Lymphoproliferative Disorders

research paper

haematologica 2002; 87:602-608
http://www.haematologica.ws/2002_06/602.htm

Prognostic significance of lymphocyte morphology in patients with advanced chronic lymphocytic leukemia treated with first line therapy of fludarabine + prednisone

FRANCESCA ROMANA MAURO, MASSIMO GENTILE, FRANCESCA MANCINI, DIANA GIANNARELLI, ANNA GUARINI, MARIA STEFANIA DE PROPRIIS, RAFFAELLA CERRETTI, ROBIN FOA

Dipartimento di Biotecnologie Cellulari ed Ematologia, University "La Sapienza" of Rome, Italy

Correspondence: Francesca Romana Mauro, Dipartimento di Biotecnologie Cellulari ed Ematologia, University "La Sapienza", via Benevento 6, 00161 Rome, Italy. Phone: international +39.06.857951. Fax: international +39.06.44241984. E-mail: mauro@bce.med.uniroma1.it

Background and Objectives. Chronic lymphocytic leukemia (CLL) is characterized by clinical, immunophenotypic and morphologic heterogeneity. The morphologic pattern of CLL lymphocytes at diagnosis has been associated with likelihood of different prognoses, while its prognostic significance at the time of disease progression is uncertain.

Design and Methods. In 69 previously untreated patients with advanced CLL the morphology of peripheral blood (PB) lymphocytes was retrospectively analyzed prior to therapy with fludarabine (FD: 25 mg/m^2 × 5 consecutive days every 4 weeks) and prednisone (P: 40 mg/m^2 × 5 consecutive days every 4 weeks). Two groups of patients were identified: the first one characterized by typical CLL morphology (T) and ≤11% of atypical lymphocytes, and the second one characterized by >11% of atypical lymphocytes (A). The second group was further subdivided into a group characterized by *prolymphocyte* prevalence (Ap) and into a group characterized by *mixed cell* morphology (Amc), with a prevalence of large-sized lymphocytes and/or small, cleaved lymphocytes and/or lymphoplasmocytoid cells with or without shaped nucleus.

Results. Forty-two patients (61%) showed a T morphology and 27 (39%) an A morphology. The latter group included 14 patients with an Ap morphology and 13 with an Amc morphology. Two thirds of patients with A morphology showed an immunophenotypic score of 3-4. No significant differences in the distribution of clinical features prior to therapy were observed within the three morphologic groups (T, Ap, Amc), except for a higher lymphocyte count in the Ap group (p<0.05). The morphologic pattern did not have a significant impact on the response rate or on the duration of response. Patients with A morphology did, nonetheless, have a significantly shorter survival than patients with T morphology (p=0.05). However, in multivariate analysis we failed to demonstrate an independent prognostic effect of the lymphocyte morphology observed prior to therapy, while age (≤55 vs >55 years) and CLL duration (≤12 vs >12 months) emerged as significant and independent prognostic factors of survival probability.

Interpretation and Conclusions. The results of this study indicate that about one third of CLL patients with advanced disease have an atypical morphology and that about two thirds of patients with A morphology also show a low immunophenotypic score. The morphologic pattern at the time of progression does not allow identification of prognostic subgroups of patients with different response rates to first line therapy with FD + P.

Key words: chronic lymphocytic leukemia; morphology; fludarabine; prednisone.

Chronic lymphocytic leukemia (CLL) is characterized by clinical, immunophenotypic and morphologic heterogeneity.[1] In 1989, the morphologic classification proposed by the FAB group defined the different lymphocyte subtypes that can be observed in CLL patients at the time of diagnosis.[2] Two main morphologic patterns, typical and atypical, were described. Various reports have suggested that the morphologic patterns of CLL at the time of presentation are associated with different prognostic likelihoods.[3-10] A correlation between typical or atypical lymphocyte morphology at CLL diagnosis, the immunophenotypic profile and the presence of cytogenetic abnormalities has been reported by some authors.[11-17] On the basis of these clinical and biological findings, it has been suggested that typical and atypical CLL may represent two closely related CLL entities with different characteristics and clinical outcomes.[10] Previous studies have mainly focused on the evaluation of the prognostic role of lymphocyte morphology at the time of CLL presentation, while little information is available on the morphologic pattern observed at the time of disease progression and on its prognostic relevance to the response to purine analog therapy. The purpose of the present study was, therefore, to analyze the distribution of the different morphologic patterns observed at the time of disease progression and to evaluate their prog-

nostic influence on the response to fludarabine (FD) therapy. With this aim in mind, we retrospectively assessed, in 69 CLL patients with progressive disease, the prognostic impact of peripheral blood lymphocyte morphology observed before first line treatment with FD + prednisone (P) on survival and response to therapy.

Design and Methods

Patients

Between 1995 and 1999, 69 consecutive previously untreated CLL patients were diagnosed and treated at our Institute. Their diagnosis were based on the criteria recommended by the National Cancer Institute (NCI)[18] and CLL stage was defined according to the classifications proposed by Rai and Binet.[19,20] All 69 patients showed active CLL requiring therapy and 46 of them had a disease duration ≥6 months. Before the start of therapy a morphologic and immunologic work-up was performed in all cases. Treatment consisted of FD (25 mg/m^2 × 5 consecutive days every 4 weeks) associated with P (40 mg/m^2 × 5 consecutive days every 4 weeks). A median number of 6 courses (range: 2-6 courses) were administered. Response was assessed according to NCI criteria.[18]

Immunophenotypic characterization

The expression of CD5, CD20, CD22, CD23, FMC-7, as well as the intensity of surface immunoglobulins (SmIg), were evaluated. The immunophenotypic scoring system proposed by Matutes *et al.* in 1994 was applied.[21] To avoid the inclusion of non-CLL leukemic B-lymphoproliferative diseases, only cases with a score ≥ 3 were included in the analysis. In the last 21 patients observed after 1997, the immunophenotypic profile was integrated by the evaluation of CD79b expression;[22] the same above mentioned score system was still applied.

Morphologic evaluation

May-Grünwald-Giemsa stained peripheral blood smears were observed by 3 examiners. The following morphologic lymphocyte subtypes were considered: small-sized and large-sized lymphocytes, small cleaved lymphocytes, lymphoplasmacytoid cells and prolymphocytes. A total of 200 lymphoid cells per patient were counted. Patients showing more than 55% atypical lymphocytes (large lymphocytes, small cleaved lymphocytes, lymphoplasmacytoid cells and prolymphocytes) were excluded from the study. Two groups of patients were identified. The first group, defined as having typical (T) morphology, was characterized by the prevalence of small and mature appearing lymphocytes with <11% of atypical lymphocytes. The second group, defined as having atypical (A) morphology, included two subgroups: a group defined as having CLL/PL, or atypical CLL with ≥ 11% of *prolymphocytes* (Ap), and a group defined as having atypical CLL with *mixed cell* morphology (Amc), characterized by the presence of large lymphocytes and/or lymphoplasmacytoid cells with or without shaped nucleus and/or small cleaved cells and less than 10% of prolymphocytes[14] (Figure 1).

The Amc subgroup has been previously defined on the basis of the presence of >15% atypical cells.[14] For the purpose of this study, to uniform the rate of atypical lymphocytes in the two subgroups with A morphology, the Amc subgroup was taken to have a proportion of atypical cells ≥11%. Only cells carrying a single and evident vesicular nucleolus, low nuclear/cytoplasmic ratio, clear and abundant cytoplasm were defined as prolymphocytes according to Melo *et al.*[23] Large lymphocytes were similar to typical CLL lymphocytes but were greater in size (>2 red blood cells), had a lower nuclear/cytoplasmic ratio and inconspicuous or small nucleolus.[2] Lymphoplasmacytoid cells showed the features of large cells with an eccentric and frequently shaped nucleus and more abundant basophil cytoplasm.[14]

Small cleaved lymphocytes were characterized by scanty cytoplasm and the presence of a shallow or deep narrow nuclear cleft.[2]

Statistical analysis

The distribution of the three morphologic patterns (T, Ap and Amc) and the response rate to therapy were related to the following parameters recorded at the time of disease progression: age (≤55 vs >55 years), gender (male vs female), time from CLL diagnosis (≤12 vs >12 months), peripheral blood lymphocytes (≤60 vs >60×10^9/L), Hb values in g/dL (≤10 vs >10), platelets count ×10^9/L (≤100 vs >100), lymphocyte doubling time (LDT, ≤12 vs >12 months) and bone marrow histology (diffuse vs non-diffuse). The actuarial survival probability was calculated from the start of FD+P therapy. The time to progression probability was calculated from the time of response to therapy. Survival probability and time to progression probability were analyzed according to the different morphologic patterns (T vs A and T vs Ap vs Amc) and the above-listed parameters. The corrected χ^2 test was applied to compare groups. Survival curves were calculated according to Kaplan and Meier,[24] and compared with the log-rank test.[25]

Results

Morphologic classification and clinical characteristics of the patients

Forty-two patients (61%) showed a T pattern, while 27 (39%) had an A pattern with an Ap profile in 13 patients and an Amc morphology in 14 (Table 1). The distribution of clinical features was not statistically different within the 3 groups, except for a higher lymphocyte count in the Ap group ($p<0.05$) (Table 2). Patients with an Ap pattern showed a median of 23% prolymphocytoid cells (range: 14-34%). In 11/13 patients of the Ap group, in addition to the prolymphocyte population, a mixture of large-sized lymphocytes, small, cleaved and lymphoplasmacytoid cells ranging between 5 and 10% was also observed. In the Amc group, large-sized lymphocytes, small cleaved and lymphoplasmacytoid cells accounted for a median of 21% (range: 14-50%) of the lymphoid population. In 9/14 patients, in addition to large-sized lymphocytes, small cleaved and lymphoplasmacytoid cells, and a proportion of prolymphocytes between 1 and 7% were also recorded. Thirty-seven of the 46 patients (67%) with an interval time ≥ 6 months from CLL diagnosis, had an evaluable peripheral blood smear at the time of presentation. Twenty-four out of the 30 patients (80%) with a T morphology at presentation maintained the same morphologic T pattern at progression, while in 6 (20%) a typical to atypical shift of the morphologic pattern was recorded with a proportional increase of the same atypical cells observed at presentation. The remaining 7 patients showed an A pattern both at CLL presentation and at progression, with a percent increase of atypical cells. The immunophenotypic scoring system proposed by Matutes *et al.*[21] identified two groups of patients: one including 46 patients (46%) with a score of 5 and another including 23 patients with scores of 3-4 (33%). An immunophenotypic score of 3-4 was present in 14% of patients with T morphology (6 patients) and in 63% of patients with A morphology (17 patients; $p<0.001$) (Table 3).

A T morphology with an immunophenotypic score of 5 was present in 52% of cases (group A); an A morphology with an immunophenotypic score of 3-4 was recorded in 23% of cases (group B), while the combination of a T morphology with an immunophenotypic score of 3-4 or of an A morphology with an immunophenotypic score of 5 was observed in 25% of cases (group C).

Response to therapy and response duration

The overall response rate to first line therapy with FD + P was 87% (60 patients). The response rate according to the morphologic pattern, T vs A and Ap vs Amc, was different, though not significantly (T vs A: 90 vs 77%, $p=0.4$; Ap vs Amc: 77 vs 86%; $p=0.9$). Furthermore, no significant differences in the rate of complete responses (CR) were observed between the three groups of patients (T vs A: 52 vs 55%, $p=0.6$; Ap vs Amc: 47 vs 64%; $p=0.7$).

The actuarial time to progression probability at 45 months for the 60 patients who obtained a

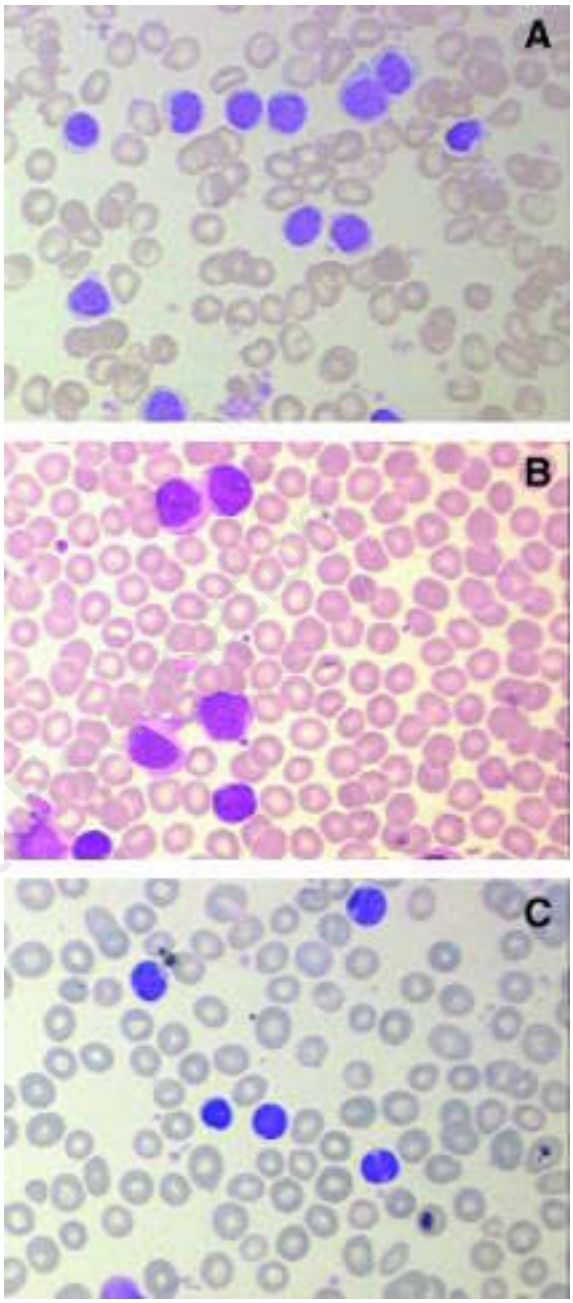

Figure 1. May-Grünwald-Giemsa stained peripheral blood smears: a) small lymphocytes; b) prolymphocytoid cells; cleaved lymphocytes.

Table 1. Distribution of clinical features before FD + P therapy according to typical or atypical morphologic features.

Clinical features	*Typical morphology (pts=42; 61%)*	*Atypical morphology (pts=27; 39%)*	*p*
Sex (%) male/female	66/34	77/23	NS
Age group (%) ≤ 55 vs > 55 years	66/34	52/48	NS
Time from CLL diagnosis (%) > 12 vs ≤ 12 months	66/34	56/44	NS
Binet's stage (%) B / C	88/12	81/19	NS
Rai's stage (%) I+II / III+IV	86/14	81/19	NS
Hemoglobin (%) > 10 vs ≤ 10 g/dL	89/11	89/11	NS
Lymphocytes (%) ≤ 60 vs > 60 $\times 10^9$/L	34/66	48/52	NS
Platelets (%) >100 vs ≤ 100 $\times 10^9$/L	93/7	93/7	NS
Bone marrow histology (%) non-diffuse vs diffuse	45/55	52/48	NS
Lymphocyte doubling time or other signs of active disease (%) > 12 vs ≤ 12 months	40/60	30/70	NS

NS: not significant.

Table 2. Distribution of clinical features before FD + P therapy according to the atypical prolymphocytic or atypical mixed cell morphologic features.

Clinical features	*Atypical prolymphocytic (pts=13)*	*Atypical mixed cell (pts=14)*	*p*
Sex (%) male/female	77/23	78/22	NS
Age group (%) ≤ 55 vs > 55 years	54/46	50/50	NS
Time from CLL diagnosis (%) > 12 vs ≤ 12 months	54/46	57/43	NS
Binet's stage (%) B / C	77/23	86/14	NS
Rai's stage (%) I+II/III+IV	77/23	86/14	NS
Hemoglobin (%) > 10 vs ≤ 10 g/dL	85/15	93/7	NS
Lymphocytes (%) ≤ 60 vs > 60 $\times 10^9$/L	23/77	71/29	p<0.05
Platelets (%) >100 vs ≤ 100 $\times 10^9$/L	92/8	93/7	NS
Bone marrow histology (%) non-diffuse vs diffuse	39/61	55/45	NS
Lymphocyte doubling time or other signs of active disease (%) > 12 vs ≤ 12 months	31/69	29/71	NS

NS: not significant.

response to FD + P therapy was 44%. Patients with T and A morphology before treatment showed a similar response duration probability (actuarial progression probability at 45 months: T vs A: 42 vs 47%; p=0.8). No differences in response rate and in quality of response were observed when the morphologic pattern and the immunophenotypic score were matched as follows: T morphology with immunophenotypic score of 5 (group A), A morphology with immunophenotypic score of 3-4 (group B) and the combination of a T morphology with an immunophenotypic score of 3-4 or of an A morphology with an immunophenotypic score of 5 (group C) (Table 4).

No significant differences in the response rate were observed in patients who maintained a T morphology at the time of presentation and progression (24 patients: 87%), in those who showed a typical to atypical shift from diagnosis to progression (6 patients: 100%) and in patients who maintained an A morphology (7 patients: 71%).

At multivariate analysis, three independent parameters appeared significantly related to response duration: 1) the time interval from CLL diagnosis and the start of FD + P therapy, 2) bone marrow histology and 3) the quality of response to therapy.

Survival probability from the start of therapy

The overall survival probability from the start of therapy was 73% at 45 months. Patients with A morphology had a significantly shorter survival than patients with T morphology (survival probability at 45 months, T vs A: 82 vs 56 %; p< 0.05; Figure 2). No significant differences in survival probability emerged when the three different morphologic groups - T, Ap and Amc - were compared (p=0.09) and when the immunophenotypic pattern and morphology were matched as reported above. In multivariate analysis, lymphocyte morphology lost its predictive significance on survival, while two parameters, age and CLL duration prior to FD + P ther-

Table 3. Patients' distribution by morphologic pattern and immunophenotypic score according to Matutes *et al.*[21]

Morphologic Pattern	*No. of patients*	*Immunophenotypic score 3-4 (%)*	*5 (%)*	*p*
Typical	42	6 (14)	36 (86)	*p*<0.001
Atypical	27	17 (63)	10 (37)	
Total	69	23 (33)	46 (67)	

Table 4. Response to FD + P therapy according to the morphologic pattern and immunophenotypic score.

	Group A (pts=36; 52%)	*Group B (pts=17; 25%)*	*Group C (pts=16; 23%)*	*p*
Overall response	34 (94)	13 (76)	14 (87)	0.1
Complete response	19 (52)	10 (59)	9 (56)	0.4
Partial response	15 (42)	3 (17)	5 (31)	

Group A: typical morphology with an immunophenotypic score of 5;
Group B: atypical morphology with an immunophenotypic score of 3-4;
Group C: typical morphology with an immunophenotypic score of 3-4 and atypical morphology with an immunophenotypic score of 5.

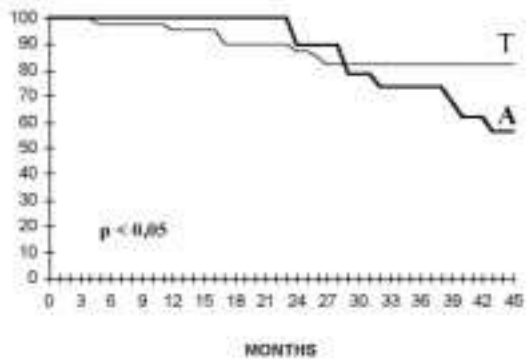

Figure 2. Survival probability at 45 months from therapy by morphology, typical (42 patients) vs atypical (27 patients) morphology: 82 vs 56%; $p < 0.05$ (T: typical morphology; A: atypical morphology).

apy, emerged as significant and independent prognostic factors.

Discussion

Over the last 20 years, the prognostic impact of lymphocyte morphology at CLL presentation has been analyzed with controversial results.[3-10;26-28] While in some studies the morphologic pattern showed no significant prognostic value,[27,28] in most studies an increased rate of atypical lymphocytes at CLL diagnosis was coupled to progressive disease, advanced stage and a shorter survival rate.[6-9;26]

In the present study, focused on the morphologic pattern observed in patients with advanced disease, 39% of patients showed an atypical morphology which was equally distributed between the Ap subtype and the Amc subtype. The rate of cases with atypical morphology was higher than that previously reported in newly diagnosed patients by Matutes *et al.* (13%) and by Criel *et al.* (23%).[6,9,21] The purpose of the present study was to evaluate the morphologic features of patients with an immunologic profile strongly indicative of a diagnosis of CLL avoiding the possible inclusion of leukemic lymphomas. Thus, only patients with an immunophenotypic score ≥3 were retrospectively included in this morphologic re-evaluation. An immunophenotypic score <5 was detected in the majority of cases with A morphology. Despite different immunophenotypic inclusion criteria, this observation confirms the higher rate of cases with a low immunophenotypic score within patients with A morphology previously reported by Matutes *et al.*[21] and by Criel *et al.*[6] In newly diagnosed patients, a higher rate of atypical cell has been related to clinical features associated with a high leukemic burden. In our study, which included only patients with progressed disease, a higher rate of atypical cells did not translate into clinical features correlated with a higher leukemic burden. The only significant difference that emerged was a higher lymphocyte count in patients in the Ap subgroup. The same finding has been previously described by Vallespi *et al.*[7] in patients with a high prolymphocyte percentage at presentation of their CLL.

In the present study, all patients were treated with first line FD + P and the presence of a T or A morphology did not have a significant impact on the response to therapy or on the response duration. It would be of interest to evaluate whether lymphocyte morphology influences response to the less expensive chlorambucil therapy.

At multivariate analysis, three independent parameters proved significantly related to response duration: 1) CLL duration prior to therapy, 2) bone marrow histology and 3) the quality of response to

therapy. As previously observed in newly diagnosed patients,[7,9] in univariate analysis the presence of a high percentage of prolymphocytes and atypical cells correlated with a poor survival of progressed patients. The presence, at diagnosis, of an A morphology has often been associated with biological features associated with an unfavorable effect on survival, such as some cytogenetic aberration.[11-17] Atypical morphology has been related with trisomy 12[8, 13-17, 29] and, in rare cases, with deletion of 13q14[11] and t(11;14)(q13;q32).[12] Deletion of 11q23, has been observed in CLL patients with Richter's transformation[30] and late, during the disease course, in patients with PLL-CLL/PL morphology at diagnosis.[31] The identification of the gene expression profile associated with 11q23 deletion, recently reported by Aalto *et al.*[32] may provide information relevant to understanding the leukemogenesis of CLL. Furthermore, the presence of an unmutated VH Ig gene profile, which characterizes a distinct subtype of poor prognosis CLL arising from naïve B cells, has been associated with the presence of A morphology and with trisomy 12.[33] Taken together, these findings suggest that an A morphology is frequently associated with genetic features related to poor prognosis and that an A morphology may reflect an aberrant genetic pattern.

The unfavorable prognostic effect of an increase in the percentages of prolymphocytes and atypical lymphocytes has been more recently reported from the Medical Research Council (MRC) CLL3 trial in a series of 645 patients observed at diagnosis.[9] However, after stratification by stage, A morphology and an increase in prolymphocyte rate lost their prognostic significance. Only within stage A was the percentage of prolymphocytes still statistically significant and correlated with lymphocyte-doubling time, which was the best dependent prognostic factor for stage A patients. In our study, which included only patients with progressive disease, the morphologic pattern lost significance in multivariate analysis. Taken together, these findings suggest that lymphocyte morphology could be a better predictor of survival for patients showing an early stage of disease at presentation than for those with an advanced stage or a progressed disease.

In conclusion, the results of our study indicate that at the time of CLL progression about one third of previously untreated patients have an A morphology and that about two thirds of patients with A morphology also show a low immunophenotypic score. In our experience, the morphologic pattern at the time of progression does not allow prognostic subgroups with a different response rates to first line FD + P to be identified. Further studies, possibly including genetic analyses, should be attempted to improve the definition of the prognostic value of lymphocyte morphology on response to therapy and survival of CLL patients.

Contributions and Acknowledgments

FRM and RF designed the study, contributed equally to this work and should be considered as the principal authors. FRM, MG and RC were responsible for the care of patients and data collection. FRM, FM and MG were responsible for the morphologic evaluations. AG and MSDP were responsible for the immunophenotypic characterizations. DG was responsible for the statistical analyses. All authors contributed to revising the manuscript. They are listed according to a criterion of decreasing individual contribution to the work, with the exception of the last author who had a major role as senior author in interpreting the data. We would like to thank Maria Grazia Nardacci and Alessandro Lisci for their support in performing all laboratory analyses.

Funding

This work was supported by RomaAIL (Italian Association Against Leukemia-Section of Rome), by Associazione Italiana per la Ricerca sul Cancro (AIRC), Milan, Italy and by Dipartimento di Biotecnologie Cellulari ed Ematologia, University "La Sapienza" of Rome, Italy.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

References

1. Catovsky D, Foa R. The Lymphoid Leukaemias. London, Butterworths, 1990.
2. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposals for the classification of chronic (mature) B and T lymphoid leukaemias. French-American-British (FAB) Cooperative Group. J Clin Pathol 1989; 42:567-84.
3. Melo JV, Catovsky D, Galton DA. The relationship between chronic lymphocytic leukaemia and prolymphocytic leukaemia. I. Clinical and laboratory features of 300 patients and characterization of an intermediate group. Br J Haematol 1986; 63:377-87.
4. Orfao A, Gonzalez M, San Miguel JF, Canizo MC, Galindo P, Caballero MD, et al. Clinical and immunological findings in large B-cell chronic lymphocytic leukemia. Clin Immunol Immunopathol 1988; 46:177-85.
5. Economopoulos T, Fotopoulos S, Hatzioannou J, Gardikas C. "Prolymphocytoid" cells in chronic lymphocytic leukaemia and their prognostic significance. Scand J Haematol 1982; 28:238-42.
6. Criel A, Verhoef G, Vlietinck R, Mecucci C, Billiet J, Michaux L, et al. Further characterization of morphologically defined typical and atypical CLL: a clinical, immunophenotypic, cytogenetic and prognostic study on 390 cases. Br J Haema-

tol 1997; 97:383-91.
7. Vallespi T, Montserrat E, Sanz MA. Chronic lymphocytic leukaemia: prognostic value of lymphocyte morphological subtypes. A multivariate survival analysis in 146 patients. Br J Haematol 1991; 77:478-85.
8. Oscier DG, Matutes E, Copplestone A, Pickering RM, Chapman R, Gillingham R, et al. Atypical lymphocyte morphology: an adverse prognostic factor for disease progression in stage A CLL independent of trisomy 12. Br J Haematol 1997; 98:934-9.
9. Matutes E, Halsey J, Morilla R. Prognostic significance of membrane antigens and morphology in chronic lymphocytic leukemia. Blood 1999; 94: 535a[abstract].
10. Criel A, Michaux L, De Wolf-Peeters C. The concept of typical and atypical chronic lymphocytic leukaemia. Leuk Lymphoma 1999; 33:33-45.
11. Dohner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullinger L, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 2000; 343:1910-6.
12. Cuneo A, Balboni M, Piva N, Rigolin GM, Roberti MG, Mejak C, et al. Atypical chronic lymphocytic leukaemia with (11;14)(q13;q32): karyotype evolution and prolymphocytic transformation. Br J Haematol 1995; 90:409-16.
13. Criel A, Wlodarska I, Meeus P, Stul M, Louwagie A, Van Hoof A, et al. Trisomy 12 is uncommon in typical chronic lymphocytic leukaemias. Br J Haematol 1994; 87:523-8.
14. Matutes E, Oscier D, Garcia-Marco J, Ellis J, Copplestone A, Gillingham R, et al. Trisomy 12 defines a group of CLL with atypical morphology: correlation between cytogenetic, clinical and laboratory features in 544 patients. Br J Haematol 1996; 92:382-8.
15. Knauf WU, Knuutila S, Zeigmeister B, Thiel E. Trisomy 12 in B-cell chronic lymphocytic leukemia: correlation with advanced disease, atypical morphology, high levels of sCD25, and with refractoriness to treatment. Leuk Lymphoma 1995; 19:289-94.
16. Woessner S, Sole F, Perez-Losada A, Florensa L, Vila RM. Trisomy 12 is a rare cytogenetic finding in typical chronic lymphocytic leukaemia. Leuk Res 1996; 20:369-74.
17. Que TH, Marco JG, Ellis J, Matutes E, Babapulle VB, Boyle S, et al. Trisomy 12 in chronic lymphocytic leukemia detected by fluorescence in situ hybridization: analysis by stage, immunophenotype, and morphology. Blood 1993; 82:571-5.
18. Cheson BD, Bennett JM, Rai KR, Grever MR, Kay NE, Schiffer CA, et al. Guidelines for clinical protocols for chronic lymphocytic leukemia: recommendations of the National Cancer Institute-sponsored working group. Am J Hematol 1988; 29:152-63.
19. Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, Pasternak BS. Clinical staging of chronic lymphocytic leukemia. Blood 1975; 46:219-34.
20. Binet JL, Auquier A, Dighiero G, Chastang C, Piguet H, Goasguen J, et al. A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer 1981; 48:198-206.
21. Matutes E, Owusu-Ankomah K, Morilla R, Garcia Marco J, Houlihan A, Que TH, et al. The immunological profile of B-cell disorders and proposal of a scoring system for the diagnosis of CLL. Leukemia 1994; 8:1640-5.
22. Moreau EJ, Matutes E, A'Hern RP, Morilla AM, Morilla RM, Owusu-Ankomah KA, et al. Improvement of the chronic lymphocytic leukemia scoring system with the monoclonal antibody SN8 (CD79b). Am J Clin Pathol 1997; 108:378-82.
23. Melo JV, Catovsky D, Galton DA. The relationship between chronic lymphocytic leukaemia and prolymphocytic leukaemia. II. Patterns of evolution of "prolymphocytoid" transformation. Br J Haematol 1986; 64:77-86.
24. Kaplan EL, Meier P. Non-parametric estimation from incomplete observations. J Am Stat Ass 1958; 53:457-81.
25. Peto R, Pike MC, Armitage P. Design and analysis of randomised clinical trials requiring prolonged observation of each patients. Analyses and examples. Br J Cancer 1977; 35:1-39.
26. Ralfkiaer E, Geisler C, Hansen MM, Hou-Jensen K. Nuclear clefts in chronic lymphocytic leukaemia. A light microscopic and ultrastructural study of a new prognostic parameter. Scand J Haematol 1983; 30:5-12.
27. Ghani AM, Krause JR. Investigation of cell size and nuclear clefts as prognostic parameters in chronic lymphocytic leukemia. Cancer 1986; 58:2233-8.
28. Molica S, Alberti A. Investigation of nuclear clefts as a prognostic parameter in chronic lymphocytic leukemia. Eur J Haematol 1988; 41:62-5.
29. Hernandez JM, Mecucci C, Criel A. Cytogenetic analysis of B cell chronic lymphoid leukemias classified according to morphologic and immunophenotypic (FAB) criteria. Leukemia 1995; 9:2140-6.
30. Zhu Y, Monni O, Franssila K, Elonen E, Vilpo J, Joensuu H, et al. Deletions at 11q23 in different lymphoma subtypes. Haematologica 2000; 85:908-12.
31. Cuneo A, Bigoni R, Rigolin GM, Roberti MG, Bardi A, Cavazzini F, et al. Late appearance of the 11q22.3-23.1 deletion involving the ATM locus in B-cell chronic lymphocytic leukemia and related disorders. Clinico-biological significance. Haematologica 2002; 87:44-51.
32. Aalto Y, El-Rifa W, Vilpo L, Ollila J, Nagy B, Vihinen M, et al. Distinct gene expression profiling in chronic lymphocytic leukemia with 11q23 deletion. Leukemia 2001; 15:1721-8.
33. Hamblin TJ, Orchard JA, Gardiner A, Oscier DG, Davis Z, Stevenson FK. Immunoglobulin V genes and CD38 expression in CLL. Blood 2000; 9:2455-7

Peer Review Outcomes

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Dr. Estella Matutes, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Dr. Matutes and the Editors. Manuscript received December 4, 2001; accepted April 10, 2002.

What is already known on this topic

Atypical morphology in CLL analyzed at diagnosis has been documented to be associated with progressive disease, advanced stages and trisomy 12 in large series of patients.

What this study adds

This study evaluates the influence of morphology analysed at the time of progression in a small group of CLL and shows on univariate analisis a significant shorter survival for the atypical CLL but morphology had no impact on response rate and duration of response to FD+P.

Potential implications for clinical practice

The cell morphology at the time of progression in CLL does not allow prediction of which patients will respond to fludarabine.

Estella Matutes, Associate Editor