (Figure 1). The survival probability of the whole series was significantly influenced by the type of BM histology ($p=0.01$) (Figure 2), but no significant differences in survival rates could be observed when the same analysis was performed within the individual stages, including B stage. Cox multivariate regression analysis was performed in order to define the relative statistical significance of the prognostic factors.

Three groups of variables were tested separately. In the first group BM histology and other clinical factors which proved to have a prognostic significance were included. In addition to the variables considered in the first group, stage according to the Rai and Binet classifications was included, respectively, in the second and third groups. BM histology lost its prognostic significance in all series of variables tested by multivariate analysis, while stage and hemoglobin value emerged as the most significant prognostic parameters (Table 3).

Progressive disease

Disease progression (DP) requiring therapy occurred in 139/294 (47%) evaluable, untreated





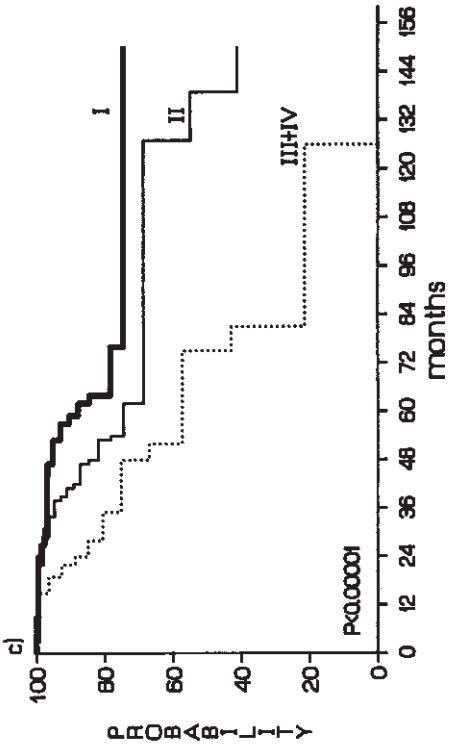


Figure 1. Actuarial survival probability by stage according to the Rai (a), Binet (b), and Mandelli (c) staging systems.

Table 3. Significance of prognostic variables: Cox multivariate regression analysis.

Prognostic variables	(*) p value	Model 1 p value	Model 2 p value	Model 3 p value
Stage RAI classification	(< 0.0001)	n.t.	0.006	n.t.
Stage BINET classification	(< 0.00001)	n.t.	n.t.	< 0.00001
Hemoglobin	(0.0005)	0.006	0.006	0.06
Lymphadenopathy	(0.01)	0.04	0.02	0.32
Platelets	(< 0.01)	0.09	0.21	0.19
Splenomegaly	(<0.001)	0.12	0.36	0.15
Lymphocytes	(< 0.05)	0.79	0.29	0.71
BM pattern	(0.01)	0.36	0.35	0.35
Symptoms	(< 0.01)	0.53	0.2	0.58

(*) = level of significance of the variables: univariate analysis; n.t. = non tested

patients with A (102/248 pts: 41%) and B (37/46 pts: 80%) stage. The overall median time from diagnosis to DP was 41 months and was significantly shorter in B stage patients (A vs B stage: 47 vs 5 months), and in patients with D pattern (ND vs D pattern: 45 vs 20 months). The DP free curve estimated according to stage revealed a significantly higher probability of progression in B stage patients than in the A stage ones (p<0.00001) (Figure 3). When the

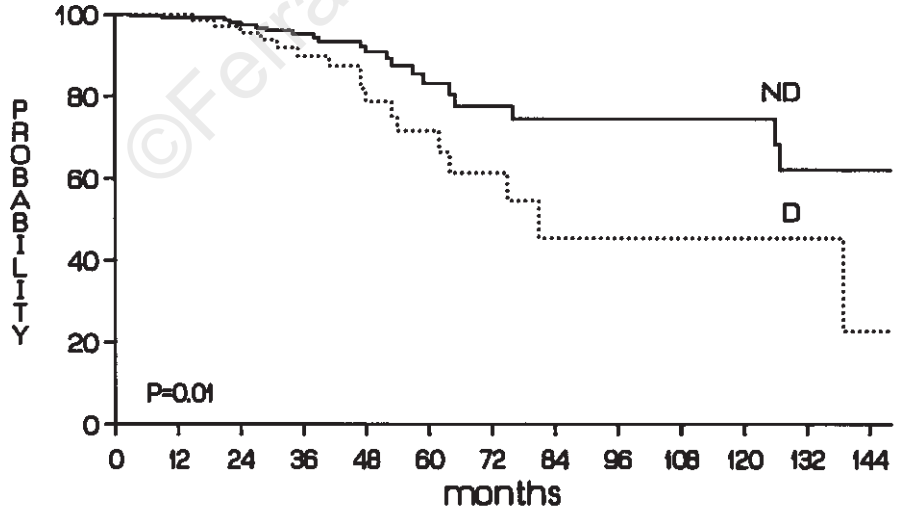


Figure 2. Actuarial survival probability according to BM histology. ND: non diffuse BM histology; D: diffuse BM histology.

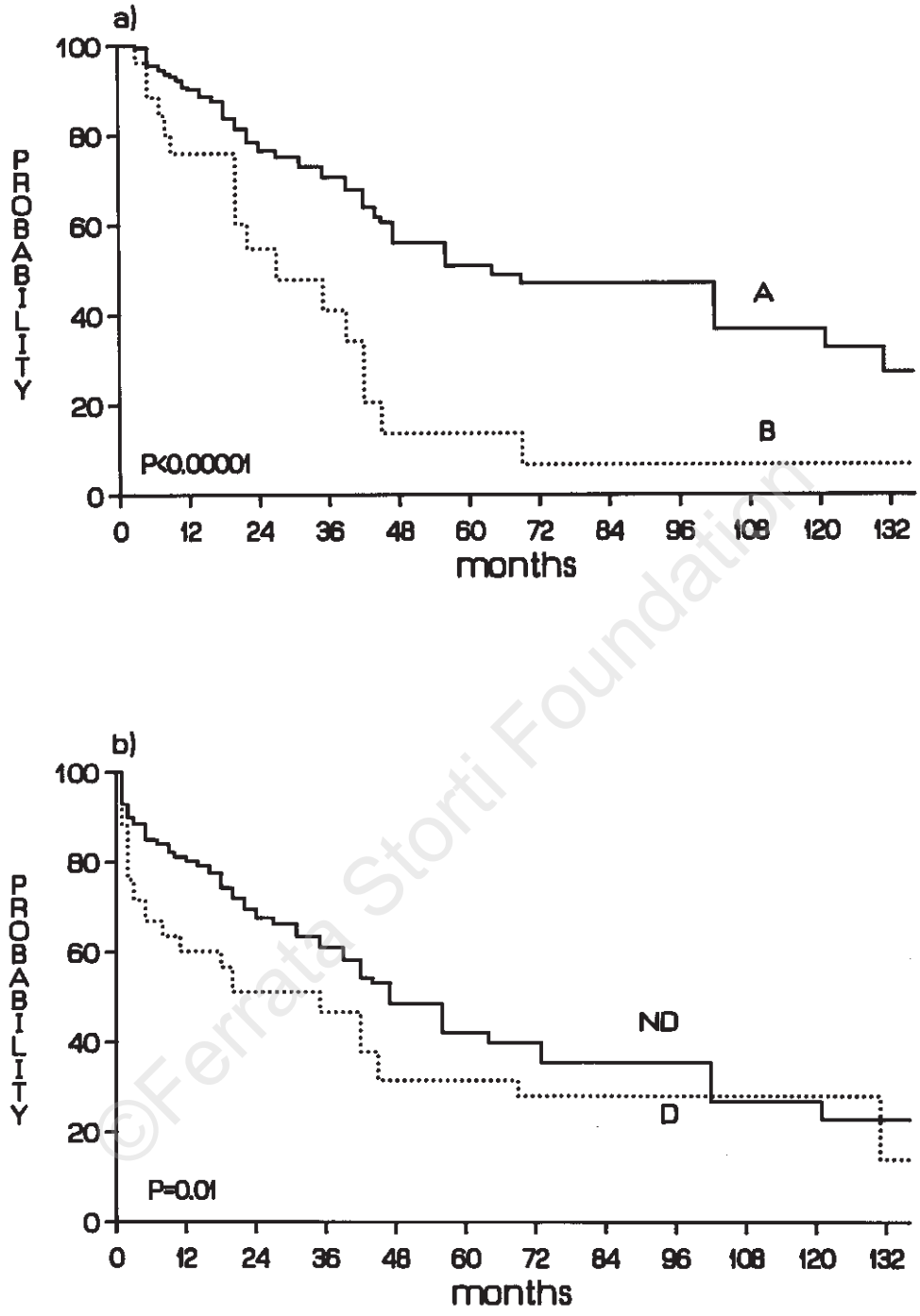


Figure 3. Overall actuarial disease free progression probability according to stage (a) and BM histology (b) in A and B stage patients.

same event was evaluated taking into account BM histology, DP appeared significantly more frequently in patients with the D pattern of involvement (p=0.01) (Figure 3).

Nevertheless, no significant difference in DP probability was found when the influence of BM histology was analyzed within the individual A and B stages. The analysis was then restricted to evaluating the incidence of only delayed DP occurring three or more months after diagnosis. Again, the delayed DP free probability was found to be closely correlated to stage (p=0.0001), while BM histology, in this case, did not show a significant effect (p=0.5).

Discussion

The prognostic value of several clinical and laboratory features was investigated to improve our ability to predict the outcome of CLL patients. Recent findings suggest the prognostic importance of the type of BM involvement at CLL diagnosis: a significant correlation between BM histology, clinical stage, survival, DP probability and incidence of infections has been demonstrated by several authors.[12-15,22,23]

Our results confirm the significant relationship between the D pattern of BM infiltration and unfavorable clinical behavior of the disease. A similar correlation between D histology and advanced stages was observed in all the different clinical staging systems considered.

As expected, the survival probability of patients with a diffuse BM pattern was significantly lower than for those with ND histology.

The Spanish Cooperative Group for CLL has demonstrated that the survival probability of B stage patients with a ND pattern was significantly higher as compared with the survival probability of those with the D pattern.[13] In our series we did not observed the same discriminating prognostic effect for the type of BM histology within any of the individual stages, including B stage. In the Spanish study multivariate analysis demonstrated that the BM histologic pattern was the best single prognostic parameter. In our series, Cox multivariate analysis failed to confirm the independence of the prognostic value of BM histology, while stage and hemoglobin value emerged as the best prognostic parameters.

The discrepancy between our results and those of the Spanish study may probably be attributed to some differences regarding the criteria employed for the diagnosis of CLL, different therapeutic management, and perhaps to a higher proportion of stage A patients in our series.

Moreover, the Spanish study was multicentric, while all the patients included in our study belonged to a single institution; this is another important factor that has probably influenced the different results of the two series.

Pangalis et al. reported that DP in 150 untreated A and B stage patients could be predicted by BM histology but not by stage. In the Greek study patients with D histology showed a higher and earlier incidence of DP and required therapy more frequently than those with a ND pattern. Furthermore, the predictive value of the BM pattern was also of statistical significance when calculated within stages A and B.[15]

Our results confirm the unfavorable effect of the D pattern on overall DP free probability. Moreover, our data suggest that the D pattern seems to exert a negative influence, especially on early progression of CLL. In contrast with the Greek study, DP probability was found to be closely related to stage, and we were unable to detect a predictive effect of BM histology on DP probability within the individual stages. However, it is important to note that different criteria were used by the Greek group and by us to establish DP. In the Greek study DP was defined only by the occurrence of a more advanced stage, while in our series DP comprised an increase in stage or when one or more signs of progressive disease were present. Our findings suggest that B-CLL patients with a D pattern of BM involvement are characterized by more advanced and earlier progressive disease. However, despite the clear correlation between BM histology and degree of CLL malignancy, we observed neither a discriminating prognostic influence for the BM pattern within the individual stages, nor any independent prognostic effect in predicting survival.

Although this study did not demonstrate the

independent prognostic value of BM histology, BM biopsy should always be considered the best method for evaluating CLL at diagnosis, and afterwards for evaluating residual disease or marrow failure.

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