

A multicenter retrospective clinical study of CD5/CD10-negative chronic B cell leukemias

Maria Goldaniga,^{1*} Andrea Ferrario,¹ Sergio Cortelazzo,² Andrea Guffanti,³ Enzo Pavone,⁴ Achille Ambrosetti,⁵ Luigi Marcheselli,⁶ Francesca Rossi,¹ Stefano Luminari,⁶ Andrea Rossi,² Lilla Cro,⁷ Massimo Federico,⁶ Giorgio Lambertenghi Delilieri,¹ and Luca Baldini¹

CD5-negative chronic B cell lymphoproliferative disorders in leukemic phase (B-CLPD) are heterogeneous and relatively uncommon pathologies that often lack a histopathological definition because of the absence of accessible pathological tissue. We describe the clinical features and evolution-related variables of 156 patients with CD5/CD10-negative B-CLPD (median age 66 years, range 25–86). The median follow-up was 51 months (range 6–216), and overall 3- and 5-year survival was respectively 87 and 76%; 50 patients needed therapy at diagnosis, 56 during follow-up, and 50 remained untreated until the last control. A combined clinical, histological, cytomorphological, immunophenotypic, and cytogenetic diagnostic approach allowed the complete classification of only a minority of patients as being affected by splenic marginal zone or lymphoplasmacytic lymphoma; the majority of cases remained unclassifiable. Multivariate analysis showed that the clinicohematological variables adversely related to overall survival were serum LDH levels and age, whereas high serum LDH levels, hemoglobin levels of <11 g/dl, and splenomegaly related to treatment-free time (in “wait and see” cases); only splenomegaly related to time to progression (in treated patients). In conclusion, our retrospective study describes the clinical features and variables related to evolution in a large group of patients with CD5/CD10-negative chronic B-cell lymphoid leukemias and underlines the fact that a probable lymphoplasmacytic or marginal zone normal cell origin can be supposed in such leukemic forms, but never surely demonstrated. *Am. J. Hematol.* 83:349–354, 2008. © 2008 Wiley-Liss, Inc.

Introduction

Chronic B cell lymphoproliferative disorders in leukemic phase (B-CLPD) are a frequent and biologically heterogeneous group of neoplastic disorders characterized by different clinical behaviors and therapeutic responses, whose first-line definition is based on cell morphology, immunophenotype findings, and clinical presentation [1]. Histopathological analysis is very helpful for attributing the leukemic picture to a specific clinicopathologic entity according to the REAL/WHO classification [2], but is not always available because of the absence of easily accessible pathological tissue, except for bone marrow whose examination is not always sufficient for a precise diagnostic definition.

Finally, as the development of cytogenetic and molecular studies has allowed the identification of specific markers for only some subsets of these disorders, more specific diagnostic evaluations are left to flow cytometry analysis. Among the different surface antigens, CD5 can be considered a quite reliable marker for classifying B-CLPD into two main groups: CD5-positive forms, which are mainly represented by classical and atypical B-cell chronic lymphocytic leukemias (CLL), mantle cell lymphomas (MCL) in leukemic phase, and rare cases of splenic marginal zone lymphomas (SMZL), and CD5 negative cases, which mainly consist of non-Hodgkin lymphoma (NHL) in leukemic form as recently described by Ugo et al. [3], particularly lymphoplasmacytic lymphoma (LPL) in leukemic phase, SMZL with (>10%) or without villous lymphocytes, and hairy cell leukemia (HCL) and, less frequently, other NHLs such as follicular lymphoma (FL).

HCL has a particular morphological and immunophenotypic presentation, and the leukemic phase of FL and LCL is scarcely frequent, and there are also specific markers (e.g., the expression of CD10 and the presence of t(14;18) in FL), but it is not always possible to define the

diagnosis of the other CD5-negative forms. Consequently, the aim of this study was to improve our knowledge of the clinical features of this latter subset by retrospectively analyzing a broader series of cases referred to various Italian Hematological Institutes. In addition to evaluating their main clinical features, we also tried to identify the variables at diagnosis that may relate to overall and failure free-survival.

Results

Cytomorphology analysis

In terms of morphology, 42/156 (27%) patients had typical CLL or CLL-like disease, 19 (12%) LPL in leukemic phase, 30 (19%) SMZL without villous lymphocytes, and 15 (9.6%) SMZL with villous lymphocytes, 19 (12.1%) CLL/

¹UO Ematologia e Centro Trapianti di Midollo, Ospedale Maggiore, IRCCS, University of Milan, Milan, Italy; ²Divisione di Ematologia, Ospedali Riuniti, Bergamo, Italy; ³Divisione di Medicina I, Ospedale Fatebenefratelli e Ottalmico, Milan, Italy; ⁴Divisione di Ematologia, University of Bari, Italy; ⁵Dipartimento di Medicina Clinica e Sperimentale, University of Verona, Italy; ⁶Dipartimento di Oncologia e Ematologia, University of Modena and Reggio Emilia, Italy; ⁷UO Ematologia 2, Ospedale Maggiore, IRCCS, Milan, Italy

Contract grant sponsor: Italian Ministry of Health.

*Correspondence to: Maria Cecilia Goldaniga, MD, Unità Malattie Linfoproliferative, Unità Operativa Ematologia 1, Centro G. Marcora, Ospedale Maggiore, I.R.C.C.S., Via F. Sforza 35, 20122, Milano, Italy. E-mail: mariacecilia.goldaniga@virgilio.it

Received for publication 8 May 2007; Accepted 1 August 2007

Am. J. Hematol. 83:349–354, 2008.

Published online 10 January 2008 in Wiley InterScience (www.interscience.wiley.com).

DOI: 10.1002/ajh.21065

TABLE I. Clinical and Hematological Characteristics of the Study Population

Variable	N (%)			P value
	Total cases N = 156	"W&W" N = 106	Treatment at diagnosis N = 50	
M/F	85/71 (54/46)	58/48 (55/45)	27/23 (54/46)	>0.50
Median age (years; range)	66 (25–87)	66 (25–84)	68 (45–87)	0.151
B symptoms	20 (12.8)	9 (8.5)	11 (22.0)	0.037
Performance status (ECOG): ≥ 2	23 (14.7)	13 (12.3)	7 (14.0)	>0.5
Splenomegaly	110 (70.5)	63 (59)	47 (94)	0.005
Nodal disease	29 (18.6)	12 (11.3)	17 (34.0)	0.002
N. extranodal sites > 2	6 (3.8)	2 (1.9)	4 (8.0)	0.084
Bulky disease	20 (12.8)	9 (8.5)	11 (22.0)	0.037
Hb < 11 g/dl	37 (23.7)	18 (17.0)	19 (38.0)	0.008
Lymphocytes > $10 \times 10^9/l$	85 (54.5)	50 (47.2)	35 (70.0)	0.010
PLT < $100 \times 10^9/l$	14 (9.0)	7 (6.6)	7 (14.0)	0.144
Serum LDH value upper normal	46 (29.3)	23 (21.4)	23 (46.0)	0.003
ESR > 30 mm/h	53 (33.9)	35 (32.5)	18 (36.8)	>0.50
$\beta 2$ -microglobulin > 2.6 mcg/ml*	53 (67.1)	33 (58.9)	20 (87.0)	0.018
Albumina > 3.5 g/dl	140 (89.8)	101 (95.3)	39 (78.0)	0.003
Bone marrow infiltration pattern*:				
Interstitial	20 (16.8)	18 (23.0)	2 (4.9)	0.017
Nodular	11 (9.2)	4 (5.1)	7 (17.1)	
Mixed	69 (58.0)	43 (55.1)	26 (63.4)	
Diffuse	19 (16.0)	13 (16.7)	6 (14.6)	
Serum monoclonal component	42 (26.9)	28 (26.4)	14 (28.0)	>0.50
HCV antibodies*	6 (5.3)	3 (3.7)	3 (9.7)	0.343

*Not available for assessment: 77 cases for B2M, 37 for infiltration pattern and 43 for HCV antibodies.

chronic prolymphocytic leukemia and 65 (42%) heterogeneous (polymorphic) forms.

Cytogenetic features

Conventional cytogenetic studies were available for 92 of the 156 patients, 44 (47.8%) of whom had a normal karyotype. Deletions (11q) or (13q) were found in 2 patients each. Trisomy 12, +3, and +18 were detected in respective 8, 9, and 11 patients, and were more frequently associated with other cytogenetic abnormalities. The involvement of chromosome 17 was detected in eight cases, and a complex karyotype in 20 cases.

Clinicohematological features

The main clinicohematological features at diagnosis are shown in Table I. The majority of patients had a quite good performance status and 20 (12.8%) had B symptoms, 37 patients (23.7%) were anemic (Hb <11 g/dl), 46 (29.3%) had increased LDH levels, and 53 out of 79 analyzed cases (67.1%) had increased serum $\beta 2$ -microglobulin levels. Serum monoclonal gammopathy was present at diagnosis in 42 patients (26.9%; IgM class in 28/42). Lymph node involvement was absent or minimal (<3 sites and not conspicuous) in 127 patients (81.4%), but 29 (18.6%) had ≥ 3 lymph node sites, mainly with deep involvement; splenomegaly was present in 110 patients (70.5%).

In all of the cases, bone marrow histology showed lymphoproliferative disorders sustained by mature B cell/lymphocytes: the infiltration pattern (described in 119 cases, 76.3%) was interstitial in 20 patients (16.8%), mixed in 69 (58%, with an intrasinusoidal component in nine cases), nodular in 11 (9.2%), and diffuse in 19 (16%). Eight patients underwent tissue biopsy during follow-up, leading to histological diagnoses of marginal zone NHL in four patients (one nodal and three splenic subtypes), small lymphocytic lymphoma in two, and LPL in two.

Diagnostic definition

On the basis of their clinical presentation, peripheral blood cytomorphology and karyotype, bone marrow histopathology, and the presence or absence of serum MC, a possible diagnosis of SMZL or LPL could be postulated in a number of cases. Thirty of the 156 patients (19.2%) could reasonably be considered as having SMZL because they had splenomegaly plus a compatible karyotype or cytomorphology or an intrasinusoidal bone marrow pattern; furthermore, 42/156 (27%) had a monoclonal component, 19 of whom could be considered LPL because of the presence of a serum monoclonal component and clear lymphoplasmacytic leukemic cell differentiation. In the remaining 107 cases (unclassifiable group), no precise diagnostic definition was possible retrospectively.

Survival analysis

As shown in Table I, there were statistically significant differences in a number of clinical variables between the patients who were treated or not at diagnosis. Overall survival (OS) after 3 and 5 years was respectively 87 and 76% (see Fig. 1). Fifty-six of the 106 initially untreated patients required therapy after a median time freedom to treatment (TFTT) of 55 months, and 24/50 initially treated patients showed relapse/progression after a median time to progression (TTP) of 31 months (see Fig. 2). The clinical and hematological features of the whole group at diagnosis were evaluated in terms of their prognostic relevance for OS, TFTT, and TTP by means of univariate and multivariate analyses.

At univariate analysis (Table II), age, high serum LDH levels, hemoglobin levels of <11 g/dl, low serum albumin, and high $\beta 2$ -microglobulin levels, splenomegaly and treatment at diagnosis significantly correlated with OS, and high serum LDH levels, hemoglobin levels of <11 g/dl, splenomegaly, lymphocytosis ($>15 \times 10^9/l$), and performance sta-

tus >1 significantly correlated with TFFT. Only splenomegaly correlated with TTP.

At multivariate analysis (Table III), only serum LDH levels and an age of >60 years retained their prognostic relevance for OS (hazard ratios [HR] 3.00 and 6.82, respectively), but the factor “no treatment at diagnosis” (HR 2.32, $P = 0.008$ at univariate analysis) did not ($P = 0.244$). In relation to TFFT, serum LDH, hemoglobin and splenomegaly (HRs 4.21, 3.66, and 2.27 respectively) retained their prognostic relevance.

We subsequently constructed a simple prognostic scoring system for OS by assigning 1 point to an age of >60 years and 1 point to high LDH levels (class I, low risk, score 0; class II, intermediate risk, score 1; class III, high risk, score 2) (Table IV). Our scoring system identified three subsets of patients characterized by different clinical outcomes (Fig. 3).

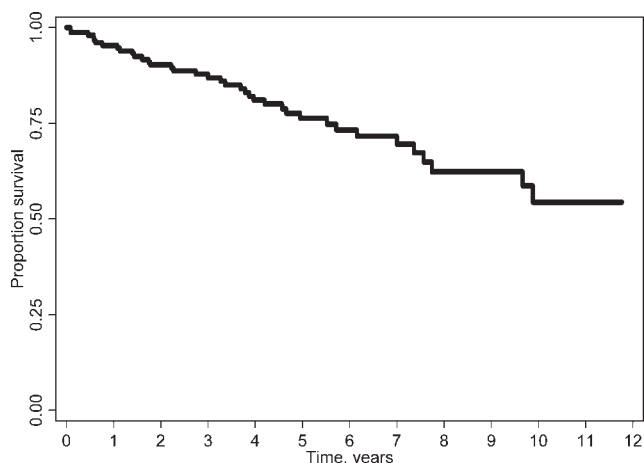


Figure 1. OS of the study population (156 cases).

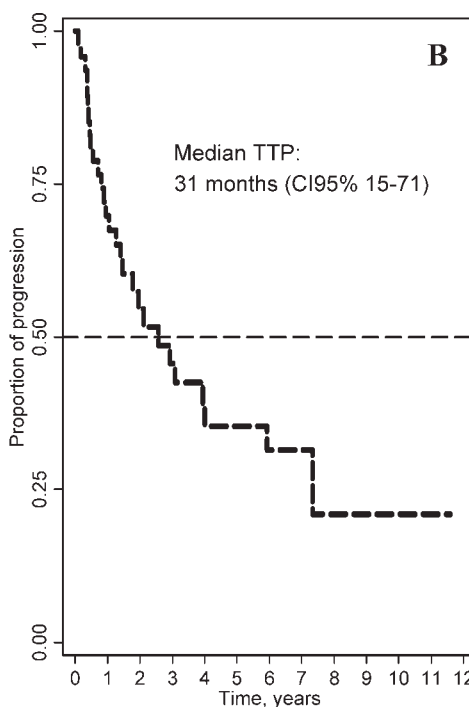
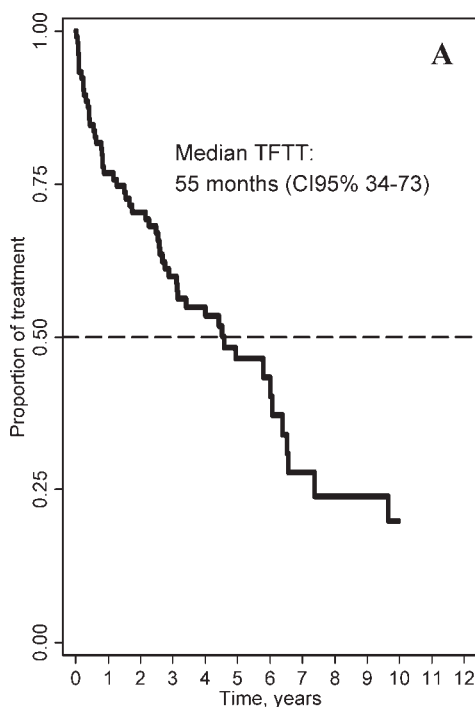


Figure 2. TFFT (A) 106 “W&W” patients and TTP (B) 50 upfront treated patients.

Finally, on the basis of our diagnostic hypotheses, we statistically analyzed dividing our patients into three subgroups: there were no significant differences in terms of clinical presentation, OS, TTP, or TFFT between the MZL (30 patients), LPL (19), and unclassifiable group (107), but there was a slight prevalence of splenomegaly in MZL; at the time of the analysis, median OS in the three groups was respectively 119, 170, and 122 months ($P > 0.50$) (data not shown).

Discussion

B-CLPDs are a heterogeneous group of neoplastic disorders whose appropriate diagnostic definition comes from the concurrence of clinical, cytomorphological, histological, cytofluorimetric, cytogenetic, and molecular findings; however, as a histopathological analysis is not available in many cases because of the absence of a suitable bioptical approach, the diagnosis is often left to a immunocytomorphological evaluation of leukemic cells in addition to bone marrow histology.

A simple and routinely used marker such as CD5 is often capable of separating B-CLPD cases into two main groups on the basis of its functional and anatomic distribution. The CD5-positive group mainly includes classical and atypical B-CLL and MCL in leukemic phase, although rare cases of MZL and LPL have also been reported [3,4]; in this sense, an RMH score of 4–5 or less helps in the correct definition of typical B-CLL versus other pathological situations [5,6]. The group of CD5-negative disorders include heterogeneous entities such as polymorphocytic leukemia, HCL, FL, SMZL, LPL in leukemic phase, and rare cases of LCL [2,5–7]. HCL, FL, and LCL are often easily diagnosed on the basis of their cytomorphology, bone marrow histology, immunophenotype features, and/or specific genetic markers, and in some of the other cases, a definite WHO diagnosis may be made if lymph node or splenic tissue is examined (as they are easily recognized, these lymphoid neoplasias are often included in specific clinical trials) [3,4].

TABLE II. Clinical Parameters Influencing Overall Survival (OS), Time Freedom To Treatment (TFTT) and Time To Progression (TTP) at Univariate Analysis

Variable	P-value		
	OS	TFTT	TTP
No of patients	156	106	50
Age > 60 years	<0.001	>0.50	>0.50
Age std form (mean 66 yrs, SD 10)	0.001	>0.50	>0.50
LDH ratio > normal	<0.001	<0.001	0.285
Haemoglobin < 11 g/dl	0.015	<0.001	>0.50
Serum albumin < 3.5 g/dl	0.021	>0.5	0.400
β 2-microglobulin ratio > normal	0.028	0.127	0.382
Splenomegaly	0.050	0.010	>0.50
Bulky disease	0.073	0.170	>0.50
Lymphocytosis > $15 \times 10^3 \mu\text{l}^{-1}$	0.086	0.039	0.218
B symptoms	0.087	0.061	>0.50
Bone marrow infiltration (%)	0.094	0.190	>0.5
ESR > 30 mm/h	0.463	0.167	>0.50
Sex F	>0.50	0.237	0.182
PS 2-3	>0.50	0.013	>0.5
No treatment at diagnosis	0.008	-	-

TABLE III. Clinical Parameters Influencing Overall Survival (OS) and Time Freedom to Therapy (TFTT) at Multivariate Analysis

Variable	Coeff. (SE)	HR (CI 95%)	P value
OS			
Age > 60 yrs	1.92 (0.60)	6.82 (2.08-22.3)	0.001
LDH ratio > normal	1.10 (0.33)	3.00 (1.56-5.76)	0.001
TFTT			
LDH ratio > normal	1.44 (0.33)	4.21 (2.20-8.07)	<0.001
Hb < 11 g/dl	1.30 (0.37)	3.66 (1.78-7.51)	<0.001
Splenomegaly	0.820 (0.313)	2.27 (1.23-4.20)	0.009

The remaining forms of CD5-negative B-CLPD are characterized by atypical and various morphological features, undistinctive immunophenotype patterns, and the absence of any pathognomonic molecular markers [8-13]. The possibility of performing a lymphoid tissue biopsy has allowed some of these CD5-negative B-CLPDs to be classified within WHO-defined nosological entities, such as variants of FL and nodal or splenic MZL or LPL. In the absence of a histological definition, these forms are frequently excluded from clinical trials, and our knowledge of their clinical characteristics and outcomes is limited. In comparison with B-CLL, it seems that they are characterized by similar survival rates, more frequent splenic involvement, and systemic symptoms; it is less clear whether or not their clinical presentation is more advanced at diagnosis [7,9,10,14-16].

We systematically analyzed a large series of patients in an attempt to contribute towards better defining CD5-negative B-CLPDs, but as described above, a probable diagnosis at clinical presentation was possible in only 49 patients. As underlined by other authors [3], morphological aspect alone was inadequate distinguishing CLL from the other B-CLPDs as 42 patients (27%) had a CLL/CLL-like morphology despite an RMH score of ≤ 3 . The cytogenetic analysis showed a normal karyotype in many patients, and the documented chromosomal abnormalities were not useful for better defining this subgroup, with the exception of some cases in which the presence of +3 or +18 suggested a diagnosis of marginal zone cell lymphoma. Bone

TABLE IV. Prognostic Scoring System for Overall Survival Based on Age > 60 Years and/or LDH Ratio > Normal

Score risk	N (%)	Median (months)	P value
OS			
Low - Score 0	35 (22)	-	
Intermediate - Score 1	89 (50)	91	I vs. L <0.001
High - Score 2	33 (21)	59	H vs. I 0.006
Total	156		

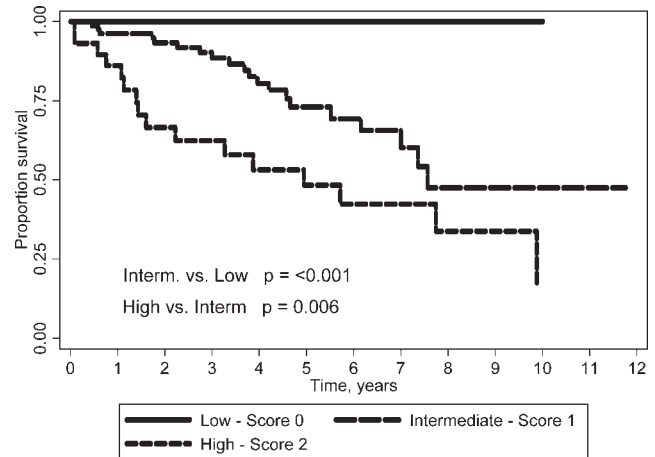


Figure 3. OS stratified by proposed prognostic score (age > 60 year and/or LDH ratio > normal).

marrow histology patterns were available for 119 patients, but only nine had a diagnostic "intrasinusoidal pattern" and only 10 showed lymphoplasmacytic differentiation.

When a diagnosis could be made on the basis of this multiparametric diagnostic approach, it was hypothesized that these leukemic forms had a lymphoplasmacytic or marginal zone normal origin.

As in the case of all low-grade lymphoproliferative disorders, our patients experienced an indolent phase during which no treatment was required: only 50 of the 156 patients started treatment at the time of diagnosis, mainly because of the presence of B symptoms and/or bulky disease and/or anemia; the remaining 106 remained untreated for a median of 33 months.

Multivariate analysis showed that the relationships between OS and LDH levels and age were statistically significant. We compared our cases with mortality due to any cause in the Italian population ("La mortalità per causa in Italia 1980-1994", Istituto Superiore Italiano di Sanità; ICD9-CM <http://www.mortalita.iss.it>), and found that age remained statistically significant, especially in patients aged 70-80 years: older than that, age tended to become less predictive, but this may have been due to the small sample size (data not shown). On the basis of these two simple variables, we could identify three patient categories characterized by a different prognosis.

Finally, serum LDH levels, hemoglobin levels, and splenomegaly could be considered variables related to the need for treatment in a patient affected by CD5/CD10-negative B-CLPD following a "wait and see" regimen.

In conclusion, we believe that CD5/CD10-negative B-CLPDs are a heterogeneous group of conditions in which it is frequently complex to recognize a neoplastic cell origin, especially for institutions that cannot apply advanced immu-

nophenotypical and molecular methods. Like other authors, we believe that a large proportion of these forms represent the leukemic presentation of lymphoproliferative disorders classifiable in the WHO categories of marginal zone or LPLs, although only a minority of cases can be diagnosed certainly at the time of clinical presentation. Our prognostic features may be useful for evaluating the need for treatment and predicting the tendency to treatment failure in individual patients.

Materials and Methods

Patients

The study involved 156 patients with newly diagnosed CD5-negative B-CLPD (male/female ratio = 85/71; median age at diagnosis 66 years, range 25–87), who were referred to 10 Italian Institutions between 1990 and 2003. Five of the institutions belonged to the “Gruppo Italiano Studio Linfomi” (GISL: 66 patients) and four to the “Gruppo Multiregionale” (45 patients); the 10th was the Division of Hematology, Ospedale Maggiore, Bergamo, Italy (45 patients). The data were managed and analyzed in accordance with the ethical rules of the Modena Cancer Registry and the Helsinki Declaration of 1964 (as revised in 2000). All of the cases had light chain restricted, CD19-positive and CD5-negative lymphocytosis ($\geq 5 \times 10^9/l$) diagnosed on the basis of conventional procedures, including the usual laboratory tests (serum LDH, $\beta 2$ microglobulin, HCV-Ab status, liver, and kidney function). All of the patients underwent bone marrow aspiration and a trephine biopsy at diagnosis and whenever considered necessary during follow-up. CD10-positive leukemic follicular lymphomas (FLs), HCL and large B-cell lymphomas in leukemic phase were excluded.

A preliminary database of 172 patients was established, from which 16 cases were excluded because they did not respect the inclusion criteria or their clinical data were incomplete; 24 of the included patients have been described in a previously published study [17]. At the time of analysis, the median follow-up was 51 months (range 6–216), which was not significantly different from that of the patients who were still alive (55 months, range 6–216). Thirty-eight patients had died: 23 (60.5%) because of disease progression, four (10.5%) because of treatment toxicity, and 11 (29%) for other reasons.

Treatment

A “watch and wait” policy was adopted at diagnosis in the case of 106 patients (67.9%), the remaining 50 (32.1%) received chemotherapy. Fifty-six of the patients in the “watch and wait” group started therapy during follow-up (median time to treatment 19 months, range 3–117), giving a total of 106 treated patients.

Although this was a retrospective study, the study centers had a common policy concerning the need for treatment (due their well-established cooperation in clinical trials), which was considered appropriate if there was at least one of the following active disease criteria: systemic symptoms, bulky disease, progressive marrow failure with the development or worsening of anemia (Hb < 2 g/dl below the lower normal limit or < 10 g/dl), and/or thrombocytopenia < $100 \times 10^9/l$, a lymphocyte doubling time of < 12 months, progressive splenomegaly, and/or lymph adenopathy.

The first-line treatments included monochemotherapy (alkylating agents) in 55 cases (51.8%), polychemotherapy with anthracyclines in 21 (19.9%), polychemotherapy without anthracyclines in four (3.8%), and purine analogues in 26 (24.5%). Purine analogues were prevalently used for the patients treated after an indolent phase because they started therapy more recently. Responses were evaluated on the basis of the guidelines proposed by the International Workshop and the Working Group for Chronic Lymphocytic Leukemia [18,19].

Cytomorphological analysis

Peripheral blood films stained with May-Grunwald–Giemsa were morphologically evaluated using the FAB criteria (1) and the WHO’s revised REAL classification (2), and the cases were grouped on the basis of morphology into: (a) typical CLL, (b) LPL in leukemic phase (>15% of cells showing lymphoplasmacytic differentiation), (c) SMZL with (>10%) or without villous lymphocytes, (d) CLL/prolymphocytic leukemia (prolymphocytic cells 1,154%), and (e) heterogeneous forms (polymorphic forms).

Immunophenotype analysis of leukemic cells

The specimens were analyzed by means of a flow cytometer using a panel of monoclonal antibodies defined by each single institution but always including CD19, CD3, CD5, CD10, FMC7, and CD79b/CD22. All of the cases had an RMH score of < 4. B clonal lymphocytosis was diagnosed when the surface immunoglobulin k/λ ratio was more than 5 for k and less than 0.2 for λ light-chains. CD5 expression was considered negative when the number of cells showing coexpression after dual CD5/CD19 labeling was less than 10% of the number of cells expressing CD19. Secondary phenotype markers (CD23, CD11c, CD49c, CD1c, CD103, CD25, and CD49d) were analyzed in many specimens. The results are not included here because of the different techniques used by the different laboratories.

Cytogenetic analysis

The karyotypes were analyzed by means of QFD binding as previously described [20], and the chromosomes were classified according to the International System for Human Cytogenetic Nomenclature [21]. A clone was defined as two cells having the same structural rearrangement, or a gain of the same chromosome, or three cells with the loss of the same chromosome.

Statistical methods

Descriptive statistics were calculated for the quantitative (mean, standard deviation, minimum, maximum, and median in the case of asymmetric distributions) and qualitative variables (absolute and relative frequencies), together with their pertinent 95% confidence intervals. The diagnostic classes were compared using Kruskal–Wallis nonparametric analysis of variance for quantitative variables, and the χ^2 test or exact Fisher’s test for qualitative variables. The significance level of the multiple comparisons was corrected using Bonferroni’s procedure [22]. OS was defined as the time between the date of diagnosis and the date of death due to any cause, or the date of the last clinical examination. TFFT was defined in the group of patients in the “watch and wait” group as the time between the date of diagnosis and the date therapy was started, or date of the last clinical examination. TTP was defined in the group of patients treated from the beginning as the time between the date of diagnosis and the date of relapse/progression, or the date of the last clinical examination. The cumulative probability of failures of in the “watch and wait” group (TFFT), progression (TTP), and survival (OS) was evaluated according to Kaplan and Meier [23], with the differences between the survival curves being assessed using the log-rank test [24].

For the Cox proportional hazards regression analysis [25], we used the covariates with P values of < 0.1 in the univariate analysis; the best subset of prognostic factors was obtained from the full-model using Wald tests to identify covariates that might be deleted from the model, and the partial likelihood ratio test to identify the nonsignificance of the deleted covariate [26]. The proportional hazard assumption was tested using the method of Grambsch–Therneau [27]. Cox–Snell [28] residuals were used to assess the fit of a model based on the Cox proportional hazard assumption. Goodness of fit was checked by means of the Hosmer–Lemeshow test [29], and the presence of outliers by means of deviance residuals versus linear predictor. The stability of the model was verified using the bootstrap technique [30].

All of the P values were computed from two-sided tests, and those that were < 0.05 were considered statistically significant. All of the data were analyzed using Stata 8/SE statistical packages [31].

References

1. Bennet JM, Catovsky D, Daniel M-T, et al. The French-American-British (FAB) cooperative group. Proposals for the classification of chronic (mature) B and T lymphoid leukemias. *J Clin Pathol* 1989;42:567–584.
2. Harris NL, Jaffe ES, Diebold J, et al. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting–Airlie House, Virginia, November 1997. *J Clin Oncol* 1999;12:3835–3849.
3. Ugo V, Leporrier N, Salaun V, et al. Deciphering leukemic B-cell chronic lymphoproliferative disorders. *Leuk Lymphoma* 2006;47:2088–2095.
4. Zent CS. Chronic B-cell lymphoproliferative disorders: How many diseases? *Leuk Lymphoma* 2006;47:2006–2007.
5. Muller-Hermelink HK, Catovsky D, Montserrat E, Harris NL. Chronic lymphocytic leukemia/small lymphocytic lymphoma. In: Jaffe E, Harris N, Stein H, Vardiman J, editors. *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon: IARC Press; 2001. pp 127–130.
6. Nowakowski GS, Dewald GW, Hoyer JD, et al. Interphase fluorescence in situ hybridization with an IGH probe is important in the evaluation of patients with

- a clinical diagnosis of chronic lymphocytic leukaemia. *Br J Haematol* 2005;130:36–42.
7. Cro L, Guffanti A, Colombi M, et al. Diagnostic role and prognostic significance of a simplified immunophenotypic classification of mature B cell chronic lymphoid leukemias. *Leukemia* 2003;17:125–132.
 8. Huang JC, Finn WG, Goolsby CL, et al. CD5- small B-cell leukemias are rarely classifiable as chronic lymphocytic leukaemia. *Am J Clin Pathol* 1999;111:123–130.
 9. Geisler CH, Larsen JK, Hansen NE, et al. Prognostic importance of flow cytometric immunophenotyping of 540 consecutive patients with B-cell chronic lymphocytic leukaemia. *Blood* 1991;78:1795–1802.
 10. Shapiro JL, Miller ML, Pohlman B, et al. CD5- B-Cell lymphoproliferative disorders presenting in blood and bone marrow. *Am J Clin Pathol* 1999;111:477–487.
 11. Wang C, Amato D, Fernandes BCD. -5 negative phenotype of monoclonal B-lymphocytosis of undetermined significance (MLUS). *Am J Hematol* 2002;69:147–149.
 12. Sheikh SS, Kallakury BV, Al-Kuraya KA, et al. CD5-negative, CD10-negative small B-cell leukemia: Variant of chronic lymphocytic leukemia or a distinct entity? *Am J Hematol* 2002;71:306–310.
 13. Ikematsu W, Ikematsu H, Okamura S, et al. Surface phenotype and Ig heavy-chain gene usage in chronic B-cell leukemias: Expression of myelomonocytic surface markers in CD5- chronic B-cell leukaemia. *Blood* 1994;83:2602–2610.
 14. De Rossi G, Mauro FR, Lo Coco F, et al. CD5 negative lymphocytosis mimicking typical B-chronic lymphocytic leukaemia. Description of 26 cases. *Nouv Rev Fr Hematol* 1993;35:451–455.
 15. Cartron G, Linassier C, Bremond JL, et al. CD5 negative B-cell chronic lymphocytic leukemia: Clinical and biological features of 42 cases. *Leuk Lymphoma* 1998;31:209–216.
 16. Salomon-Nguyen F, Valensi F, Merle-Beral H, Flandrin G. A scoring system for the classification of CD5-B CLL versus CD5+ B CLL and B PLL. *Leuk Lymphoma* 1995;16:445–450.
 17. Arcaini L, Lazzarino M, Colombo N, et al. Splenic marginal zone lymphoma: A prognostic model for clinical use. *Blood* 2006;107:4643–4649.
 18. Chronic lymphocytic leukemia: Recommendations for diagnosis, staging, and response criteria. International Workshop on Chronic Lymphocytic Leukemia. *Ann Intern Med* 1989;110:236–238.
 19. Cheson BD, Bennett JM, Grever M, et al. National Cancer Institute-sponsored Working Group guidelines for chronic lymphocytic leukemia: Revised guidelines for diagnosis and treatment. *Blood* 1996;87:4990–4997.
 20. Baldini L, Fracchiolla NS, Cro LM, et al. Frequent p53 gene involvement in splenic B-cell leukemia/lymphomas of possible marginal zone origin. *Blood* 1994;84:270–278.
 21. Mitelman F. Guidelines for Cancer Cytogenetics. Supplement to an International System for Human Cytogenetic Nomenclature. Basel, Switzerland: Karger; 1991.
 22. Armitage P, Berry G. *Statistical Methods in Medical Research*. Oxford: Blackwell Scientific Publications; 1987. pp 408–420.
 23. Kaplan EI, Meier P. Non parametric evaluation from incomplete observations. *J Am Stat Assoc* 1958;53:457–481.
 24. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. *Cancer Chemother Rep* 1966;50:163–170.
 25. Cox DR. Regression models and life-tables. *J R Stat Soc [B]* 1972;34:187–220.
 26. Hosmer DW, Lemeshow S. *Applied Survival Analysis*. New York: Wiley Interscience; 1999. pp 158–180.
 27. Grambsch PM, Therneau TM. Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika* 1994;81:515–526.
 28. Cox DR, Snell EJ. A general definition of residuals. *J R Stat Soc [B]* 1968;30:248–275.
 29. Hosmer DW, Lemeshow S. *Applied Survival Analysis*. New York: Wiley Interscience; 1999. pp 225–230.
 30. Sauerbrei W, Schumacher M. A bootstrap resampling procedure for model building: Application to the Cox regression model. *Stat Med* 1992;11:2093–2109.
 31. StataCorp. *Stata Statistical Software: Release 8*. College Station, TX: Stata-Corp; 2003.