Brief Report: Natural History of Individuals With Clinically Recognized Monoclonal B-Cell Lymphocytosis Compared With Patients With Rai 0 Chronic Lymphocytic Leukemia

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The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

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ABSTRACT

Purpose

The diagnosis of monoclonal B-cell lymphocytosis (MBL) is used to characterize patients with a circulating population of clonal B cells, a total B-cell count of less than $5 \times 10^9/L$, and no other features of a B-cell lymphoproliferative disorder including lymphadenopathy/organomegaly. The natural history of clinically identified MBL is unclear. The goal of this study was to explore the outcome of patients with MBL relative to that of individuals with Rai stage 0 chronic lymphocytic leukemia (CLL).

Patients and Methods

We used hematopathology records to identify a cohort of 631 patients with newly diagnosed MBL or Rai stage 0 CLL. Within this cohort, 302 patients had MBL (B-cell counts of 0.02 to $4.99 \times 10^9/L$); 94 patients had Rai stage 0 CLL with an absolute lymphocyte count (ALC) $\leq 10 \times 10^9/L$; and 219 patients had Rai stage 0 CLL with an ALC more than $10 \times 10^9/L$. Data on clinical outcome were abstracted from medical records.

Results

The percentage of MBL patients free of treatment at 1, 2, and 5 years was 99%, 98%, and 93%, respectively. B-cell count as a continuous variable (hazard ratio [HR] = 2.9, $P = .04$) and CD38 status (HR = 10.8, $P = .006$) predicted time to treatment (TTT) among MBL patients. The likelihood of treatment for MBL patients was lower (HR = 0.32, $P = .04$) than that of both Rai stage 0 CLL patients with an ALC less than $10 \times 10^9/L$ ($n = 94$) and Rai stage 0 CLL patients with an ALC more than $10 \times 10^9/L$ ($n = 219$; $P = .0003$).

Conclusion

Individuals with MBL identified in clinical practice have a low risk for progression at 5 years. Because B-cell count seems to relate to TTT as a continuous variable, additional studies are needed to determine what B-cell count should be used to distinguish between MBL and CLL.

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INTRODUCTION

The diagnosis of monoclonal B-cell lymphocytosis (MBL) is currently used to characterize patients who have a circulating population of clonal B cells, a total B-cell count of less than $5 \times 10^9/L$, and no other features of a lymphoproliferative disorder including lymphadenopathy or organomegaly.¹ In most patients, the immunophenotype is similar to that of chronic lymphocytic leukemia (CLL).^{1,2}

The natural history of CLL-phenotype MBL is unclear and could differ depending on whether patients are identified through population screening using highly sensitive assays²⁻⁴ or recognized in rou-

tine clinical practice when undergoing evaluation of low-grade lymphocytosis.⁵⁻⁷ Because population screening for MBL is not used outside of epidemiologic research studies, the issue oncologists currently encounter is how to counsel individuals with MBL identified in routine practice.⁵⁻⁸ There are minimal data on the natural history of clinically identified MBL,^{5,9} and there is even less information regarding whether it differs from Rai stage 0 CLL.⁶ To develop relevant information on the latter, we used the Mayo Clinic hematopathology records to identify patients with CLL-phenotype MBL diagnosed in routine clinical practice along with a comparison group of concurrently diagnosed patients with Rai stage 0 CLL to compare clinical outcomes.

PATIENTS AND METHODS

Patients

With the approval of the Mayo Clinic Institutional Review Board, we used hematopathology records to identify a cohort of 1,815 patients who had peripheral-blood flow cytometry at Mayo Clinic between January 2000 and December 2006 that detected a clonal B-cell population of CLL phenotype.^{10,11} After elimination of patients with more advanced-stage (Rai stages I to IV) CLL, previously treated CLL, or an established diagnosis of CLL/MBL more than 1 year before the flow cytometric study, 631 unique patients were identified.

On the basis of our prior analysis⁸ demonstrating that nearly all patients with an absolute lymphocyte count (ALC) more than $10 \times 10^9/L$ also have a B-cell count more than $5 \times 10^9/L$, of 631 patients, we classified the 219 patients with an ALC more than $10 \times 10^9/L$ as Rai 0 CLL with ALC more than $10 \times 10^9/L$ but pursued additional analysis in the 396 patients who had an ALC less than $10 \times 10^9/L$ and the necessary data from flow cytometry analysis to determine the B-cell count. All peripheral-blood samples were evaluated by multicolor flow cytometric immunophenotyping analysis on a FACS Calibur flow cytometer (BD Biosciences, San Jose, CA) within 24 hours of blood sampling according to previously described methods using fluorochrome-conjugated antibodies.¹¹ The proportions of B cells, T cells, and natural killer cells were determined in each patient and normalized to 100% lymphocytes, and then an absolute count of each lymphocyte subset (B, T, and natural killer cells) was calculated using a simultaneously run (± 10 days) CBC with differential cell counts. In addition to the number of total B cells, the number of clonal B cells (CD19, CD5 coexpression) was determined among patients with an absolute B-cell count less than $1.5 \times 10^9/L$ using the same methods to assess how the proportion of B cells that are clonal relates to outcome among low-count MBL patients. The threshold of less than $1.5 \times 10^9/L$ was chosen for this analysis because it was the upper limit of the B-cell range in the early studies of MBL among the normal population² (we have previously shown that as the B-cell count increases, nearly all B cells are clonal B cells¹²). On the basis of current criteria, patients with a total B-cell count less than $5 \times 10^9/L$ were classified as having MBL.^{1,10} Patients with a total B-cell count more than $5 \times 10^9/L$ but an ALC less than $10 \times 10^9/L$ were classified as low-count, Rai 0 CLL (Appendix Fig A1, online only).

Clinical information regarding date of diagnosis, physical examination, prognostic parameters, treatment history, and disease-related complications were abstracted from clinical records. Prognostic testing performed as part of clinical or research studies, including evaluation of *IgHV* gene mutation analysis, ZAP-70 status, CD38 status, and cytogenetics abnormalities by interphase fluorescent in situ hybridization (FISH), was performed as previously described by our group.¹³⁻¹⁵

Statistical Methods

Time to treatment (TTT) was defined as the time between date of diagnosis and date of initiation of first treatment or date of last follow-up at which patient was known to be untreated. Overall survival (OS) was defined as the time between date of diagnosis and date of death or date of last follow-up. The accepted indications to initiate treatment for CLL were based on the National Cancer Institute Working Group 1996 criteria during the study interval.¹⁶ Estimates of TTT and OS were calculated using the Kaplan-Meier method. Cox proportional hazards models were used to model the relationship of B-cell count or other prognostic parameters with TTT and OS. Likelihood ratio tests were used to test the effects of each individual factor. Hazard ratios (HRs) and 95% CIs were calculated from the Cox models. $P < .05$ was considered significant.

RESULTS

We identified 631 patients seen at Mayo Clinic between January 2000 and December 2006 who had a circulating clonal population of CLL phenotype on flow cytometry and no other characteristics of a lymphoproliferative disorder (eg, palpable lymphadenopathy, organo-

megaly, cytopenia caused by marrow infiltration, and so on). Of these, 219 patients had an ALC more than $10 \times 10^9/L$ and were classified as Rai 0 CLL with ALC more than $10 \times 10^9/L$. Among the 396 patients with an ALC less than $10 \times 10^9/L$, 302 patients (76%) had a B-cell count less than $5 \times 10^9/L$ at the time of flow cytometry analysis and met the current criteria for the diagnosis of MBL.¹ The remaining 94 patients (24%) had a B-cell count more than $5 \times 10^9/L$ (with an ALC $< 10 \times 10^9/L$) and were considered low-count, Rai 0 CLL.¹⁰ Lymphocytosis (ALC $> 3 \times 10^9/L$) was present in 263 (87%) of the 302 patients with MBL, and the median B-cell count was ($2.76 \times 10^9/L$), which is slightly lower than that reported in the only other large MBL series (median, $3.3 \times 10^9/L$)⁵ reporting these data. Median age at the time of MBL diagnosis was 69 years (range, 34 to 93 years). The age, sex distribution, and hemoglobin levels of patients with MBL were similar to those of patients with low-count, Rai 0 CLL, although the median platelet count was slightly higher among MBL patients (Table 1).

Because CD38, ZAP-70, *IgHV* gene mutation status, and FISH analysis were not routinely performed during the entirety of the study period, results were not available for all patients. In MBL patients, CD38 status was available for 274 patients (91%), ZAP-70 status was available for 120 patients (40%), *IgHV* gene mutation status was available for 109 patients (36%), and cytogenetic analysis by FISH was available for 126 patients (42%). Results of prognostic testing are listed in Table 1. No significant differences in prognostic test parameters were observed between patients with MBL and those with low-count, Rai 0 CLL.

A follow-up lymphocyte count more than 3 months (range, 3 to 95 months) after the baseline count was available for 210 MBL patients (70%). Although a persistent absolute lymphocytosis ($> 3 \times 10^9/L$) was present in 182 (95%) of 192 patients with lymphocytosis at baseline, only 66 patients (36%) experienced a doubling in lymphocyte count during follow-up.

Patients underwent sequential monitoring with a median follow-up time of 18 months (range, 0 to 97 months). For individuals with MBL, the proportion of patients free of treatment was 99% (95% CI, 98% to 100%) at 1 year, 98% (95% CI, 96% to 100%) at 2 years, and 93% (95% CI, 86% to 100%) at 5 years (Fig 1A). During the first 5 years of follow-up, the rate of progression requiring treatment for the MBL patients in our series was approximately 1.4% per year. Primary indications for treatment among MBL patients were progressive lymphadenopathy ($n = 2$), marrow failure/cytopenia ($n = 3$), progressive lymphocytosis ($n = 1$), and autoimmune hemolytic anemia ($n = 1$).

We next evaluated how total B-cell count and clonal B-cell count related to clinical outcome. Total B-cell count as a continuous variable (HR = 2.9; 95% CI, 1.1 to 8.2; $P = .04$) was associated with TTT. Although we have previously shown that nearly all B cells among individuals with MBL are clonal as the B-cell count increases,¹² clonal B cells may represent a small component of the total B-cell count in some MBL patients with lower B-cell counts. To evaluate this aspect, we measured the clonal B-cell count among the 64 individuals with MBL whose total B-cell count was less than $1.5 \times 10^9/L$ and who had treatment information available. The clonal B-cell percentage ranged from 51.9% to 99.7% (median, 93.9%) in these individuals (absolute B-cell counts of 0.015 to $1.46 \times 10^9/L$; median, $0.76 \times 10^9/L$). Despite this variability in the proportion of clonal B cells, only one (1.5%) of 64 individuals whose total B-cell count was less than $1.5 \times 10^9/L$ required

Table 1. Patient Demographics and Clinical and Laboratory Characteristics of Patients With ALC $\leq 10 \times 10^9/L$ (n = 396)

Characteristic	MBL (n = 302; B cell $< 5 \times 10^9/L$)		Low-Count, Rai 0 CLL (n = 94; B cell $\geq 5 \times 10^9/L$; ALC $\leq 10 \times 10^9/L$)		P
	No. of Patients	%	No. of Patients	%	
Age, years					.23
Median	69		70		
Range	34-93		45-92		
< 50	11	4	2	2	
50-60	61	20	20	21	
61-70	90	30	26	28	
> 70	140	46	46	49	
Male	175	58	58	62	.52
ALC, $\times 10^9/L$					< .0001
Median	5.4		8.0		
Range	0.3-9.6		5.7-10.0		
B-cell count, $\times 10^9/L$					< .0001
Median	2.76		6.06		
Range	0.02-4.99		5.0-9.29		
Hemoglobin, g/dL					.86
Median	13.8		13.9		
Quartile 1	12.7		12.8		
Quartile 3	15.0		14.6		
Platelets, $\times 10^9/L$					.01
Median	226		208		
Quartile 1	184		177		
Quartile 3	283		252		
CD38					.33
Positive ($\geq 30\%$)	60	22	15	17	
Negative ($< 30\%$)	214	78	73	83	
Missing	28		6		
ZAP-70					.47
Positive ($\geq 20\%$)	23	19	10	24	
Negative ($< 20\%$)	97	81	31	76	
Missing	182		53		
<i>IgHV</i> gene mutation status					.56
Unmutated	25	23	7	18	
Mutated	84	77	31	82	
Missing	193		56		
FISH result					.58
13q-	56	44	19	46	
Normal	39	31	15	37	
+12	23	18	4	10	
11q-	2	2	2	5	
17p-	4	3	1	2	
Other (6q-)	2	2	0	0	
Missing	176		53		
Sequential lymphocyte count monitoring available	210	70	69	73	.47
Doubling lymphocyte count during follow-up	74	35	31	45	.15
Developed lymphadenopathy	46	15	20	21	.17
Received chemotherapy for CLL	7	2	9	10	—*
Deceased	44	15	20	21	.15

Abbreviations: ALC, absolute lymphocyte count; MBL, monoclonal B-cell lymphocytosis; CLL, chronic lymphocytic leukemia; FISH, fluorescent in situ hybridization.
*See Figure 1.

treatment. Similarly, no difference in survival was observed based on the percentage of clonal B cells among individuals whose total B-cell count was less than $1.5 \times 10^9/L$ ($P = .71$). These results indicate that having a high proportion of clonal B cells does not necessarily indicate a higher risk for progression among individuals with a total B-cell count less than $1.5 \times 10^9/L$.

With respect to other demographic and prognostic parameters, CD38 status (HR = 10.8; 95% CI, 2.0 to 59.5; $P = .006$) was associated with TTT (Fig 2), but age ($P = .62$) and sex ($P = .99$) were not. Because of an insufficient number of patients with ZAP-70, *IgHV* gene mutation status, or FISH analysis, we could not accurately evaluate the correlation of these variables with TTT or OS.

Fig 1. Time to treatment/overall survival of 302 individuals with clinically recognized monoclonal B-cell lymphocytosis. CLL, chronic lymphocytic leukemia.

Likelihood of treatment for MBL patients was substantially lower ($HR = 0.32$, $P = .04$) than that of low-count, Rai 0 CLL patients with B-cell counts between 5.0 and $10.0 \times 10^9/L$ ($n = 94$) as well as patients with Rai stage 0 CLL who had an ALC more than $10.0 \times 10^9/L$ ($n = 219$; $P = .0003$; Fig 3). To date, no difference in OS has been observed between these three patient groups ($P = .45$).

DISCUSSION

Because it is only a recently defined diagnostic entity,¹ there is little clinical information on the natural history of individuals with MBL. We observed a low rate of progression among individuals with CLL-phenotype MBL identified in our routine clinical practice. Our study is only the third series of MBL patients to include more than 50 individuals^{5,9} and, to our knowledge, is the largest reported series of molecular/biologic prognostic parameters in MBL patients. Notably, the rate of progressive lymphocytosis and need for treatment in our series are similar to those reported by Rawstron et al⁵ in a cohort of 185 patients with clinically identified MBL who underwent sequential monitoring.

Importantly, our study is the first, to our knowledge, to compare the outcome of a large series of clinically identified MBL patients to a contemporaneous cohort of patients with newly diagnosed, Rai stage 0 CLL with comparable clinical follow-up. The MBL patients in our

Fig 2. Time to treatment among 274 monoclonal B-cell lymphocytosis patients based on whether the clonal cell population was CD38 positive ($n = 60$) or CD38 negative ($n = 214$; $P = .006$).

Fig 3. Time from diagnosis to treatment for patients with monoclonal B-cell lymphocytosis (MBL; absolute lymphocyte count [ALC] $\leq 10 \times 10^9/L$; B-cell count $< 5.0 \times 10^9/L$, low-count, Rai 0 chronic lymphocytic leukemia (CLL; ALC $\leq 10 \times 10^9/L$; B-cell count of 5.0 to $10.0 \times 10^9/L$, and Rai 0 CLL with ALC more than $10 \times 10^9/L$. The difference in time to treatment (TTT) between patients with MBL and low-count, Rai 0 CLL was statistically significant ($P = .03$). Patients with MBL also had a significantly longer TTT than Rai 0 CLL patients with an ALC more than $10 \times 10^9/L$ ($P < .001$).

cohort had longer TTT than not only individuals with Rai stage 0 CLL in general, but also patients with low-count, Rai stage 0 disease as defined by having a B-cell count more than $5 \times 10^9/L$ but an ALC less than $10 \times 10^9/L$. The only previous studies that compared the outcome of individuals with clinically identified MBL with that of patients with Rai stage 0 CLL were two small analyses (our own pilot study [$n = 47$]⁸ and a series from British Columbia [$n = 46$]⁶), both of which had little statistical power and neither of which found a difference in TTT between MBL and Rai stage 0 CLL. The findings of the current study provide the first clinical evidence to support the recently proposed revisions to the CLL diagnostic criteria, which recommend basing the diagnosis of CLL on a B-cell count of more than $5 \times 10^9/L$ rather than an ALC of more than $5 \times 10^9/L$.

Despite the fact that a B-cell threshold of $5 \times 10^9/L$ does stratify patients with different rates of progression requiring treatment, B-cell count seems to relate to TTT as a continuous variable. Additional studies are needed to determine what B-cell threshold optimally distinguishes the clinical outcome between MBL and Rai stage 0 CLL. In a separate recent study of 459 patients with an ALC more than $5 \times 10^9/L$ who met the 1996 criteria for a diagnosis of CLL¹⁶ and who were referred to a hematologist, we found that a B-cell count of $11 \times 10^9/L$ may be the optimal threshold for stratifying a patient's risk of treatment and death.¹² Although 146 individuals in the previous report are included in the present study as a result of overlap between the 1996 CLL diagnostic criteria¹⁶ and the 2005 MBL diagnostic criteria,¹ the present study includes 156 additional individuals who were not seen by a hematologist at our center and/or had an ALC less than $5 \times 10^9/L$ (ie, could not be classified as CLL by either the 2008¹⁰ or 1996¹⁶ criteria). The results of the two studies are complementary, with both suggesting that a higher B-cell count may better stratify patients' risk of adverse clinical outcome. It is also worthy of emphasis that, in the present study, having a higher proportion of clonal B cells did not predict a shorter TTT among individuals with MBL whose total B-cell count was less than $1.5 \times 10^9/L$. This finding suggests the total B-cell count is an adequate measure of risk among individuals with MBL and that having a large proportion of clonal B cells does not increase the

risk of progression among those whose total B-cell count is less than $1.5 \times 10^9/L$.

Although numerous studies have demonstrated that biologic features of CLL B cells, such as ZAP-70, CD38, *IgHV* gene mutation status, and cytogenetic abnormalities on FISH, are powerful predictors of TTT and OS in patients with Rai stage 0 CLL,^{15,17,18} there is limited information on the prognostic value of these characteristics in individuals with MBL. However, among the individuals with MBL in our series, we found that CD38 status was strongly related to TTT.

It is important to note that our study is limited by its retrospective nature, and longer follow-up will be necessary to more precisely characterize patients' long-term outcome. Additional studies are also needed to determine what biologic characteristics best predict outcome among individuals with MBL and to identify the biologic events that contribute to disease progression in the hope that such events can be prevented or reversed.

In summary, MBL seems to be a distinct clinical entity with a clinical behavior that differs from that of Rai stage 0 CLL. In this regard, the relationship between MBL and CLL may be akin to that of monoclonal gammopathy of undetermined significance and myeloma. Individuals with MBL identified in clinical practice have a low risk of requiring treatment in the next 5 years. B-cell count and CD38 status seem to predict TTT among MBL patients. Additional studies are needed to determine the optimal criteria to distinguish between MBL and CLL. Differentiating between CLL and MBL based on a patient's risk of adverse clinical outcome could reduce the psychological distress caused by classifying asymptomatic individuals at low risk for adverse clinical consequences as having CLL.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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