CD5⁻ B-Cell Lymphoproliferative Disorders Presenting in Blood and Bone Marrow

A Clinicopathologic Study of 40 Patients

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Abstract

We studied 40 patients with CD5⁻ B-cell lymphoproliferative disorders (B-LPDs) presenting in blood or bone marrow and 28 control patients with CD5⁺ B-cell chronic lymphocytic leukemia (CLL). Fifteen study patients had morphologic features typical of CLL. The 15 patients with CD5⁻ CLL were older and had lower absolute lymphocyte counts and more advanced-stage disease at diagnosis than controls. Ten study patients had morphologic features suggesting mantle cell lymphoma (MCL); 3 were later given a diagnosis of MCL based on lymph node biopsy results. The 10 patients with CD5⁻ MCL were older and at a more advanced stage than CLL control patients. The remaining 15 study patients were given the following diagnoses: circulating non-Hodgkin lymphoma, 5; splenic lymphoma with villous lymphocytes, 5; lymphoplasmacytoid lymphoma, 3; and CLL/pro-lymphocytic leukemia, 2. For the patients with CD5⁻ B-LPDs with morphologic features and manifestations resembling CLL, we prefer the term CD5⁻ CLL variant because of clinical and immunophenotypic differences. Patients with CD5⁻ B-LPDs with atypical nuclear morphologic features may represent the leukemic phase of MCL. Since CD23 is expressed in most patients with CD5⁻ B-LPD, its use in subclassifying these disorders seems limited.

The increasing use of flow cytometric analysis to evaluate B-cell lymphoproliferative disorders (B-LPDs) has led to greater diagnostic accuracy of these entities. B-LPDs are a heterogeneous group of indolent lymphoid neoplasms that can be classified based on clinical, morphologic, and immunophenotypic attributes. Current classification schemes recognize several disorders under the general heading of "peripheral B-cell neoplasms."[1] Two disorders, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and mantle cell lymphoma (MCL), characteristically coexpress CD5, a glycoprotein normally found on T cells, and CD19, a marker of B-cell differentiation. The CD5⁻ B-LPDs include lymphoplasmacytoid lymphoma, marginal zone B-cell lymphoma, follicle center lymphoma, hairy cell leukemia, and splenic marginal zone lymphoma with or without villous lymphocytes (SLVL). "Classic" cases of each of these disorders represent the majority encountered in clinical practice. However, we have identified several cases of CD5⁻ B-LPDs in which lymphocyte morphologic features and clinical findings are consistent with CLL. In addition, cases of CD5⁻ B-LPD with lymphocyte morphologic features suggestive of MCL occasionally are encountered that express CD23, a marker typically negative in MCL but positive in CLL.[2] These cases are difficult to classify using the Zukerberg model[3] for low-grade lymphoproliferative disorders or the revised European-American classification of lymphoid neoplasms (REAL).[1]

To further define these unusual cases, we reviewed the clinical, morphologic, and immunophenotypic characteristics of 40 cases of CD5⁻ B-LPD. The study was designed to answer the following questions: (1) What percentage of suspected B-LPDs presenting in the blood or bone marrow are CD5⁻? (2) Can these cases be placed into recognized diagnostic categories? (3) Among patients with CD5⁻ B-LPDs that morphologically resemble CLL or MCL, is CD23 useful for

subclassification? (4) Do the clinical manifestations of CD5⁻ B-LPDs differ from "typical" CLL manifestations?

Materials and Methods

Study Population

All cases of B-LPDs initially diagnosed in blood or bone marrow and having concomitant flow cytometric analysis performed between January 1, 1994, and January 1, 1998, were identified. Patients with a previous diagnosis of non-Hodgkin lymphoma were excluded. Patients with T-cell lymphoproliferative disorders and hairy cell leukemia also were excluded. Flow cytometry reports for the remaining patients then were reviewed to identify the cases with absent expression of CD5 in the abnormal lymphocyte population. The study population was defined as patients with newly diagnosed CD5⁻ B-LPDs involving primarily blood or bone marrow.

Control Population

A control population of patients with CLL was identified in the same manner and for the same period as the study population. These patients had peripheral smear morphologic features and an immunophenotypic profile (ie, CD5⁺, CD19⁺, CD23⁺, and surface light chain restriction) typical for CLL[4] and met all criteria defined by the 1996 National Cancer Institute–CLL Working Group.[5]

Clinical Data

Clinical data were obtained for each of the study and control patients by medical record review. The variables studied were as follows:

- Age
- Sex
- Reason for referral to hematologist
- Presence of lymphadenopathy, splenomegaly, or both as noted by physical or radiologic examination
- Total WBC count
- Hemoglobin concentration
- Platelet count
- Absolute lymphocyte count
- Serum protein electrophoresis and immunofixation
- Flow cytometric analysis of peripheral blood and/or bone marrow specimen
- Peripheral smear morphologic features
- Bone marrow aspirate morphologic features and biopsy specimen histologic features
- Cytogenetic analysis of peripheral blood or bone marrow specimen
- Clinical staging (modified Rai system)
- Treatment and follow up

Serum monoclonal protein analysis, cytogenetics, and serum immunoglobulin quantitation were performed using standard laboratory techniques. Test results were not available for all patients. The stage of disease at initial examination was assigned for patients with CLL or suspected CLL by using the modified Rai staging system.[4]

Flow Cytometry

Blood or bone marrow aspirates submitted in EDTA or heparin were processed by using standard techniques.[6] Each case was evaluated by using a panel of monoclonal antibodies (all from Becton Dickinson, San Jose, CA, unless specified) that included the T-cell markers, CD3 (Leu-4), CD5 (Leu-1), CD8 (Leu-2a); the NK-cell markers, CD16/56 (Leu-11c/NCAM16.2); and the B-cell markers, CD19 (Leu-12), CD23 (Leu-20), and κ and λ light chains (anti-κ and anti-λ immunoglobulin light chain antibodies). Additional monoclonal antibodies for CD22 (Leu-14), CD11c (Leu-M5), CD25 (anti-IL-2R), CD13 (Leu-M7), CD38 (Leu-17), and BB4 (BB4 FITC, Biotest Diagnostics, Denville, NJ) were used for analysis at the discretion of the pathologist who originally examined the case. FMC7 was not available in our laboratory during the study period. Three-color flow cytometric analysis of a lymphocyte light scatter gate then was performed as described by Segal et al.[7] Paint-a-Gate software (Becton Dickinson) was used for simultaneous multiparameter analysis. FACScan (Becton Dickinson) flow cytometer data evaluation and quality assurance techniques also were performed as described.[7] Two-dimensional dot-plot representations of the 3-color analysis were evaluated independently by 3 of us (J.L.S., M.L.M., A.J.F.).

Surface antigen expression and intensity of the abnormal B-cell (CD19⁺) lymphocyte population were determined by using quadrant statistics and categorized as follows: negative, less than 5%; plus or minus, 6% to 20%; or positive, more than 20%. Quadrant cursors were set by using isotypic negative controls. Fluorescent intensity of surface antigens was estimated by using scaled logarithmic values. Target lymphoid populations centered on the first log decade were designated *low density,* on the second log decade as *moderate density,* and on the third log decade as *high density.* A representative CD5⁻ B-LPD flow result is shown in **Figure 1**.

Assignment of CD5⁻ Subgroups

Peripheral smears, bone marrow aspirates, or both for each of the study patients were reviewed independently by 3 of us (J.L.S., M.L.M., A.J.F.), with consensus agreement made independent of the clinical data. With the exception of 1 case in which only the marrow aspirate was available for review, the

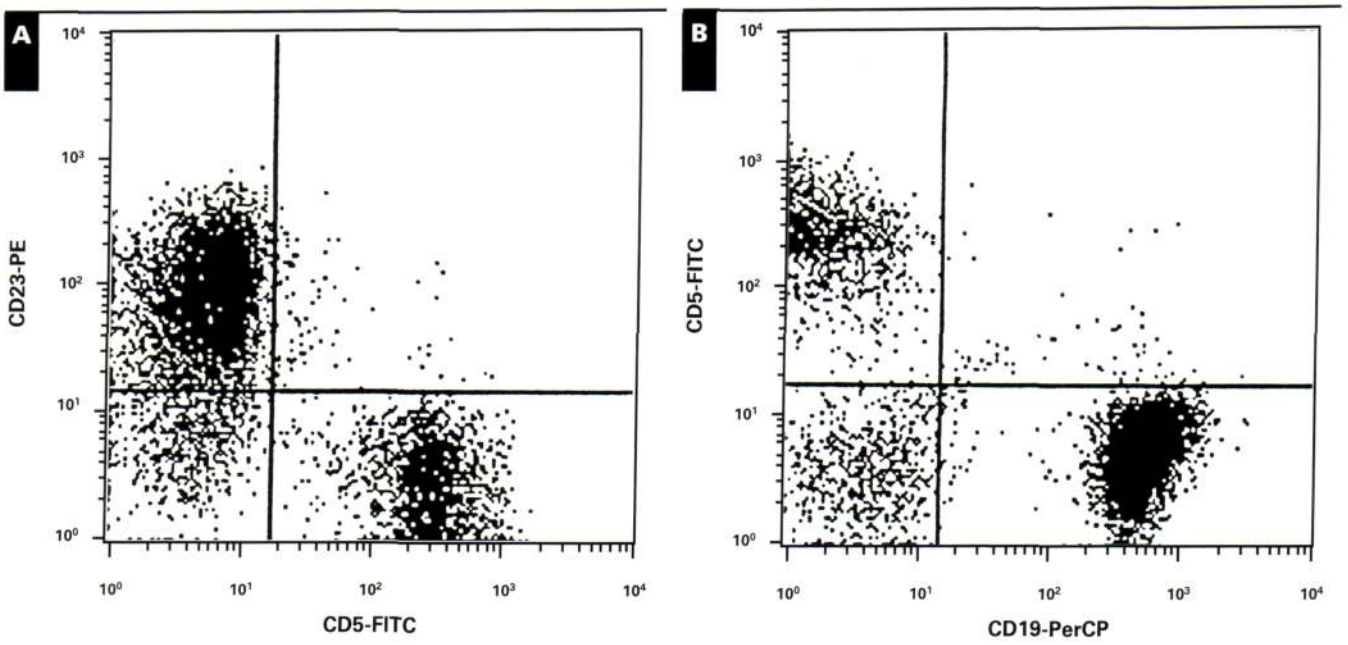


Figure 1 Dot plot histogram of flow cytometric analysis of peripheral blood in a patient with peripheral lymphocytosis. Lymphocyte light scatter gating was used in the analysis. A, Dual comparative studies of CD23 and CD5. B, Comparison of CD19 and CD5. The patient whose histogram is shown had lymphocyte morphologic features typical for CLL. The immunophenotype was interpreted as $CD19^+$, $CD5^-$, $CD23^+$. A smaller population of $CD5^+$ T cells also is present.

lymphocyte morphologic features on the peripheral smears and aspirates were similar. The study patients were assigned to 1 of 6 subgroups **Table 1**: (1) $CD5^-$ CLL variant ($CD5^-$ CLL)—lymphocytosis in the blood (absolute lymphocyte count, >5,000/μL [$>5 \times 10^3$/μL]) or bone marrow aspirate (>30% lymphocytes [0.30]); a clinical impression of CLL; a lymphocyte population that consisted of predominantly small lymphocytes with condensed chromatin, inconspicuous or absent nucleoli, and rounded nuclear contours; or a mixture of lymphocytes ("mixed pattern" of CLL as described by Bennett et al[8]) with small nuclei, condensed chromatin, and variable amounts of light blue reactive-appearing cytoplasm; (2) $CD5^-$ B-LPD resembling MCL ($CD5^-$ MCL)—lymphocytosis in the blood or bone marrow, no tissue diagnosis of MCL at the initial examination, and atypical lymphocyte morphologic features with more than 15% of lymphocytes demonstrating prominent nuclear clefts or notches and slightly immature chromatin[9]; (3) circulating non-Hodgkin lymphoma resembling follicle center lymphoma (circulating NHL)—absent or variable absolute lymphocytosis, clinical suspicion of lymphoma (based on the presence of cytopenia or constitutional symptoms such as weight loss, fatigue, or fever) without palpable adenopathy, and large, atypical lymphoid cells with high nuclear/cytoplasmic ratios, deeply cleft nuclei, and variable condensation of chromatin; (4) SLVL—clinical features (as described by Troussard et al[10]) characteristic of SLVL, slightly enlarged lymphocytes with at least 20% of the lymphocytes demonstrating clumped chromatin, small but prominent nucleoli, and short cytoplasmic villi with an uneven or polar distribution around the nucleus[11]; (5) lymphoplasmacytoid lymphoma (LPL)—lymphocytosis in blood, bone marrow, or both; the presence of increased plasmacytoid lymphocytes, plasma cells, or both in the blood or bone marrow; or the clinical syndrome of Waldenström macroglobulinemia; (6) CLL/prolymphocytic leukemia (CLL/PL)—lymphocytosis in the blood, bone marrow, or both with 11% to 54% prolymphocytes.

Table 1
Subgroups of $CD5^-$ B-Cell Lymphoproliferative Disorders

Subgroup Designation	No. of Patients
$CD5^-$ chronic lymphocytic leukemia variant	15
$CD5^-$ B-LPD resembling mantle cell lymphoma	10
Circulating non-Hodgkin lymphoma resembling follicle center lymphoma	5
Splenic lymphoma with villous lymphocytes	5
Lymphoplasmacytoid lymphoma	3
Chronic lymphocytic leukemia/prolymphocytic leukemia	2
Total	40

Statistical Analysis

The clinical and hematologic variables of interest for patients with $CD5^-$ CLL and $CD5^-$ MCL were compared statistically with those of the control patients. Comparisons on continuous or ranked data were done with the Wilcoxon rank-sum test; for categorical variables, the χ^2 or the Fisher exact test was used. The survival experience of patients was

described using Kaplan-Meier survival analysis, and groups were compared with the log-rank test. The significance of each hypothesis was .05. Since for each hypothesis 2 comparisons were made with the control group, a Bonferroni correction for multiple comparisons was made, and, thus, only P values <.025 were considered statistically significant.

Results

During the study interval, flow cytometric analysis of peripheral blood or bone marrow was performed on specimens from 362 patients with a suspected B-LPD but no previous diagnosis. Analysis of the flow cytometric data showed that 58 (16.0%) had a CD5⁻ immunophenotypic profile. Clinical information, the results of peripheral blood smears, bone marrow aspirates, or both, and follow-up data were available for 40 patients.

Subgroups

The study patients were assigned to 1 of 6 subgroups based on lymphocyte morphologic features, laboratory data, and clinical impression. Of the 40 study patients, 15 had morphologic features typical of CLL **Image 1A** and were assigned to the CD5⁻ CLL group. Included in this group were 4 patients having a "mixed" morphologic pattern. Ten patients were classified as CD5⁻ MCL **Image 1B**. The remaining patients were divided as follows: circulating NHL,

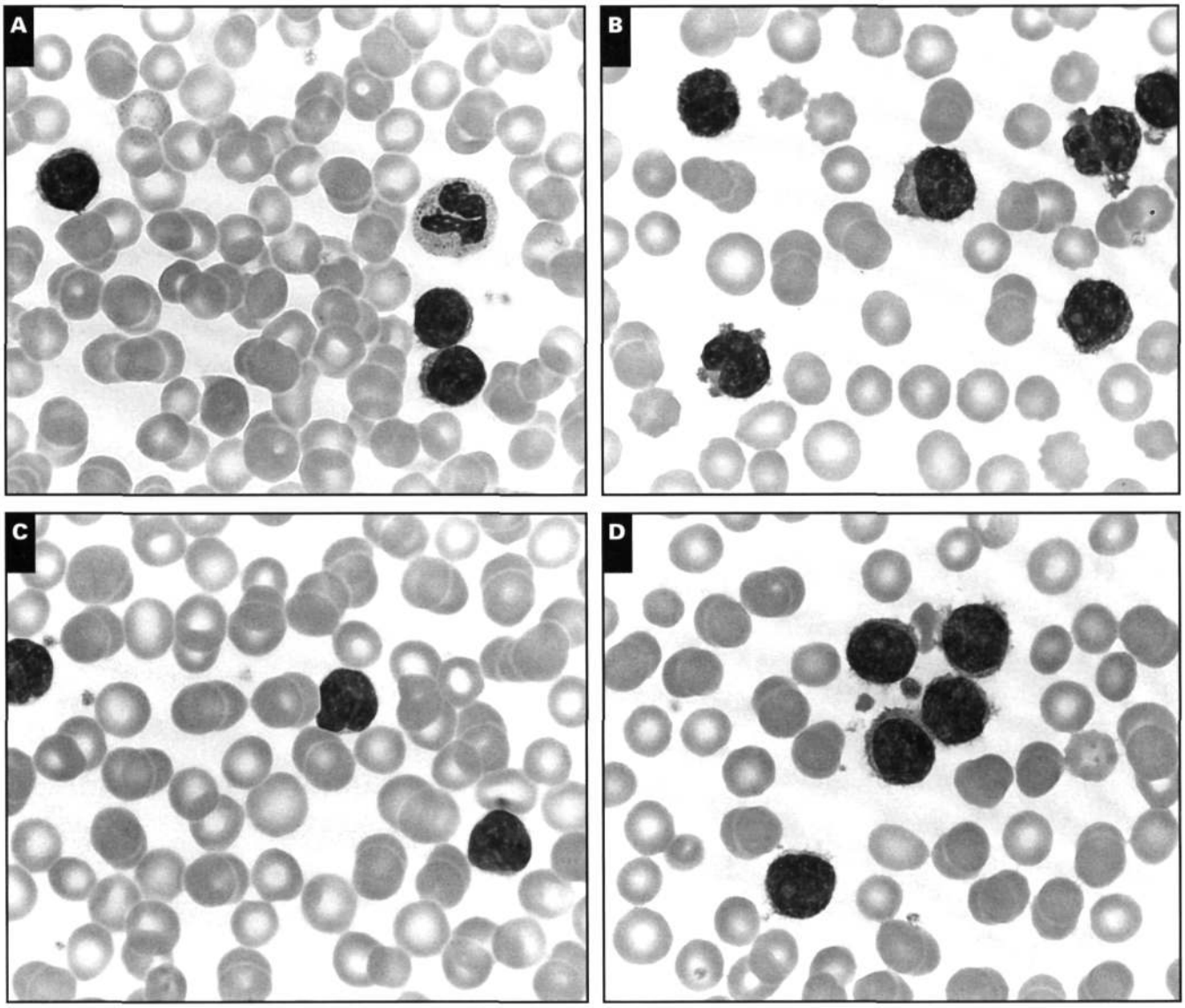


Image 1 Morphologic appearance of CD5⁻ B-cell lymphoproliferative disorder (B-LPD) subgroups. A, CD5⁻ chronic lymphocytic leukemia variant. B, CD5⁻ B-LPD resembling mantle cell lymphoma. C, Circulating non-Hodgkin lymphoma. D, Splenic lymphoma with villous lymphocytes (Wright stain, ×1,000).

5 patients ▮Image 1C▮; SLVL, 5 patients ▮Image 1D▮; LPL, 3 patients; and CLL/PL, 2 patients. By study design, all 28 patients in the control group had morphologic features that met National Cancer Institute-CLL Working Group guidelines[5] for CLL.

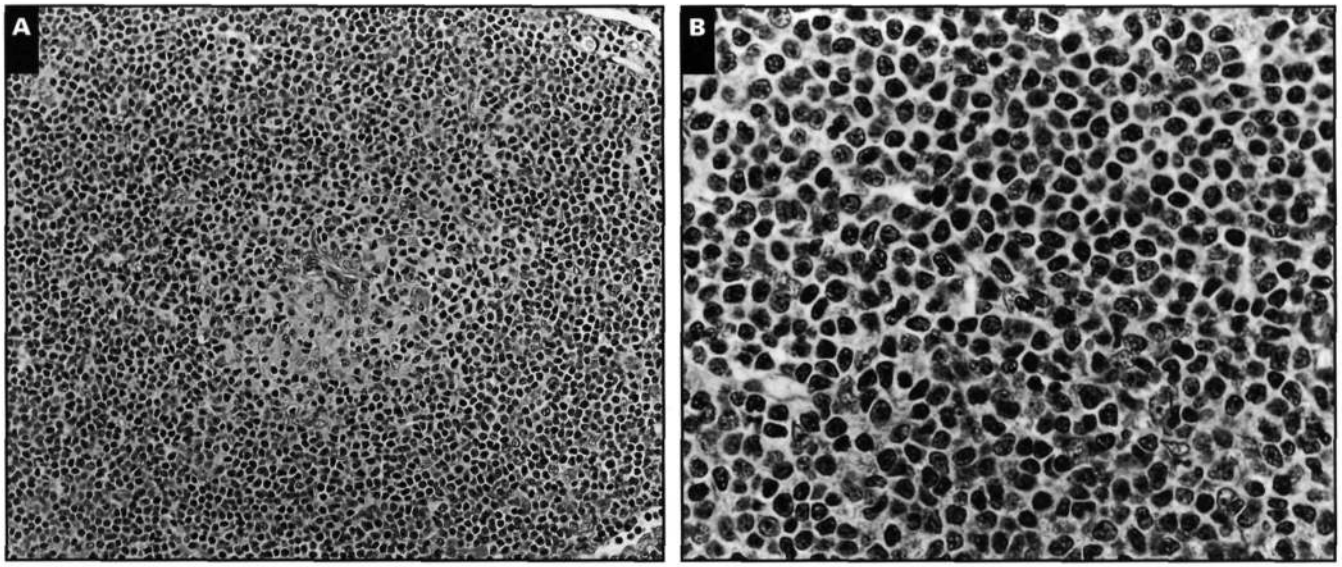
Bone marrow biopsies were performed on 31 of 40 study patients and 7 of 28 control patients. A diffuse pattern of marrow involvement was observed in 6 of 31 bone marrow specimens from study patients and 3 of 7 from control patients. The remaining patients had variable combinations of nodular and interstitial bone marrow involvement. There was no correlation between the pattern of marrow involvement and the diagnostic subgroup. In 2 patients with circulating NHL, the bone marrow specimens had paratrabecular and nonparatrabecular lymphoid aggregates without an interstitial lymphocytosis and lymphocytes with cleaved and irregular nuclear contours.

Immunohistochemical staining for κ and λ light chains was performed on 6 bone marrow biopsy specimens. Three patients with $CD5^-$ CLL were tested, and 1 was found to have scattered plasma cells with a κ light chain predominance; the other 2 patients had scattered plasma cells with a polyclonal pattern of immunoreactivity. The 2 patients with LPL who were tested had increased numbers of plasma cells and plasmacytoid lymphocytes with κ light chain predominance. One patient with SLVL had only rare polyclonal plasma cells. Five control patients with CLL all demonstrated polyclonal immunoreactivity.

Lymph node biopsies were performed on 5 study patients and 2 control patients after the diagnosis of B-LPD was established. One patient with $CD5^-$ CLL and 2 patients in the control group were found to have histologic features consistent with SLL in the lymph node biopsy specimen. Three patients with $CD5^-$ MCL had histologic features most consistent with the mantle zone pattern of MCL ▮Image 2A▮ and ▮Image 2B▮. Immunohistochemical staining for cyclin D1 was performed on paraffin-embedded tissue for the 3 patients in the $CD5^-$ MCL group who had undergone lymph node biopsies. One of the 3 specimens demonstrated strong diffuse nuclear staining; results for the other 2 patients were negative. One patient in the circulating NHL group had a follicle center lymphoma confirmed at lymph node biopsy. Three patients with SLVL underwent splenectomy demonstrating splenic marginal zone lymphoma.

Clinical Data

The study patients ranged in age from 38 to 89 years of age; 17 were men and 23 were women. The $CD5^-$ CLL (mean ± SD, 75.9 ± 7.2 years; $P < .001$) and $CD5^-$ MCL (mean ± SD, 74.6 ± 7.4; $P = .001$) subgroups were older than the control group (mean ± SD, 62.6 ± 10.0 years). Manifesting symptoms were variable. In the $CD5^-$ CLL group, 8 of 15 patients were asymptomatic and were referred to a hematologist because of incidental findings on the CBC count or because of splenomegaly. The other 7 patients noted weakness and fatigue; 3 of these 7 also had constitutional symptoms. With the exception of 2 patients (1 with LPL and 1 with circulating NHL) who sought medical attention because of fatigue, all patients in the other subgroups were asymptomatic and referred to a hematologist for evaluation



▮Image 2▮ Lymph node histologic features in a patient with a $CD5^-$ B-cell lymphoproliferative disorder resembling mantle cell lymphoma. The lymphocytes were also $CD23^+$. A, Low magnification demonstrating expansion of mantle zones with compression of germinal centers (H&E, ×250). B, High magnification demonstrating a monotonous population of small lymphocytes with slightly irregular nuclear contours and interspersed epithelioid histiocytes (H&E, ×640).

of an abnormal CBC count. Five of 28 control CLL patients had subjective complaints; the remainder were referred because of abnormal laboratory findings. The physical examination and radiographic findings also varied significantly. Patients with CD5⁻ CLL and CD5⁻ MCL had splenomegaly more often ($P < .001$ and $P = .005$, respectively) than did the control patients with CLL **Table 2**. No difference in lymphadenopathy was found between the study groups and control patients. The patients with CD5⁻ CLL ($P < .001$) and CD5⁻ MCL ($P < .001$) had a higher median stage of disease when they were first examined than the did patients in the control group.

Thirteen of the 40 study patients were treated during the study interval with cytotoxic chemotherapy, chlorambucil and prednisone (n = 11), cyclophosphamide, vincristine, and prednisone (n = 1), or cyclophosphamide, mitoxantrone, and fludarabine (n = 1). Two patients received gamma globulin for the treatment of hypogammaglobulinemia. The remaining patients were observed during the follow-up interval without treatment. Three of the 28 control patients received chlorambucil and prednisone; 1 was treated with gamma globulin. The median follow-up interval for all patients, including CLL control patients, was 16 months (range, 1–68 months). One patient in the CD5⁻ CLL group died of unrelated causes 14 months after diagnosis. Two patients in the CD5⁻ MCL group died of unrelated causes 1 and 38 months after diagnosis. One patient in the control group died of unrelated causes 5 months after diagnosis. The Kaplan-Meier survival estimate at 2 years was 93% (95% confidence interval [CI], 83%–100%) for the 40 study patients and 96% (CI, 88%–100%) for the control patients ($P = .59$ log-rank test). The 2-year survival for the CD5⁻ CLL and CD5⁻ MCL was 86% (CI, 60%–100%) and 90% (CI < 71%–100%), respectively, with neither group having their survival experience statistically different from the control patients.

Hematologic Variables

Results of the CBC counts of the study and control patients at the time of initial examination are given in **Table 3**. The median total WBC count, hemoglobin concentration,

Table 2
Clinical Data

Subgroup	Age (y), Median (Minimum, Maximum)	Splenomegaly*	Adenopathy*	Stage,† Median (Mean)	Follow Up (mo),‡ Median (Minimum–Maximum)
Total CD5⁻ B-LPD (n = 40)	76 (38, 89)	23	9	NA	16 (1–53)
CD5⁻ CLL (n = 15)	77 (59, 86)§	10‖	4	2 (2.3)§	13 (1–53)
CD5⁻ MCL (n = 10)	77 (62, 84)§	6‖	3	3 (2.6)§	16 (1–42)
Circulating NHL (n = 5)	72 (39, 74)	2	1	NA	17 (5–21)
SLVL (n = 5)	75 (65, 82)	4	1	NA	23 (2–45)
LPL (n = 3)	66 (58, 76)	0	0	NA	17 (7–25)
CLL/PL (n = 2)	64 (38, 89)	1	0	NA	17 (1–33)
CLL control (n = 28)	63 (45, 82)	3	7	1 (1.4)	16 (1–68)

B-LPD = B-cell lymphoproliferative disorder; NA = not applicable; CLL = chronic lymphocytic leukemia; MCL = mantle cell lymphoma; NHL = non-Hodgkin lymphoma; SLVL = splenic lymphoma with villous lymphocytes; LPL = lymphoplasmacytoid lymphoma; PL = prolymphocytic leukemia.
* Indicates presence of splenomegaly or lymphadenopathy detected either by physical examination or by radiologic methods.
† Modified Rai stage. For purposes of statistical calculation, 1 = low risk; 2 = intermediate risk; and 3 = high risk. The Rai stage is applicable only to patients with or suspected of having CLL.
‡ Number of months from time of diagnosis.
§ Significantly different from control group (Wilcoxon rank-sum test).
‖ Significantly different from control group ($P < .025$; Fisher exact test).

Table 3
Hematologic Variables*

Subgroup	WBC Count ($\times 10^3/\mu L$)	Hemoglobin (g/dL)	Platelet Count ($\times 10^3/\mu L$)	ALC ($\times 10^3/\mu L$)
CD5⁻ CLL (n = 15)	10.2 (5.0, 19.9)†	11.4 (7.0, 15.4)†	155 (72, 244)†	6.7 (0.8, 14.9)†
CD5⁻ MCL (n = 10)	72.1 (16.5, 150.0)†	10.6 (5.0, 14.6)†	142 (72, 349)	65.8 (7.7, 130.5)
Circulating NHL (n = 5)	6.0 (3.3, 11.3)	11.5 (8.3, 14.4)	143 (115, 207)	1.4 (1.0, 4.9)
SLVL (n = 5)	20.2 (3.8, 49.9)	12.3 (11.9, 15.1)	167 (144, 187)	14.3 (2.2, 45.5)
LPL (n = 3)	8.9 (4.7, 9.8)	11.8 (7.6, 13.0)	331 (155, 346)	2.3 (1.8, 2.8)
CLL/PL (n = 2)	22.9 (15.8, 30.0)	10.7 (9.1, 12.2)	268 (260, 277)	16.8 (11.9, 21.6)
CLL control (n = 28)	20.9 (12.2, 59.8)	14.1 (11.5, 16.5)	191 (80, 617)	15.6 (4.4, 56.3)

ALC = absolute lymphocyte count; CLL = chronic lymphocytic leukemia; MCL = mantle cell lymphoma; NHL = non-Hodgkin lymphoma; SLVL = splenic lymphoma with villous lymphocytes; LPL = lymphoplasmacytoid lymphoma; PL = prolymphocytic leukemia.
* Data are given as median (minimum, maximum).
† Statistically different from controls ($P < .025$) using the Wilcoxon rank-sum test.

platelet count, and absolute lymphocyte count (ALC) for patients with CD5⁻ CLL and CD5⁻ MCL were compared with those of the control patients. The patients with CD5⁻ CLL had lower total WBC counts, ($P < .001$), ALCs ($P < .001$), platelet counts ($P = .021$), and hemoglobin concentrations ($P = .010$) than the CLL control group. The CD5⁻ MCL group had higher WBC counts ($P = .006$) and lower hemoglobin concentrations ($P = .002$) than did the control patients. Differences in platelet counts were not statistically significant between the CD5⁻ MCL and control groups.

Serum Immunoglobulin Laboratory Analysis

Serum protein electrophoresis and serum immunofixation data were available for 20 of the 40 study patients. Among these 20 patients, 13 had evidence of a monoclonal gammopathy: 5 of 7 with CD5⁻ CLL, 3 of 4 with CD5⁻ MCL, 1 of 4 with circulating NHL, 0 of 1 with SLVL, 3 of 3 with LPL, and 1 of 1 with CLL/PL. With the exception of 1 patient with CD5⁻ MCL and 1 patient with circulating NHL with low-level IgG gammopathy, all other patients with positive immunofixation studies demonstrated IgM gammopathy. The amount of M protein ranged from 0.20 g/dL to 0.90 g/dL in patients with CD5⁻ CLL but was not further quantitated in patients with CD5⁻ MCL. In the 3 patients with LPL, the M protein levels were 0.51, 1.26, and 5.24 g/dL. In the control group, serum protein electrophoresis data were available for 8 patients; only 1 patient had a possible M spike, but no subsequent immunofixation studies were performed.

Cytogenetics

Data for 8 patients in the study group were available from cytogenetic analysis of blood or bone marrow. Two of the 8 patients had abnormal karyotypes. One patient with CD5⁻ CLL had a partial deletion of the short arm of chromosome 7 [46,XX,del(7)(q31)]. One patient with LPL had a benign familial polymorphism [46,XX,inv(9)(p11q13)] found in approximately 1% of the general population. The remaining 6 study patients had normal karyotypes. In the control group, all 5 patients tested had normal karyotypes.

CD23 Expression and Additional Immunophenotypic Markers

Data on CD23 expression were available for all 40 study patients **Table 4**. Of the 40 patients, 34 had positive or plus or minus CD23 expression. Negative CD23 expression was found in 2 patients with CD5⁻ MCL, 1 patient with circulating NHL, 2 patients with SLVL, and 1 patient with LPL. Three patients with CD5⁻ MCL (2 CD23⁺ and 1 CD23±), were later found to have lymph node biopsy histologic features most consistent with MCL (Image 2). In 1 of the patients with CD5⁻/CD23⁺ MCL, flow cytometric analysis of the lymph node revealed a CD5⁻/CD23⁻ phenotype.

Table 4
CD23 Expression Among Study and Control Patients*

Subgroup	CD23⁻	CD23±	CD23⁺
CD5⁻ CLL (n = 15)	0	3	12
CD5⁻ MCL (n = 10)	2	1	7
Circulating NHL (n = 5)	1	2	2
SLVL (n = 5)	2	1	2
LPL (n = 3)	1	2	0
CLL/PL (n = 2)	0	1	1
CLL controls (n = 28)	0	0	28

CLL = chronic lymphocytic leukemia; MCL = mantle cell lymphoma; NHL = non-Hodgkin lymphoma; SLVL = splenic lymphoma with villous lymphocytes; LPL = lymphoplasmacytoid lymphoma; PL = prolymphocytic leukemia.
See text for the criteria for negative (–), plus/minus (±), and positive (+).

All patients in the study population demonstrated light chain restriction (26 κ, 14 λ). The majority of study patients had moderate density surface light chain antigen expression. High density surface light chain expression was present in 6 patients with CD5⁻ CLL, 6 with CD5⁻ MCL, 1 with circulating NHL, 1 with SLVL, 1 with LPL, and 1 with CLL/PL. In the control group, 25 of 28 patients demonstrated surface light chain restriction (14 κ, 11 λ); the remaining 3 patients were presumed to have shed light chains before flow cytometric analysis. None of the control patients demonstrated high density surface antigen expression; 10 of 25 had low density expression. Six patients with CD5⁻ CLL had positive or plus or minus CD22 and CD11c expression, but the degree of surface antigen intensity was variable; no patient had high density coexpression of CD11c and CD22, characteristic of hairy cell leukemia.[12] CD13 was expressed in 3 of 3 tested patients with CD5⁻ MCL and the 1 tested patient with CD5⁻ CLL. Markers suggestive of plasma cell differentiation (CD38 and BB4) were included in the profiles of 1 patient with CD5⁻ CLL and 2 patients with LPL; all 3 were CD38⁺/BB4 negative. Of 5 patients with SLVL, 4 demonstrated moderate to high density coexpression of CD22, CD11c, and CD20 and lacked CD25 expression; the remaining patient with SLVL had a similar immunophenotype but demonstrated only plus or minus CD11c expression. Three of 3 control patients tested had positive or plus or minus CD11c and CD22 expression, and in 1 of these patients, CD25 was expressed.

Discussion

Although the majority of B-LPDs manifesting in blood and bone marrow have a CD5⁺ phenotype, CD5⁻ B-LPD cases constitute an estimated 5.4%[13] to 22%[14] of cases. Among all patients with CD5⁻ B-LPDs, the distribution of patients with a well-defined clinicopathologic diagnosis, as well as the number of patients who cannot be classified by using current criteria, is unknown. Huang et al[14] reported that

35 (21.8%) of 160 cases of B-LPD had a CD5⁻ phenotype. They found a roughly equal distribution of hairy cell leukemia, follicle center lymphoma, and marginal zone lymphoma, with rare cases of MCL, hairy cell variant, and CLL/PL. They also noted 3 cases of CD5⁻ CLL with atypical morphologic or immunophenotypic features. Similarly, in the present study, we found that 16.0% (58/362) of B-LPDs were CD5⁻; however, the diseases in the majority of the patients in the present study were classified as CD5⁻ CLL or CD5⁻ MCL, depending on the lymphocyte morphologic features.

Among patients with B-CLL, several studies have noted highly diverse immunophenotypes with variable expression and staining intensity of different surface antigens. Geisler et al[13] described flow cytometric data for 540 consecutive patients with CLL. These investigators reported variability in surface antigen expression of CD5, CD20, CD21, CD22, CD23, FMC7, and surface IgM and attempted to correlate surface antigen expression with clinical variables and survival. Similar studies by Newman et al[15] and Batata and Shen[16] corroborated the immunophenotypic diversity of CLL. In addition to the presence or absence of a specific surface antigens, investigators also have studied the fluorescent intensity of surface antigens in CLL. In a study of 93 CLL patients, Tefferi et al[17] concluded that surface light chain and CD20 intensity varied substantially among patients and that these variations lacked short-term prognostic significance. These studies suggest that individual surface antigens such as CD5 may show variability among patients with CLL without affecting the diagnosis or possibly even the natural history of this disorder. Alternatively, Matutes et al[18] and Moreau et al[19] used a scoring system based on different patterns of surface antigen expression to distinguish CLL from other diseases. Thus, while immunophenotypic diversity in CLL and similar disorders is well recognized, its significance and role in diagnosis remain uncertain and controversial.

Although reports of CD5⁻ CLL have been published, many experts believe that CD5⁻ CLL is a disease other than CLL with a different natural history, response to treatment, and prognosis and that the patients should not be eligible for CLL protocols.[5] Geisler et al[13] reported that 37 (6.8%) of 540 consecutive cases of CLL were CD5⁻ and that these patients had higher Rai stage and borderline shorter survival than patients with CD5⁺ CLL. These cases tended to be FMC7 positive and CD23⁻. Salomon-Nguyen et al[20] reported similar findings in a series of 12 patients with CD5⁻ CLL. Compared with patients with CD5⁺ CLL, these patients more frequently had an advanced clinical stage of the disease, splenomegaly, a low hemoglobin concentration, high density surface immunoglobulin, negative CD23 expression, and mixed cellular morphologic features. In these 2 studies, however, the lack of CD23 expression in patients reportedly having CD5⁻ CLL suggests that a subset may have been cases of MCL instead of CLL. Kurec et al[21] described 12 patients with a CD5⁻ lymphocytosis and lower hemoglobin concentration, a more advanced disease stage, and decreased survival. DeRossi et al[22] described 359 patients with CLL, 25 (7.0%) of whom had CD5⁻ CLL. In contrast to the other reports, their patients with CD5⁻ CLL did not differ clinically from the patients with CD5⁺ CLL. With the exception of the study by Salomon-Nguyen et al[20] in which a significant percentage of patients with CD5⁻ CLL had increased prolymphocytes, lymphocyte morphologic features in the other studies was described as consistent with a diagnosis of typical CLL.

The 15 patients with CD5⁻ CLL in the present study were similar to the CD5⁻ CLL patients described by others. In contrast to other series, the majority of patients with CD5⁻ CLL in the present study demonstrated expression of CD23, which further strengthens the resemblance of this subgroup with typical CLL. However, we also found that these patients had significantly lower WBC count, lower ALCs, splenomegaly, and a more advanced stage compared with a control population of patients with CD5⁺ CLL. Thus, the differences in laboratory findings, clinical stage, and CD5 expression at manifestation suggest that these patients may have a disease process that differs from CLL. Furthermore, Ikematsu et al[23] noted that cases of CD5⁻ CLL demonstrated differences in IgV_H heavy chain gene switching and CD13 expression compared with cases of CD5⁺ CLL. Whether these differences imply a less favorable prognosis remains to be determined. A longer follow-up period is needed to reliably assess the survival experience of these disease groups.

Several of the patients in the present study were believed, based on initial clinical data, to have *atypical CLL.* This term, however, is poorly defined, and whether it represents a distinct disease process, a variant of CLL, or a heterogeneous group of cases that may include other low-grade B-LPDs is unclear. The authors of the proposed FAB classification[8] recognized that "there are always some cases with atypical features that do not fit exactly in any of the named diagnoses," but no further discussion of the diagnostic criteria or significance of atypical CLL was included. Criel et al[24] defined atypical CLL as cases in which 1 or more of the criteria for typical CLL were not met. Matutes et al[9] defined atypical CLL by the presence of more than 15% of the lymphocytes having a cleaved nucleus, lymphoplasmacytoid features, or both. In these studies, patients with atypical CLL had a higher incidence of trisomy 12, aberrant immunophenotypic patterns (including absence of CD5 expression), and a higher clinical stage at first examination. To further complicate the issue, some authors have regarded cases with a mixture of normal and reactive-appearing lymphocytes (so-called mixed appearance) as atypical CLL.[25] Whether cases that lack CD5 expression and have atypical nuclear morphologic features represent atypical CLL is also unclear. Since we could not reliably separate

atypical CLL from circulating MCL on the basis of morphologic features, we descriptively labeled these cases in our study as CD5⁻ MCL, and, in fact, examination of the lymph node biopsy specimens from 3 patients in this category later revealed histologic features suggestive of MCL.

The criteria for diagnosing MCL in the leukemic phase are not entirely clear. Weisenburger and Armitage[26] have stated that one should not make a diagnosis of MCL based on peripheral blood or bone marrow findings alone without examining a lymph node biopsy specimen. Nevertheless, the diagnosis of MCL in the leukemic phase is suggested when the neoplastic lymphocytes in the peripheral blood or bone marrow show more variability in size and nuclear morphologic features than the lymphocytes in typical CLL. The lymphocytes generally exhibit more irregularity to the nuclear outlines with nuclear clefts, lobulations, and more open chromatin than CLL cells.[27] Flow cytometric analysis of MCL generally reveals a CD5⁺, CD19⁺, CD23⁻ immunophenotype with strong surface light chain expression. Although this pattern is characteristic of MCL, cases of CD5⁻ MCL have been reported.[28] The distinction from CLL is thus suggested on the basis of morphologic features and immunophenotype. CD23, an IgE receptor linked to B-cell activation and proliferation, is advocated as an important discriminant between the 2 entities.[2,3] In earlier studies of MCL, however, flow cytometric analysis was performed only on lymph node biopsy specimens and not on peripheral blood specimens. In the present study, 7 of 10 patients with nuclear features suggestive of MCL were CD23⁺, and subsequent lymph node biopsy in 3 of 3 patients biopsied revealed histologic features consistent with MCL. However, it is unclear whether the characteristic immunophenotype of MCL in lymph nodes is found on neoplastic lymphocytes in peripheral blood or whether the use of CD23 as a discriminatory factor applies to cases of CD5⁻ B-LPDs. In our experience, it is difficult to distinguish atypical CLL from MCL, not only by clinical history and lymphocyte morphologic features, but also by the presence or absence of CD23 expression. It is therefore possible that patients with a CD5⁻/CD23⁺ lymphocytosis and nuclear morphologic features suggestive of MCL indeed have MCL. Additional studies using markers for *bcl*-1 gene rearrangement and overexpression of cyclin D1 may be necessary to confirm the diagnosis in these cases.[28] Although specimen examination for 1 patient in the present study demonstrated cyclin D1 by immunohistochemical staining, the limited number of patients in the present study with CD5⁻ MCL who underwent subsequent lymph node biopsy (n = 3) did not allow for meaningful statistical conclusions.

For the present study, we excluded cases in which a diagnosis of malignant non-Hodgkin lymphoma had been established by lymph node biopsy and bone marrow aspirate. The 5 patients in the present study who had been given a diagnosis of circulating NHL had anemia or thrombocytopenia, relatively low WBC counts and ALCs, and lack of palpable adenopathy. The lymphocyte morphologic features (Image 1C) in these patients were characterized by high nuclear/cytoplasmic ratios, deeply cleft nuclei, and scattered bizarre nuclear forms most consistent with a circulating follicle center lymphoma or other malignant non-Hodgkin lymphoma. Given the lack of an absolute lymphocytosis, the markedly atypical morphologic features of these cells, and the results of flow cytometry, these patients were believed to have a circulating NHL of B-cell origin. One patient subsequently underwent an excisional lymph node biopsy, which revealed follicle center lymphoma. It is unclear whether these patients have true primary bone marrow lymphomas or nodal-based disease that is not detected or not accessible at the time of initial examination. Similar cases have been reported by Bain et al[29] and Wong et al.[30] These patients may have a clonal proliferation of markedly atypical CD5⁻ B-cells with or without CD23 expression.

Patients with SLVL usually have splenomegaly without adenopathy, mild to moderate lymphocytosis with a characteristic morphologic features and immunophenotype,[10] and an IgM monoclonal gammopathy. Matutes et al[11] have suggested that in SLVL, more than 20% of the lymphoid cells have short, irregular, cytoplasmic villi in an uneven or polar distribution and a slightly enlarged nucleus with a small or prominent nucleolus. In addition, they report that the majority of cases are CD5⁻, CD19⁺, CD22⁺, and FMC7 positive with strong surface immunoglobulin expression. In the REAL classification,[1] a similar entity is proposed and called *splenic lymphoma without villous lymphocytes* but is not further defined. Interestingly, many of the patients with CD5⁻ CLL in the present study seem to have features similar to patients (in the present study and in the literature) with SLVL, including splenomegaly, advanced stage, and low-level monoclonal gammopathy. We speculate that some of the patients we and others describe as having CD5⁻ CLL may represent splenic lymphoma without villous lymphocytes or a variant of circulating monocytoid B-cell lymphoma.

One limitation of the present study was that peripheral blood morphologic features were used to separate patients into different subgroups. Although this approach is somewhat subjective, we know of no better way to classify these cases using the material available in the present study, especially since the majority of our cases lacked a tissue diagnosis. Although many patients underwent bone marrow biopsies, Wasman et al[31] have shown that the pattern of bone marrow involvement is inconclusive in separating known cases of MCL, SLL, and small cleaved cell lymphoma.[31] Furthermore, Wasman et al[31] state that cytologic comparisons also showed overlap among the 3 groups; however, they did not attempt to quantitate the percentage of atypical cells in individual cases. Similarly, the REAL classification states that "many B-cell neoplasms may occasionally show maturation to plasmacytoid or plasma cells containing CIg, including B-CLL . . . these

cases (should) be classified according to their major features, and not as lymphoplasmacytoid lymphoma"[1]; the criteria for separating CLL from LPL are not well established. Others have reported "borderline" cases of SLL with only a few monoclonal plasmacytoid cells or plasma cells.[32] One of the patients with CD5⁻ CLL in the present study demonstrated scattered monoclonal plasma cells; however, the patient lacked lymphoplasmacytoid differentiation as well as Waldenström macroglobulinemia. Thus, until more sensitive assays become available, we believe that morphologic features may be a useful starting point for separating the CD5⁻ B-LPD.

Many cases of CD5⁻ B-LPD are recognizable as distinct clinicopathologic entities, but occasional cases are encountered that may be difficult to classify. For the patients with "CLL-like" morphologic features and manifestations, we prefer the descriptive term *CD5⁻ CLL variant.* Although these patients share morphologic features with patients with typical CLL, the differences in clinical manifestations suggest that they represent a clinical or pathologic variant of CLL with possible implications for prognosis and treatment. A subset of CD5⁻ B-LPD with atypical nuclear morphologic features and CD23 expression may represent the leukemic phase of MCL. Since CD23 was expressed by the majority of CD5⁻ B-LPD patients, its use in subclassifying these disorders seems limited. Additional studies to identify *bcl*-1 gene rearrangements, cyclin D1 overexpression, or both in leukemic cells may be helpful with similar patients. Whether further subclassification of CD5⁻ B-LPD will eliminate diagnostic confusion or have prognostic significance is unclear. Long-term studies of treatment outcome are needed to answer these questions.

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References

1. Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood.* 1994;84:1361–1392.
2. Kilo MN, Dorfman DM. The utility of flow cytometric immunophenotypic analysis in the distinction of small lymphocytic lymphoma/chronic lymphocytic leukemia from mantle cell lymphoma. *Am J Clin Pathol.* 1996;105:451–457.
3. Zukerberg LR, Medeiros LJL, Ferry JA, et al. Diffuse low grade B cell lymphomas: four clinically distinct subtypes defined by a combination of morphologic and immunophenotypic features. *Am J Clin Pathol.* 1993;100:373–385.
4. Rozman C, Montserrat E. Chronic lymphocytic leukemia. *N Engl J Med.* 1995;333:1052–1056.
5. 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.
6. Jackson AL, Warner NL. Preparation, staining, and analysis by flow cytometry of peripheral blood leukocytes. In: Rose NR, Friedman H, Fakey JL, eds. *Manual of Clinical Laboratory Immunology.* 3rd ed. Washington, DC: ASM Press; 1986:226–235.
7. Segal GH, Edinger MG, Owen M, et al. Concomitant delineation of surface Ig, B-cell differentiation antigens, and HLADR on lymphoid proliferations using three-color immunocytometry. *Cytometry.* 1991;12:350–359.
8. Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of chronic (mature) B and T lymphoid leukaemias. *J Clin Pathol.* 1989;42:567–584.
9. Matutes E, Oscier D, Garcia-Marco J, 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;92:382–388.
10. Troussard X, Valensi F, Duchayne E, et al. Splenic lymphoma with villous lymphocytes: clinical presentation, biology, and prognostic factors in a series of 100 patients. *Br J Haematol.* 1996;93:731–736.
11. Matutes E, Morilla R, Owusu-Ankoma K, et al. The immunophenotype of splenic lymphoma with villous lymphocytes and its relevance to the differential diagnosis with other B-cell disorders. *Blood.* 1994;83:1558–1562.
12. Miller ML, Fishleder AJ, Tubbs RR. The expression of CD22 (Leu14) and CD11c (LeuM5) in chronic lymphoproliferative disorders using two-color flow cytometric analysis. *Am J Clin Pathol.* 1991;96:100–108.
13. Geisler CH, Larson JK, Ebbe Hansen N, et al. Prognostic importance of flow cytometric immunophenotyping of 540 consecutive patients with B-cell chronic lymphocytic leukemia. *Blood.* 1991;78:1795–1802.
14. Huang JC, Finn WG, Variakoijis D, et al. CD5 negative (–) chronic B cell leukemias are rarely classifiable as B cell chronic lymphocytic leukemia (B-CLL) [abstract]. *Mod Pathol.* 1997;10:127A.
15. Newman RA, Peterson B, Davey FR, et al. Phenotypic markers and BCL-1 gene rearrangements in B-cell chronic lymphocytic leukemia: a cancer and leukemia group B study. *Blood.* 1993;82:1239–1246.
16. Batata A, Shen B. Immunophenotyping of subtypes of B-chronic (mature) lymphoid leukemia. *Cancer.* 1992;70:2436–2443.
17. Tefferi A, Bartholmai BJ, Witzig TE, et al. Heterogeneity and clinical relevance of the intensity of CD20 and immunoglobulin light chain expression in B-cell chronic lymphocytic leukemia. *Am J Clin Pathol.* 1996;106:457–461.
18. Matutes E, Owusu-Ankomah KA, Morilla R, et al. The immunologic profile of B-cell disorders and proposal of a scoring system for the diagnosis of CLL. *Leukemia.* 1994;8:1640–1645.

19. Moreau EJ, Matutes E, A'Hern RP, et al. Improvement of the chronic lymphocytic leukemia scoring system with the monoclonal antibody SN8 (CD79b). *Am J Clin Pathol.* 1997;108:378–382.
20. Salomon-Nguyen F, Valensi F, Merle-Beral H, et al. A scoring system for the classification of $CD5^-$ B-CLL *versus* $CD5^+$ B CLL and B PLL. *Leuk Lymphoma.* 1995;16:445–450.
21. Kurec AS, Threatte GA, Gottlieb AJ, et al. Immunophenotypic subclassification of chronic lymphocytic leukaemia (CLL). *Br J Haematol.* 1992;81:45–51.
22. DeRossi 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.
23. 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 leukemia. *Blood.* 1994;83:2602–2610.
24. Criel A, Wlodarska I, Meeus P, et al. Trisomy 12 is uncommon in typical chronic lymphocytic leukaemias. *Br J Haematol.* 1996;87:523–528.
25. Bonato M, Pittaluga S, Tierens A, et al. Lymph node histology in typical and atypical chronic lymphocytic leukemia. *Am J Surg Pathol.* 1998;22:49–56.
26. Weisenburger DD, Armitage JO. Mantle cell lymphoma: an entity comes of age. *Blood.* 1996;87:4483–4494.
27. Brunning RD, McKenna RW. Small lymphocytic leukemias and related disorders. In: *Tumors of the Bone Marrow. Atlas of Tumor Pathology.* Fascicle 9, 3rd series. Washington, DC: Armed Forces Institute of Pathology; 1993:275.
28. Kaptain S, Zukerberg LR, Ferry JA, et al. BCL-1/Cyclin $D1^+$ $CD5^-$ mantle cell lymphoma [abstract]. *Mod Pathol.* 1998;11:133A.
29. Bain B, Matutes E, Robinson D, et al. Leukaemia as a manifestation of large cell lymphoma. *Br J Haematol.* 1991;77:301–310.
30. Wong KF, Chan JKC, Ng CS, et al. Large cell lymphoma with initial presentation in the bone marrow. *Hematol Oncol.* 1992;10:261–271.
31. Wasman J, Rosenthal NS, Farhi DC. Mantle cell lymphoma: morphologic findings in bone marrow involvement. *Am J Clin Pathol.* 1996;106:196–200.
32. Papadimitriou CS, Muller-Hermelink U, Lennert K. Histologic and immunohistochemical findings in the differential diagnosis of chronic lymphocytic leukemia of B-cell type and lymphoplasmacytic/lymphoplasmacytoid lymphoma. *Virchows Arch A Pathol Anat Histol.* 1979:384:149–158.