ORIGINAL ARTICLE

Monoclonal B-Cell Lymphocytosis and Chronic Lymphocytic Leukemia

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ABSTRACT

BACKGROUND

A diagnosis of chronic lymphocytic leukemia (CLL) requires a count of over 5000 circulating CLL-phenotype cells per cubic millimeter. Asymptomatic persons with fewer CLL-phenotype cells have monoclonal B-cell lymphocytosis (MBL). The goal of this study was to investigate the relation between MBL and CLL.

METHODS

We investigated 1520 subjects who were 62 to 80 years of age with a normal blood count and 2228 subjects with lymphocytosis (>4000 lymphocytes per cubic millimeter) for the presence of MBL, using flow cytometry. Monoclonal B cells were further characterized by means of cytogenetic and molecular analyses. A representative cohort of 185 subjects with CLL-phenotype MBL and lymphocytosis were monitored for a median of 6.7 years (range, 0.2 to 11.8).

RESULTS

Monoclonal CLL-phenotype B cells were detected in 5.1% of subjects (78 of 1520) with a normal blood count and 13.9% (309 of 2228) with lymphocytosis. CLL-phenotype MBL had a frequency of 13q14 deletion and trisomy 12 similar to that of CLL and showed a skewed repertoire of the immunoglobulin heavy variable group (*IGHV*) genes. Among 185 subjects presenting with lymphocytosis, progressive lymphocytosis occurred in 51 (28%), progressive CLL developed in 28 (15%), and chemotherapy was required in 13 (7%). The absolute B-cell count was the only independent prognostic factor associated with progressive lymphocytosis. During follow-up over a median of 6.7 years, 34% of subjects (62 of 185) died, but only 4 of these deaths were due to CLL. Age above 68 years and hemoglobin level below 12.5 g per deciliter were the only independent prognostic factors for death.

CONCLUSIONS

The CLL-phenotype cells found in the general population and in subjects with lymphocytosis have features in common with CLL cells. CLL requiring treatment develops in subjects with CLL-phenotype MBL and with lymphocytosis at the rate of 1.1% per year.

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The incidence of chronic lymphocytic leukemia (CLL) is approximately 6 per 100,000 persons per year in North America.[1] The National Cancer Institute–sponsored Working Group guidelines for the diagnosis of CLL require a lymphocyte count of 5000 or more per cubic millimeter and a characteristic cell-surface phenotype of B cells: the presence of CD19, CD5, and CD23, weak expression of CD20 and CD79b, and either kappa or lambda immunoglobulin light chains.[2] High-sensitivity flow cytometry allows for the detection of B cells with a CLL phenotype in numbers as low as 1 per 10,000 normal leukocytes.[3] With this method, CLL-phenotype cells have been found in over 3% of adults with otherwise normal blood counts.[4,5]

The term monoclonal B-cell lymphocytosis (MBL) indicates the presence in the blood of monoclonal B cells in numbers below 5000 per cubic millimeter with no other features of a B-cell lymphoproliferative disorder. The term has been used to designate an expansion of monoclonal B cells of uncertain clinical significance. In subjects with MBL, the B cells usually have a CLL-phenotype, although MBL without a CLL phenotype has been noted.[6]

The International Workshop on Chronic Lymphocytic Leukemia has revised the diagnostic criteria for CLL to require 5000 or more CLL-phenotype B cells per cubic millimeter because the National Cancer Institute Working Group guidelines could permit a diagnosis of CLL in a subject with a reactive T-cell lymphocytosis.[7] Lymphocytosis with fewer than 5000 CLL-phenotype B cells per cubic millimeter and an absence of symptoms of CLL is defined as CLL-phenotype MBL. It is not known whether CLL-phenotype MBL is associated with any of the characteristic abnormalities of CLL, such as the 13q14 deletion[8,9] or biased usage of specific immunoglobulin heavy variable group (*IGHV*) genes.[10]

We investigated two cohorts with CLL-phenotype MBL. The first consisted of subjects with entirely normal blood counts who were screened for CLL-phenotype MBL. The second consisted of subjects who were shown to have CLL-phenotype MBL after referral for the investigation of lymphocytosis between 1995 and 2000 and for whom long-term follow-up data were available. The aim of the study was to determine whether chromosomal abnormalities of CLL also occur in CLL-phenotype MBL and to estimate the probability that CLL requiring treatment will develop in someone with CLL-phenotype MBL.

Methods

Selection of Subjects

Table 1 lists the characteristics of the two cohorts. The first cohort comprised 890 women and 630 men, all outpatients who met the following criteria: age between 60 and 80 years, normal leukocyte and differential counts, a normal platelet count, and a normal hemoglobin level; a blood sample less than 24 hours old; and visiting a general-practice, ophthalmology, gynecology, cardiology, dermatology, or orthopedic preoperative clinic or the emergency department. Subjects were excluded if they had been visiting a hematology, oncology, or transplantation clinic or had had a sample sent for investigation of a cancer in the past. All subjects meeting these criteria during the study period were investigated for the presence of MBL.

The second cohort comprised 2228 subjects who were referred for investigation of a current or previous lymphocytosis between April 18, 1995, and December 12, 2000. The normal range for lymphocytes at our institution is 1000 to 4500 cells per cubic millimeter, but the upper limit of the normal range varies at referring centers, from 4000 to 4800 cells per cubic millimeter. In some subjects, the lymphocytosis had resolved by the time a sample from a referring institution was analyzed.

Oral informed consent was provided for blood analysis. Approval was granted by the Leeds Teaching Hospitals Research Ethics Committee to perform biologic studies on discarded blood specimens from anonymous patients and to review the outcomes of subjects presenting with lymphocytosis. Subjects from centers that performed follow-up or blood counts only on selected subjects were excluded from the outcome review. There were no significant differences in age, sex, B-cell count, or other blood-count values between the 185 subjects studied in the outcome review and all 309 subjects with CLL-phenotype MBL and lymphocytosis ($P>0.10$ for all values by the Wilcoxon–Mann–Whitney U test).

Flow Cytometry and Cell Purification

Leukocytes were prepared using ammonium chloride as reported previously.[5] To screen for MBL in samples with a normal blood count, 5×10^5 cells

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Table 1. Characteristics and Outcomes of Subjects with CLL-Phenotype MBL, According to Cohort.*

	Subjects with Normal Blood Count	Subjects with Lymphocytosis
CLL-phenotype MBL — no./total no. (%)	78/1520 (5.1)	309/2228 (13.9)
Male:female ratio	0.71:1	0.93:1
Age — yr		
Median	74	71
Range	62–80	39–99
B-cell count — per mm^3		
Median	140	3300
Range†	15–1248	100–4990
Lymphocyte count — per mm^3		
Median	2700	6000
Range‡	1300–4400	1100–16,800
Hemoglobin — g/dl		
Median	13.6	13.4
Range	10.9–16.0	6.2–20.3
Platelets — per mm^3		
Median	259,000	221,000
Range	142,000–492,000	67,000–487,000
Sequential monitoring — no./total no.		
Yes	0/78	185/309
No	78/78	124/309
Reason for exclusion from sequential monitoring	Material analyzed from anonymous subjects; follow-up not possible	Subjects monitored in hospitals with selective follow-up or by family doctor with no further blood sampling
Years of follow-up		
Median		6.7
Range		0.2–11.8
Progressive lymphocytosis — no./total no. (%)		51/185 (28)
Evidence of progressive CLL — no./total no. (%)		28/185 (15)
Lymphadenopathy or splenomegaly		17/28
Anemia or thrombocytopenia		4/28
Lymphocyte doubling time <6 mo		6/28
Sweats or infection		5/28
Chemotherapy for CLL — no./total no. (%)		13/185 (7)
Death — no./total no.		
From any cause		62/185
With progressive CLL		13/62
CLL recorded as a cause of death		4/62

* The 1520 subjects with entirely normal blood counts were screened for CLL-phenotype MBL. The 2228 subjects with lymphocytosis (defined as a lymphocyte count of >4000 per cubic millimeter), detected between 1995 and 2000, were tested for monoclonal B cells as part of routine investigation of lymphocytosis and had long-term follow-up data available. CLL denotes chronic lymphocytic leukemia, and MBL monoclonal B-cell lymphocytosis.

† The reference range for B-cell count at our institution is 25 to 490 per cubic millimeter.

‡ The reference range for lymphocyte count at our institution is 1000 to 4500 per cubic millimeter; at referring centers, the upper limits vary between 4000 and 4800. In some patients referred for investigation of lymphocytosis, the lymphocyte count had normalized by the time immunophenotyping was performed.

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were incubated for 30 minutes with 5 μl each of anti-CD19 phycoerythrin–cyanin 5.5, anti-CD5 allophycocyanin, anti-kappa fluorescein isothiocyanate, and anti-lambda phycoerythrin. Subjects with a clonal B-cell excess (kappa:lambda ratio <1:1 or >2.1:1) were assessed with the use of an extended panel (described in the Supplementary Appendix, available with the full text of this article at www.nejm.org).

B cells were purified from separated leukocytes obtained from subjects with CLL-phenotype MBL, with the use of anti-CD19–coated magnetic beads and a cell separator (autoMACS, Miltenyi Biotec). In samples with more than 95% CD19 expression on selected cells, cytospin slides were prepared, air-dried overnight, and stored at –20°C; if a sufficient sample was available, genomic DNA was isolated (QIAamp DNA blood mini kit, Qiagen).

FLUORESCENCE IN SITU HYBRIDIZATION (FISH)

Cytospin slides were fixed in methanol acetic acid and glacial acetic acid (3:1) and pretreated in 2× saline sodium citrate (SSC) and 0.1% Nonidet P-40 and dehydrated in an ethanol series. Five microliters of probe in 50% formamide hybridization buffer was applied. Probes were either a mixture of 13q14 and 13q34 or a set of CLL multicolor probes (05J81-001, 05J80-001, and 05J83-001; Abbott Molecular). Cells and probe were codenatured at 73°C for 3 minutes and hybridized overnight at 37°C. Post-hybridization stringency washing was carried out at 69°C during two 2-minute cycles in 0.4× SSC and 0.3% Nonidet P-40, followed by 2 minutes in 2× SSC and 1% Nonidet P-40. Cells counterstained with 4′,6-diamidine-2-phenylidole dihydrochloride were examined under a fluorescence microscope (Axioplan 2, Zeiss); images were captured by a camera (IMAC-CCD S30, Sony) and processed with the use of ISIS3 software (MetaSystems).

IGHV GENE ANALYSIS

DNA was amplified with the use of BIOMED-2 *IGHV* 5-carboxyfluorescein–labeled primers for fragment analysis, as described previously,[5] by means of an ABI 377 sequencer and GENESCAN software (Applied Biosystems). Monoclonal samples were reamplified with the use of nonfluorescent primers, the products were purified on 2% agarose gels (Qiaquick Gel Extraction kit, Qiagen), and direct sequencing was performed by means of a BigDye Terminator cycle sequencing kit on an ABI 377 sequencer (Applied Biosystems). Rearranged *IGHV* segments were identified by comparison with the germ-line sequences by means of IgBlast (www.ncbi.nlm.nih.gov/igblast) and IMGT/V-QUEST (http://imgt.cines.fr). The percent mutation from the closest germ-line *IGHV* sequence was calculated from the number of nucleotide differences between the 5′ end of framework 1 and the 3′ end of framework 3, as a percentage of total nucleotides.

STATISTICAL ANALYSIS

We determined the degree of association between the presence of chromosomal abnormalities and *IGHV* gene usage using Fisher's exact test and the likelihood-ratio chi-square analysis. Univariate log-rank outcome analysis was performed on groups with the use of cutoff points defined by the highest Youden's J value (a measure of the receiver-operating-characteristic curve that enables the selection of an optimal cutoff point) for predicting the development of progressive lymphocytosis or death. Progressive lymphocytosis was defined as a lymphocyte count that was more than twice the count at presentation and remained at this level or increased at subsequent assessments. Multivariate analysis was performed with the use of the Cox proportional-hazards model, and assumptions were tested on the basis of Schoenfeld residuals. All variables were tested for intercorrelation (by Spearman's rank test with Bonferroni correction) and those that generated interference in the multivariate analysis were excluded; CD38 expression by B cells was also excluded from multivariate analysis because it was not significant in the univariate analysis and because data were not available for all subjects. All P values were two-sided and calculated with the use of Stata 9.0 software (Statacorp); P values less than 0.05 were considered to indicate statistical significance.

RESULTS

PREVALENCE OF CLL-PHENOTYPE MBL

Among the 1520 subjects who were 62 to 80 years of age and had normal blood counts and no history of cancer, CLL-phenotype MBL was detected in 78 (5.1%) and non–CLL-phenotype MBL (i.e.,

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light-chain–restricted CD19+ B cells with no CD5 expression and strong CD20 expression) was identified in 27 (1.8%). In most subjects, the absolute B-cell count was within the normal range of 25 to 490 per cubic millimeter. Of the 2228 subjects referred for review for lymphocytosis who had a current or previous lymphocyte count above 4000 per cubic millimeter, CLL-phenotype MBL was detected in 309 (13.9%). CLL was diagnosed in 1031 of the 2228 subjects (46.3%), and a non-CLL B-cell abnormality or reactive lymphocytosis in 888 (39.9%).

CHROMOSOMAL ABNORMALITIES

Interphase FISH analysis was performed in 71 subjects with CLL-phenotype MBL in either cohort (Table 2). The proportion of subjects with a 13q14 deletion or trisomy 12 was similar to that seen among the subjects with CLL described by Döhner et al.[9]; 13q14 deletion was detected in 48% of our subjects and trisomy 12 in 20% (vs. 55% and 16%, respectively, in the study by Döhner et al.). Markers associated with poor prognosis (deletion of the ataxia–telangiectasia mutated gene *ATM* or the tumor protein p53 gene *TP53*) were detected in none of the subjects with CLL-phenotype MBL and a normal blood count and in only 3 of the 33 subjects (9%) with CLL-phenotype MBL and lymphocytosis.

IGHV GENE REPERTOIRE AND MUTATION STATUS

Direct *IGHV* sequencing was performed in 40 subjects with CLL-phenotype MBL (Table 2). The presence of more than 2% mutation from the germ-line sequence in the *IGHV* gene is a good prognostic factor for CLL, occurring in 55% (796 of 1447) of reported subjects with CLL (range, 47 to 58).[10-15] A total of 88% (35 of 40) subjects with CLL-phenotype MBL had more than 2% *IGHV* mutation. The *IGHV* repertoire was skewed, with over half the 40 subjects having rearranged *IGHV3-07*, *IGHV3-23*, or *IGHV4-34* segments, which account for less than one quarter of rearranged *IGHV* genes in normal B cells.[11] There was no significant difference in the *IGHV* repertoire between subjects with a normal blood count and those with lymphocytosis (P=0.46 by the likelihood-ratio chi-square analysis). In comparison with previously reported subjects with CLL,[10] the *IGHV* repertoire in all subjects with CLL-phenotype MBL was similar to that seen in subjects with CLL associated with a favorable prognosis, as defined by the presence of more than 2% *IGHV* mutation from the germ-line sequence (P=0.14 by the like-

Table 2. Chromosomal Abnormalities and *IGHV* Gene Usage and Mutation in Subjects with CLL-Phenotype MBL.

Abnormality or Usage	CLL-Phenotype MBL and Normal Blood Count	CLL-Phenotype MBL and Lymphocytosis*	CLL in Previously Reported Series†
Chromosomal abnormalities — no./total no. (%)			
13q14 Deletion	15/38 (39)	19/33 (58)	178/328 (54)
Trisomy 12	4/22 (18)	7/33 (21)	53/325 (16)
11q23 Deletion	0/21	2/33 (6)	58/325 (18)
17p Deletion	0/10	1/33 (3)	23/325 (7)
Percent *IGHV* mutation from germ-line sequence			
Median	6.6	6.5	
Range	0.5–13.7	1.1–11.8	
More than 2% *IGHV* mutation — no./total no. (%)	17/20 (85)	18/20 (90)	534/927 (58)
Predominant *IGHV* genes used			
>2% Mutation	*3-07, 3-23, 4-34*	*3-07, 3-23, 4-34*	*3-07, 3-23, 4-34*
≤2% Mutation	Not applicable	Not applicable	*1-69, 4-39*

* Lymphocytosis was defined as a lymphocyte count of more than 4000 per cubic millimeter.
† Data for chromosomal abnormalities are from Döhner et al.,[9] and data for *IGHV* mutation from the germ-line sequence are from Stamatopoulos et al.[10]

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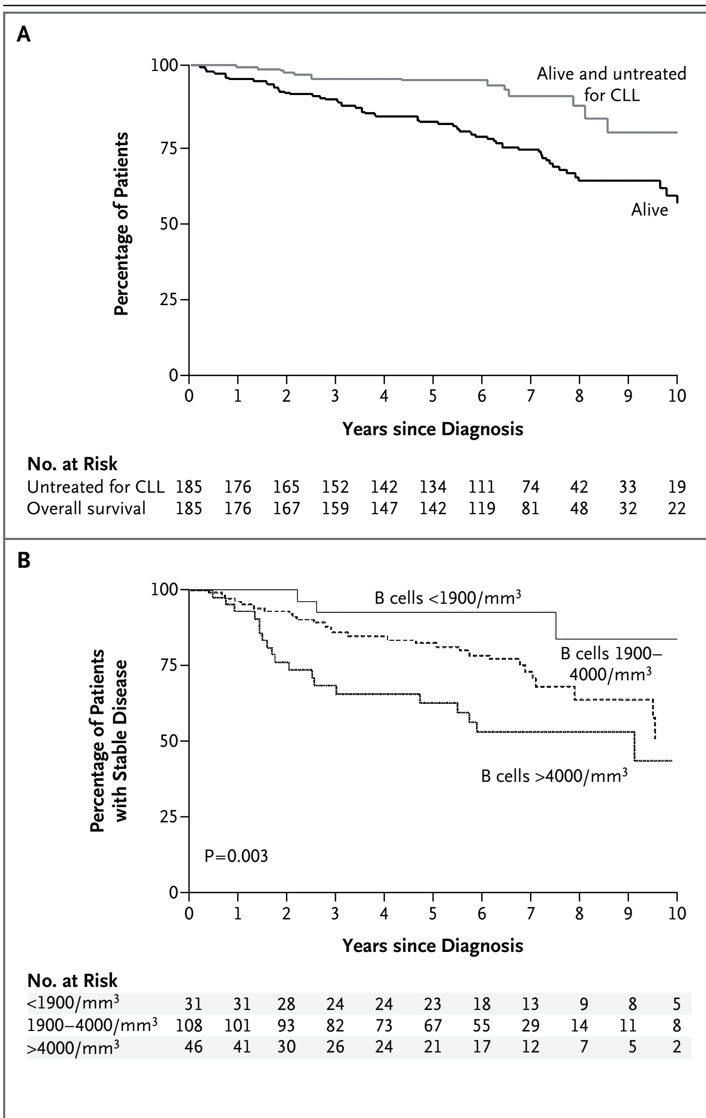

Figure 1. Kaplan–Meier Estimates of Outcomes among Subjects with CLL-Phenotype MBL and Lymphocytosis.

Panel A shows the proportion of subjects remaining alive and the proportion who were alive and remained free from treatment for CLL. Panel B shows the proportion of subjects with stable CLL-phenotype MBL, defined as the absence of symptoms or features of CLL and the maintenance of a stable lymphocyte count (a count less than twice that at presentation).

lihood-ratio chi-square analysis) but significantly different from the *IGHV* repertoire seen in subjects with CLL associated with a poor prognosis, as defined by the presence of *IGHV* mutation of less than or equal to 2% ($P<0.001$ by the likelihood-ratio chi-square analysis).

OUTCOMES IN SUBJECTS PRESENTING WITH LYMPHOCYTOSIS

Sequential monitoring with a median follow-up of 6.7 years (range, 0.2 to 11.8) was performed in 185 subjects with CLL-phenotype MBL referred for investigation of a current or previous lymphocyte count above 4000 per cubic millimeter. Figure 1 shows their Kaplan–Meier estimates of outcome, Table 1 lists their outcome data, and Table 3 lists the risk factors for progressive lymphocytosis and for death.

Progressive lymphocytosis was observed in 51 of the 185 subjects (28%) during the follow-up period, with a lymphocyte count above 30,000 per cubic millimeter occurring in 31 of the 51 subjects. The total lymphocyte count at presentation was a significant risk factor for progressive lymphocytosis in the univariate analysis but not the multivariate analysis. The previously used cutoff point of 5000 lymphocytes per cubic millimeter was not predictive of progressive lymphocytosis or the development of CLL. The B-cell count at presentation was the only significant independent factor that predicted progressive lymphocytosis, whether assessed using cutoff points or as a continuous variable. After a median of 6.9 years of follow-up (range, 0.2 to 11.5), there was little or no change in the total lymphocyte count in subjects with a B-cell count below 1900 per cubic millimeter at presentation. The hazard ratio for the development of progressive lymphocytosis was 1.46 (95% confidence interval [CI], 1.12 to 1.91) for each increase of 1000 B cells per cubic millimeter at presentation ($P=0.005$ by Cox proportional-hazards model).

Of the 51 subjects with progressive lymphocytosis, further evidence of progressive CLL, predominantly lymphadenopathy, developed in 28 (55%), and 13 of these 51 subjects eventually required chemotherapy (Table 1). Treatment was initiated at a median of 4.0 years (range, 1.1 to 10.1) after initial diagnosis. The estimated rate of progression to CLL requiring treatment among subjects with CLL-phenotype MBL presenting with lymphocytosis was 1.1% per year (95% CI, 0.7 to 1.9). None of the factors assessed — including age, sex, hemoglobin level, total lymphocyte count, T-cell count, B-cell count, and B-cell CD38 expression — predicted an increased risk of disease progression or the requirement for

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treatment. Of the 13 treated subjects, 7 are alive, with a median of 1.9 years (range, 0.0 to 8.6) of follow-up since the initiation of treatment.

There were 62 deaths among the subjects with CLL-phenotype MBL and lymphocytosis during follow-up. The age and hemoglobin level at diagnosis of CLL-phenotype MBL were the only independent factors associated with overall survival. By definition, anemia at the time of diagnosis of CLL-phenotype MBL was not autoimmune or due to bone marrow infiltration by CLL. Of the 62 subjects who died, 13 had progressive CLL, but this was noted as a cause of death in only 4.

DISCUSSION

MBL is a relatively new diagnostic category, which reflects the ability of high-sensitivity flow cytometry to detect CLL-phenotype cells at low levels during routine investigations for unrelated disorders or health screening. Separating CLL-phenotype MBL from CLL is important, because CLL-phenotype MBL does not necessarily evolve into CLL.

CLL-phenotype cells are detectable in over 3% of adults[4,5] and in over 10% of persons with more than two first-degree relatives affected by CLL.[16,17] The CLL phenotype is always associated with monoclonal surface immunoglobulin.[4,5,17] Preliminary reports indicate that proteins such as CD81 and messenger RNA such as lymphoid enhancer-binding factor 1, which are aberrantly expressed in CLL, show a similar pattern in CLL-phenotype MBL.[18,19] Our study found that CLL-phenotype cells can have chromosomal abnormalities, most notably the deletion of 13q14, which occurs at similar frequencies in CLL-phenotype MBL and in CLL. *IGHV* gene usage in CLL-phenotype MBL is skewed, with more than 87% of subjects having mutated *IGHV* genes. Therefore, even at the lowest levels of detection in persons with a normal blood count, CLL-phenotype B cells are, according to the currently available methods, biologically indistinguishable from CLL B cells.

Outcome data for persons with CLL-phenotype MBL have been limited to two studies involving fewer than 50 subjects and less than 5 years of follow-up.[20,21] In our study, during a median follow-up of 6.7 years, progressive CLL (characterized by lymphadenopathy, splenomegaly, anemia, thrombocytopenia, lymphocyte doubling time <6 months, persistent infection, or drench-

Table 3. Risks of Progressive Lymphocytosis and Death among Subjects with CLL-Phenotype MBL and Lymphocytosis, According to Feature at Presentation.*

Feature at Presentation	Progressive Lymphocytosis (N=51)				Death (N=62)			
	Cutoff Point or Category (no. of subjects)†	Univariate (Log-Rank) P Value	Multivariate (Cox) P Value	Hazard Ratio (95% CI)‡	Cutoff Point or Category (no. of subjects)†	Univariate (Log-Rank) P value	Multivariate (Cox) P Value	Hazard Ratio (95% CI)‡
Age — yr	68 (72/113)	0.59	0.47	1.24 (0.69–2.22)	68 (72/113)	<0.001	<0.001	3.15 (1.67–5.92)
Sex	M/F (89/96)	0.40	0.66	0.88 (0.49–1.56)	M/F (89/96)	0.24	0.38	1.28 (0.74–2.19)
Hemoglobin — g/dl	12.5 (38/147)	0.25	0.10	0.54 (0.26–1.13)	12.5 (38/147)	<0.001	<0.001	0.34 (0.20–0.59)
Platelet count — per mm³	295,000 (144/41)	0.35	0.48	0.75 (0.34–1.65)	216,000 (77/108)	0.02	0.49	0.83 (0.49–1.40)
Lymphocyte count — per mm³	6800 (143/42)	0.008	0.61	1.23 (0.56–2.66)	5700 (88/97)	0.10		
B-cell count — per mm³	1900/4000 (31/108/46)	0.005	0.01	2.03 (1.17–3.54)	3200 (96/89)	0.04	0.07	1.60 (0.96–2.68)
T-cell count — per mm³	1700 (131/54)	0.40	0.36	1.45 (0.66–3.16)	2600 (116/69)	0.002	0.06	0.53 (0.28–1.01)
B cells expressing CD38 — %§	2 (58/116)	0.54			5 (145/29)	0.14		

* The 5-year risk of progressive lymphocytosis was 26% (95% confidence interval [CI], 19 to 34) and of death 26% (95% CI, 20 to 33). Optimal cutoff points for outcome prediction were defined according to Youden's J value. These cutoff points were used for variables entered into the Cox proportional-hazards analysis of the defined groups.

† With the exception of sex and B-cell count among subjects with progressive lymphocytosis, the numbers in parentheses are the number of subjects with a value at or above the cutoff point and the number of subjects with a value below the cutoff point, respectively. For sex, the numbers in parentheses are the number of men and the number of women, respectively. Two cutoff points for B-cell count were identified for the risk of progressive lymphocytosis, since the J value peaked multiple times between the counts of 1900 and 4000 per cubic millimeter. The numbers in parentheses are the numbers of subjects with B-cell counts below 1900, between 1900 and 4000, and above 4000, respectively.

‡ Hazard ratios were calculated for having a value at or above the cutoff point as compared with a value below the cutoff point, except for sex, for which the hazard ratios were calculated for being male as compared with being female, and except for progressive lymphocytosis according to B-cell count, for which the hazard ratios were calculated for having a count at or below 4000 as compared with a value above 4000.

§ Data for B-cell expression of CD38 were not available for all subjects.

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ing night sweats) developed in 15% (28 of 185) of subjects with CLL-phenotype MBL with an initial lymphocyte count of more than 4000 per cubic millimeter. The annual risk of developing CLL requiring chemotherapy among subjects with CLL-phenotype MBL presenting with lymphocytosis was 1 to 2%, which is similar to the rate of progression to myeloma seen in patients with monoclonal gammopathy of undetermined significance (MGUS).[22] A further similarity to MGUS is that the majority of deaths of subjects with CLL-phenotype MBL are not due to CLL but to unrelated causes. Age and hemoglobin level were the only independent factors predicting death in our series. Anemia was not caused by the CLL-phenotype cells, because such a cause would exclude a diagnosis of MBL. The B-cell and T-cell counts at presentation were of borderline significance, and the relevance of these factors may become apparent in larger series.

Predicting outcome in subjects with CLL-phenotype MBL is difficult, because few subjects express markers associated with an adverse outcome in CLL, such as unmutated *IGHV* genes.[12,23] Moreover, progression can occur in subjects with CLL-phenotype MBL in whom B cells express markers associated with a good outcome in CLL. Lymphocyte doubling time is uninformative, because CLL-phenotype cells usually do not represent the majority of lymphocytes. Kaplan–Meier curves of disease progression showed no plateau over time, indicating that, as with MGUS, indefinite periodic monitoring is indicated for subjects with CLL-phenotype MBL presenting with a lymphocytosis.

Our data support the use of the B-cell count rather than lymphocyte count for the diagnosis of CLL or MBL. The cutoff point of 5000 lymphocytes per cubic millimeter does not predict outcome and allows for a diagnosis of CLL in someone with a reactive T-cell lymphocytosis. The age-standardized incidence rate in our region, since the diagnosis of CLL-phenotype MBL was introduced in 2005, is 2.4 cases per 100,000 per year, as compared with 5.8 cases of CLL. The incidence rate for CLL-phenotype MBL with a B-cell count above 1900 cells per cubic millimeter is 2 cases per 100,000 per year. The incidence rate is less than 1 case per 100,000 per year among persons younger than 40 years of age, rising steadily to 30 cases per 100,000 per year among adults over 70 years of age.[24]

In summary, we have found a biologic relation between CLL-phenotype MBL and CLL. The majority of deaths in persons with CLL-phenotype MBL are due to unrelated causes, but progressive CLL requiring chemotherapy will develop in a clinically significant proportion of subjects presenting with lymphocytosis.

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