



## CLINICO-PROGNOSTIC EVALUATION OF BONE MARROW INFILTRATION (BIOPSY VERSUS ASPIRATE) IN EARLY CHRONIC LYMPHOCYTIC LEUKEMIA. A SINGLE CENTER STUDY

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### ABSTRACT

**Background and Objective.** Recently published studies dealing with chronic lymphocytic leukemia (CLL) patients in early clinical stage reported that bone marrow (BM) biopsies and aspirates can be considered complementary methods to evaluate the extent of BM involvement. Consequently, we designed the present study to investigate clinico-prognostic implications of BM biopsies and aspirates in a series of stage A CLL patients followed-up in a single center.

**Patients and Methods.** BM biopsy sections and aspirate smears obtained at the time of diagnosis from 102 CLL stage A patients were retrospectively evaluated. Results were correlated with clinico-hematological features as well as with survival and disease-progression risk.

**Results.** Diffuse (D) BM histology was detected in 10 patients (9.8%) while 21 patients (20.5%) displayed lymphocyte infiltration (LI) > 80%. Twenty-six patients (25.4%) died with a 5- and 10-year sur-

vival probability of 85% and 50%, respectively. Survival of patients with D-BM histology was significantly shorter than that of patients with non-diffuse (non-D) histology ( $p < 0.05$ ). Interestingly, when considering only CLL-related deaths (i.e. leukemia-progression, infections) there was an increase in statistical significance of BM histology ( $p = 0.01$ ). There was no difference in life-expectancy in cases with LI both using different cutoff levels (i.e. 70% and 80%) and excluding non-CLL related deaths. According to our experience, disease-progression could only be predicted by BM histology ( $p = 0.008$ ) while LI was not useful to predict progression to more advanced stages ( $p = \text{NS}$ ).

**Interpretation and Conclusions.** In patients with early CLL BM histology provides more reliable information regarding clinical outcome of disease than LI. ©1997, Ferrata Storti Foundation

*Key words:* BM infiltration, biopsy, aspirate, prognosis

The clinical course of chronic lymphocytic leukemia (CLL) patients is highly variable; overall survival ranging from a median survival > 12 years for early stages to 2 years for advanced stages.<sup>1</sup> Although current clinical staging systems are useful tools for assessing prognosis of CLL patients;<sup>2,3</sup> clinical outcome of patients in different prognostic subgroups is heterogeneous. Within Binet stage A, patients without lymph node enlargement (Rai 0) present a significantly longer survival than those with lymph node and/or spleen or liver involvement (Rai I/II).<sup>4</sup> Furthermore, clinical studies investigating the natural history of Binet stage A, identified additional parameters [namely, bone marrow (BM) histology, lymphocyte doubling time (LDT), peripheral blood (PB) lymphocyte count, hemoglobin value] leading to a better prognostic assessment of patients.<sup>5,6</sup> This is of clinical relevance because such patients should not be treated unless progression is observed.

The prognostic role of BM involvement in stage A CLL has been recently analyzed by different authors who evaluated the impact of either BM histology or aspirate on overall survival and disease-free pro-

gression.<sup>7,9</sup> These studies reveal that both the pattern of BM histology and the degree of lymphocyte infiltration (LI) separate CLL patients into different groups with different clinical outcome, although only LI retained its prognostic value in the multivariate survival analysis.<sup>7,9</sup> We designed a study to investigate clinico-prognostic implications of BM histology and aspirate in a random series of 102 stage A CLL patients observed during a 10-year period in a single center.

### Materials and Methods

#### Patients

The study included 102 patients (70 males and 32 females, mean 65.1 yrs, SD 10.3) with diagnosis of B-cell CLL in our center from 1985 and 1995. Diagnostic criteria of CLL were those generally recommended by *International Workshop on CLL*.<sup>11</sup> In all instances the predominant leukemic population shared B cell markers (CD19 and/or CD20) and CD5 antigen. B cells were monoclonal expressing either  $\kappa$  or  $\lambda$  light chain surface immunoglobulin.<sup>12</sup> From June 1988 cellular immunological studies were carried out in flow cytometry.

Sixty-three (61.7%) patients were in Rai stage 0, 7 (6.8%) in I, 29 (28.4%) in II, 3 (2.9%) in III. LDT was retrospectively evaluated according to previous described methods.<sup>10</sup> Two different proposals for subclassification of stage A patients were applied

to our population. Briefly, *smoldering* CLL was defined on the basis of lymphocyte peripheral blood count  $< 30 \times 10^9/L$ , hemoglobin level  $\geq 13$  g/dL, LDT  $> 12$  months, and a non diffuse pattern of BM involvement.<sup>5</sup> The definition of 'A<sup>71</sup>' disease corresponded to peripheral blood lymphocyte count  $< 30 \times 10^9/L$ , lymphoid areas involved  $< 2$ , hemoglobin level  $\geq 13$  g/dL, and LI  $< 80\%$ .<sup>6</sup>

#### BM aspirate and biopsy

BM aspirates and biopsies were performed in all instances at the time of CLL diagnosis. BM aspirates were obtained from sternum or the postero-superior iliac crest and smears were stained by Giemsa. LI was assessed by counting 200 nucleated BM cells.

BM biopsies, upon informed consent, were taken from the posterior iliac crests. Each BM biopsy was classified on the basis of widely accepted classification proposed by the Spanish Group<sup>13,14</sup> which included 4 histological patterns of BM involvement: nodular, interstitial, mixed (nodular + interstitial) and diffuse. For the purpose of this retrospective study biopsy sections together with aspirate smears were independently assessed by 2 physicians (L.T. and S.M.) without knowledge of patients' identities or disease clinical status and data were subsequently correlated by the authors. Nodular, interstitial and mixed (i.e. interstitial + nodular) subtypes were put together and formed the non-diffuse group.

#### Treatment and follow-up data

All patients were treated according to the conventionally accepted methods. Treatment indications included clear evidence of disease-progression (i.e. from A to C or symptomatic B), a LDT  $< 6$  months, appearance of B-symptoms (fever, night sweats, or weight loss not resulting from other causes) or autoimmune cytopenias. Sixty-five (63.7%) patients did not receive any treatment after periods ranging from 7 to 110 months. An alkylating agent, usually chlorambucil associated with low doses of corticosteroids, was the drug chosen for the remaining 37 patients after periods ranging from 3 to 84 months (median, 23 months). Median duration of follow-up for the whole series was 52 months (range, 6 to 154 months). At the time of the present study 26 patients (25.4%) died. Death could be related to CLL (i.e., leukemia progression, infections) in 20 patients (77%). Six patients died as a consequence of apparently CLL unrelated causes (i.e., non-lymphoid secondary neoplasm, 3; cardiovascular disease, 3).

#### Statistical methods

Progression and survival were the end points considered in this study. Survival curves and curves of disease-progression were plotted according to the method of Kaplan and Meier<sup>15</sup> and compared by using the log-rank test.<sup>16</sup> Survival curves were

plotted with and without inclusion of CLL-unrelated deaths with these latter mainly including vascular complications and non-lymphoid secondary neoplasms. The hazard function analysis was performed as suggested by Simes and Zelen<sup>17</sup> to evaluate how the risk of each event varied over time.

## Results

#### BM histology and LI.

A non-D pattern of BM infiltration was found in 92 (90.1%) patients and a D pattern in 10 (9.8%). As compared to patients with non-D BM histology those with a D pattern had higher absolute lymphocyte counts ( $p = 0.003$ ), shorter LDT ( $p = 0.0001$ ), and a trend toward a lower hemoglobin level ( $p = 0.08$ ) (Table 1).

LI was estimated to be  $64.4 \pm 14.5\%$  (range, 32-94%) and 21 patients (20.5%) displayed a LI  $\geq 80\%$ . Patients with LI  $\geq 80\%$  had lower hemoglobin levels ( $p = 0.04$ ), and higher lymphocyte counts ( $p < 0.05$ ) (Table 1). Another weak correlation was detected ( $r = 0.203$ ;  $p < 0.05$ ) when both LI and number of PB lymphocytes were analyzed as continuous variables.

LI was higher ( $84.2 \pm 7.2\%$ ; range, 72-94%) in patients with D BM histology than in those with a non-D type ( $62.3 \pm 13.5\%$ ;  $p = 0.0001$ ). Although the correlation between the BM infiltration pattern and LI was confirmed also after setting an 80% cutoff for LI ( $p = 0.0001$ ), the conformity between the two methods for assessing BM infiltration was far from absolute. Indeed, 13 out of 92 (14.1%) patients with non-D histology had LI  $\geq 80\%$  while 2 out of 10 (20%) with D BM histology had LI  $< 80\%$ . These discrepancies only marginally affected the conformity between Montserrat's and French Cooperative Group's criteria<sup>5,6</sup> of *indolent* CLL. Only 3 out of 58 (5.1%) patients fulfilling criteria of *smoldering* CLL would have been misclassified on the basis of LI  $\geq 80\%$  while no patient with A<sup>71</sup> substage had D BM histology.

Table 1. Patient characteristics according to the histologic pattern of bone marrow (BM) involvement and degree of lymphocyte infiltration (LI).

	Bone marrow		p-value	Lymphocyte infiltration		p-value
	Non-diffuse (n = 92)	Diffuse (n = 10)		< 80% (n = 81)	> 80% (n = 21)	
Sex (M/F)	66/26	6/4	0.683	58/23	12/9	0.313
Age (yrs), mean $\pm$ SD	64.8 $\pm$ 10.3	70.3 $\pm$ 8.1	0.106	65.4 $\pm$ 10.4	64.5 $\pm$ 11.1	0.728
Hb (g/dL) mean $\pm$ SD	13.7 $\pm$ 1.4	12.9 $\pm$ 1.3	0.087	13.7 $\pm$ 1.4	13 $\pm$ 1.4	0.044
Lymphocytes ( $10^9/L$ )	19.8 $\pm$ 12	32.2 $\pm$ 12.2	0.003	19.9 $\pm$ 12.8	25.9 $\pm$ 10.7	0.051
PLT ( $10^9/L$ )	183.8 $\pm$ 62.7	171 $\pm$ 39.9	0.428	187.7 $\pm$ 63.3	176.3 $\pm$ 54.3	0.454
Rai substages (0 / I - III)	58/34	6/4	0.877	52/29	12/9	0.739
LDT (> 12 mo. / < 12 mo.)	73/5	3/6	0.0001	66/5	12/4	0.09

LDT = Lymphocyte Doubling Time.

### Survival

Twenty-six patients (25.4%) died with a 5- and 10-year survival probability of 85% (95% CI: 74.8-91.6%) and 50% (95% CI: 33.1-66.9%), respectively. More detailed information on the pattern of failure was obtained by the hazard function analysis. The risk of dying increases progressively as a linear function of time ( $r = 0.843$ ;  $p < 0.01$ ). Such a pattern of survival due, at least in part, to an age-related phenomenon reflects an excess of non-CLL related deaths (i.e. cardiovascular complications, epithelial cancers).

Survival of patients with D BM histology was significantly shorter [8-year survival rate 20% (95% CI: 12.7-27.3%)] than that of patients with non-D histology [8-year survival rate 54% (95% CI: 33-75%); chi square = 4.40; d.f. = 1;  $p < 0.05$ ]. Interestingly, the value of BM histology increased in significance after removing 6 non-CLL related deaths from survival analysis (all belonging to the non-D subgroup) (chi square = 5.56; d.f. = 1;  $p = 0.01$ ) (Figure 1A).

No difference in the life-expectancy could be detected using either an 80% (chi square = 0.029; d.f. = 1;  $p = \text{NS}$ ) or a 70% cutoff value (chi square = 0.208; d.f. = 1;  $p = \text{NS}$ ). The same applied when survival analysis was carried out after excluding non-CLL related deaths (chi square = 0.473; d.f. = 1;  $p = \text{NS}$ ) (Figure 1B).

### Disease progression

Thirty patients (29.4%) progressed to a more advanced clinical stage with an actuarial risk to progress of 32% (95% CI: 26-38%) at 5 years and 47% (95% CI: 29.6-64.4%) at 10 years, respectively. Despite a linear trend toward an increasing risk ( $r = 0.99$ ;  $p = 0.001$ ), the hazard function analysis suggests that such an event is not correlated with time ( $r = 0.373$ ;  $p = \text{NS}$ ).

According to our experience, disease-progression was predicted by BM histology. The 8-year actuarial risk to progress to a more advanced clinical stage was 82.5% for patients with a D BM histology and 52.1% for those with a non-D type (chi square = 6.91; d.f. = 1;  $p = 0.008$ ) (Figure 2A). In contrast, LI failed to discriminate patient subgroups having different outcomes with respect to the risk of disease-progression (chi square = 0.207; d.f. = 1;  $p = \text{NS}$ ) (Figure 2B).

### Discussion

Although BM aspiration and biopsy are not required to CLL diagnosis, they can localize a major site of disease.<sup>18</sup> Furthermore, a BM examination is a useful prognostic tool distinguishing between diffuse or non-diffuse involvement.<sup>14,19-23</sup>

Results of the present study which accounts for 102 stage A patients are in keeping with a good correlation between BM histology and LI. This association, however, was far from absolute. Indeed, 13 out of 92 patients (14.1%) with non-diffuse histology had a LI  $\geq 80\%$  while 2 out of 10 (20%) with diffuse histology had LI  $< 80\%$ . Interestingly, discrepancies between different methods of BM evaluation only marginally affected the conformity between Montserrat's<sup>5</sup> and French Cooperative Group's<sup>6</sup> criteria for defining *smoldering* CLL. Only 3 out of 58 (5.1%) patients fulfilling criteria of *smoldering* CLL would have been misclassified on the basis of a LI  $\geq 80\%$  while no patient belonging to A<sup>u</sup>1 subtype had a diffuse histology.

Comorbidity-related deaths significantly affect overall survival of CLL patients, and, as would be expected, they are more frequent in stage A and decrease in stage B or C.<sup>24,25</sup> In our series of Binet stage A patients, the hazard survival analysis curve

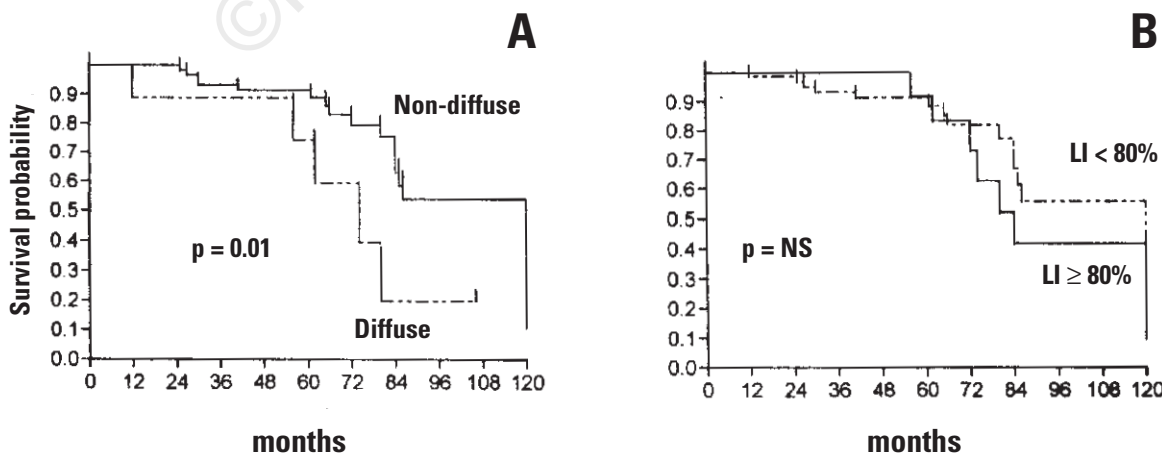


Figure 1. Survival of stage A patients analyzed according to the histopathologic pattern of bone marrow (BM) involvement (A) and the degree of lymphocyte infiltration (LI) in BM aspirate (B).

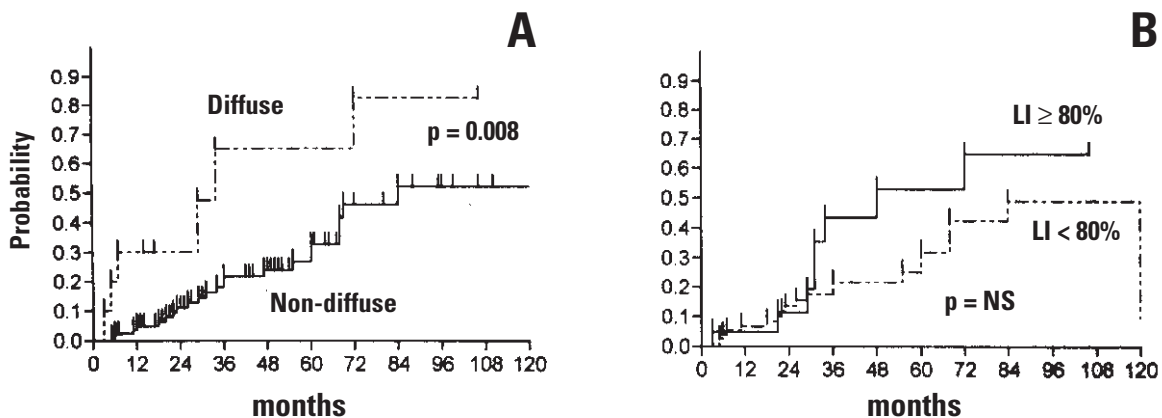


Figure 2. Disease progression risk of stage A patients according to the pattern of BM involvement (A) and the degree of LI in BM aspirate (B).

reveals a pattern of increasing risk which reflects an age-related phenomenon. Such a finding, attributed to an excess of CLL-unrelated deaths, prompted us to carry out survival analyses separately for each type of death (i.e., CLL-related and CLL-unrelated). When the background mortality was removed by considering only CLL related deaths, BM histology increased in significance. A significant prognostic value could not be determined for such a variable even when considering only CLL-related deaths or using different cutoff values of LI (i.e. 70 or 80%).

Results of the present study basically contrast with those of two recently published reports which examined the prognostic value of BM biopsy in relationship to LI.<sup>7,8</sup> Thus, in stage A aspirates but were useful in predicting survival while BM histology was not.<sup>7,8</sup> Potential reasons for these discrepancies rely on the excess of cases (i.e., about 90%) displaying a non-D BM histology as well as on the relatively low number of events in our series as in that of Montserrat *et al.*<sup>7</sup> In the prospective multicentric study of Geisler *et al.*<sup>8</sup> the impact of timely therapy given to most patients at the time of disease-progression to stage B or C may explain the lack of prognostic value of BM histology. Thus, it seems that an effective and prolonged therapy may reduce the prognostic value of well-established clinical parameters.<sup>8</sup> This is not the cases of our series of stage A patients treated at the time of disease-progression according to common clinical practice in CLL (see *Materials and Methods*).

Possible prediction of disease progression in B-cell CLL is of great importance.<sup>4</sup> In agreement with other studies, we demonstrated that such a risk is significantly higher in patients with diffuse BM histology.<sup>7-9, 26,27</sup> In contrast, LI failed to identify subsets of stage A patients with a different clinical outcome.

In conclusion, our results indicate that BM histology should be used in the prognostic assessment of early CLL patients. In comparison to LI, BM biopsy is a more reliable and reproducible method to assess extent of BM involvement thus adding prognostic information to stage. LI can be variable and percentage of lymphocytes may change as a function of the site of aspiration.

Furthermore, the intra- and inter-observer reproducibility has been shown to be higher for BM biopsy than for LI.<sup>7</sup> Whether these results can be translated into a policy of immediate treatment of stage A patients with D BM and consequently high risk of disease progression is still matter of debate.<sup>28</sup> Therapeutic advantages of treating patients with *active* CLL at the time of diagnosis are questionable,<sup>29-32</sup> in the setting of clinical practise progression to a more advanced clinical stage (from A to B or C) is the widely accepted criterion for starting therapy.<sup>18</sup>

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