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Monoclonal B-cell Lymphocytosis (MBL): Biology, Natural History, and Clinical Management

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Abstract

Chronic lymphocytic leukemia (CLL) and the other low grade non-Hodgkin lymphomas (NHL) are among the most common lymphoid malignancies. Recent studies suggest that more than 4% of the general population over age 40 harbor a population of clonal B-cells with the phenotype of either CLL or another B-cell malignancy, a condition now designated monoclonal B-cell lymphocytosis (MBL). Although all cases of CLL appear to be preceded by MBL, the majority of individuals with MBL will not develop a hematologic malignancy. The biologic characteristics and clinical implications of MBL appear to differ based on whether it is identified during the diagnostic evaluation of lymphocytosis or incidentally discovered through screening of individuals with normal lymphocyte counts as part of research studies using highly sensitive detection methods. In the present manuscript we provide a state of the art review on the prevalence, nomenclature, biology, natural history, and clinical management of MBL.

Keywords

monoclonal B-cell lymphocytosis (MBL); chronic lymphocytic leukemia (CLL); prognosis; biology; management

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is the most common leukemia in the Western world and accounts for approximately 11% of all hematologic malignancies.(1) CLL affects approximately 3–5 out of every 100,000 individuals in the U.S. and is presently considered a treatable but incurable illness with conventional therapies. Remarkably, while conducting a public health study in 1991, the U.S. Center for Disease Control (CDC) identified a clonal population of B-cells with CLL phenotype in 9/1499 (0.6%) individuals in the general population age 45 or older.(2, 3) This prevalence of roughly 1 out of every 170 individuals

was several hundred times more common than that of CLL. Subsequent studies found that clonal B-cell populations with the phenotype of other low grade, non-Hodgkin lymphoma (NHL) subtypes (e.g. marginal zone) were also prevalent in the general population.(4, 5) To standardize characterization and promote future scientific investigation, an international panel of experts designated the presence of such clones “monoclonal B-cell lymphocytosis” (MBL) and proposed formal criteria for the diagnosis of MBL in 2005.(6) These criteria also sub-classified individuals with MBL as having i) CLL-like phenotype ii) atypical CLL phenotype, or iii) non-CLL phenotype based on surface protein expression (Table 1). (6)

Since the prevalence of MBL is several hundred times more common than CLL or the NHLs of corresponding phenotype, the above studies raised the possibility that CLL and the low grade NHLs may have highly prevalent precursor states analogous to the relationship between monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma or adenomatous polyps and colon cancer. This discovery has raised a host of clinical and biologic questions that have been the focus of intense investigation over the last decade. In the present manuscript we provide a state of the art review on the prevalence, biology, natural history, nomenclature/classification, and clinical management of MBL.

POPULATION PREVALENCE OF MBL

Several studies have investigated the prevalence of MBL in the general population. The proportion of individuals with MBL and the absolute numbers of abnormal cells detected in these studies are closely associated with the methods used for detection. A summary of published studies is shown in Table 2.

The earliest population studies were performed in the U.S. in the 1990s as part of a larger investigation into the potential health impact of living around hazardous waste sites.(2) These studies included an immune biomarker panel with basic lymphocyte immunophenotyping but not B-cell kappa:lambda analysis. As discussed in the introduction, the overall prevalence from the initial study and two follow-up analyses using this approach was 9/1499 (0.6%) individuals.(3) Later studies performed independently in the UK and Italy assessed the prevalence of clonal B-cells in individuals with normal blood counts using four-color flow cytometry with a sensitivity of detection commonly used for detection of minimal residual disease in patients with CLL (1 clonal cell per 1×10^5 events).(7) These studies demonstrated a much higher prevalence of CLL-like MBL which was detected in more than 5% of adults aged over 60.(4, 5) The UK study(4) involved hospital outpatients with no history or suspicion of cancer and the Italian study(5) involved individuals from a rural community referred for routine blood tests (e.g. blood glucose, blood lipids). CLL-phenotype cells in these patients typically represented <10% of total B-cells and absolute CLL-like cell counts were usually below 15 CLL-like cells/ μ L. In both studies, the prevalence increased with age and a higher proportion of males were affected.(4, 5) In addition to CLL-like clones, non-CLL phenotype MBL (CD5⁻) cases were detected in 1–2% of individuals based on a perturbation of kappa:lambda ratio.

Both the UK and Italian groups have subsequently performed further studies of MBL in the general population with a focus on biological investigations.(4, 5) Of note the second Italian study utilized a higher sensitivity flow cytometry approach and identified a higher prevalence (7.4%) of MBL in the general population than in the primary care cohort.(8) An even more recent study from Salamanca utilized the highest sensitivity flow cytometry approaches available to analyze 5 million peripheral blood cells per individual. Using this strategy the investigators identified a very high prevalence of CLL phenotype cells in the general population which were detectable in more than 1 in 5 individuals over 60 years old. (9) As indicated by the latter two studies, the differences in reported prevalence of MBL

across series are most likely due to differences in the sensitivity of the flow cytometry approach applied. In particular, the Salamanca group screened at least ten times more cells (5 million per case vs. 200,000 – 500,000) and used 8- color staining panels as compared to the previously used 4-color(5) and 5-color(8) protocols. The percentage of aberrant/clonal B cells was below the maximum sensitivity of the previous studies (<0.01%) in more than half of the cases (62%) identified by the Salamanca study.(9) However, it should be noted that clonality was only confirmed by additional methods in 18 cases, all except one with >0.01% aberrant/clonal B-cells.(9)

The highest reported prevalence of CLL-like MBL occurs in first-degree relatives of CLL patients. All epidemiological studies that have investigated the prevalence of CLL and other lymphoproliferative disorders in relatives of CLL patients have reported elevated risks of CLL in relatives with the largest study showing a 8-fold increase in risk.(10, 11) The first reports of MBL came from studies of apparently unaffected family members in the early 1990s.(12) More recently studies in both the UK(13) and U.S.(12) demonstrated a very high prevalence of CLL-like MBL in individuals with a family history of CLL who had normal blood counts (prevalence=13.5–18%). The UK group had used the same methodology for their studies of both CLL families and the general population and found a 4-fold increase prevalence of MBL in families with a genetic predisposition to CLL.(4, 13) For young adults aged 16–40 years the relative risk is 17-fold.(13) Although these studies(12, 13) clearly demonstrate a higher risk of MBL among unaffected individuals from CLL families relative to the general population, other recent studies suggest that first-degree relatives of individuals with sporadic CLL may also be at increased risk of MBL if they are over age 60. (14)

As noted, the sensitivity of the flow cytometry approach used for detection has a direct impact on the absolute numbers of CLL-phenotype cells that can be identified and hence the proportion of individuals with detectable CLL-like MBL. The initially proposed diagnostic criteria for MBL shown in Table 1 did not specify a flow cytometry analysis method, and were simply based on detection of a monoclonal B-cell population in the peripheral blood with an overall kappa:lambda ratio >3:1 or <0.3:1, or greater than 25% of B cells lacking or expressing low level surface immunoglobulin in conjunction with a disease-specific immunophenotype.(6) These criteria are primarily intended for diagnostic laboratories undertaking evaluation of samples referred for investigation of a suspected lymphoproliferative disorder. The ability to detect very small abnormal B-cell populations representing less than 100 cells/ μ L is generally not required and high-sensitivity approaches are not needed for routine diagnostic purposes.

It should be noted, however, that it is important to discriminate between CLL-like MBL, which has CD5 and CD23 expression along with weak CD20, CD79b and surface immunoglobulin, and other sub-types of MBL. The two most common non-CLL types are CD5⁻ MBL (classified as “non-CLL phenotype MBL”) and CD5⁺ MBL with a phenotype that is not typical for CLL, i.e. strong CD20, CD79b or immunoglobulin expression (classified as “atypical-CLL phenotype MBL”). Individuals with a CD5⁺, CD23 negative phenotype must have the diagnosis of mantle cell lymphoma excluded by FISH evaluation for the t(11;14) before being classified as having atypical-CLL phenotype MBL.

The optimal flow cytometry analysis approach for biologic studies aimed at investigating the earliest stages of leukemogenesis will depend on the numbers of abnormal cells required for validation and the planned downstream analysis. Studies investigating CLL-like MBL designed to detect at least 1 CLL cell/ μ L will require analysis of at least 200,000 total events using a minimum of five color analysis (CD5, CD19, CD20, kappa, lambda). In many cases the detection of an abnormal population can be confirmed by consensus IGH-PCR on

separated B-cells. Analysis of CLL cells below this level will typically require analysis of more than 500,000 total cells with six or more fluorescent channels (CD3, CD5, CD19, CD20, kappa, lambda). Such studies need to be carefully controlled and validation of the abnormal population requires fluorescent cell sorting of the abnormal population coupled with FISH analysis and IGHV gene sequencing after cloning or extraction of DNA from single sorted cells.(15)

BIOLOGY AND NOMENCLATURE

CLL-like MBL has been so-named because of its close phenotypic resemblance with clinical CLL. Shared markers used in the diagnostic work-up include, beside CD5 and CD20, also CD23, low levels of surface Immunoglobulins (Ig) and Ig-related molecules (CD79b), and low to negative expression of FMC7.(4, 5) Further analyses have been performed to define specific protein expression that may distinguish MBL from CLL. One study evaluated 18 additional B-cell markers by flow cytometry in individuals with CLL, MBL, other B-cell lymphoproliferative disorders, and individuals without B-cell malignancy.(16) This analysis confirmed the distinct phenotype of CLL compared to other B-cell disorders, but found individuals with CLL and CLL-like MBL clustered together when an unsupervised analysis was performed. Expression of no specific protein was able to distinguish between CLL and CLL-like MBL.

A similar conclusion was reached using gene expression profiling analysis. Although a single gene; lymphoid enhancer binding factor 1 (LEF1), was capable of distinguishing CLL cells from controls and all other B-cell disorders evaluated, the expression in CLL and CLL-like MBL was similar.(17)

While these findings suggest a significant biologic overlap between CLL and CLL-like MBL, it is important to emphasize that all these studies were performed on MBL cases with a high number of monoclonal cells to facilitate these analyses. In routine clinical practice the vast majority (~85%) of MBL cases are identified following investigation of lymphocytosis and have an abnormal B-cell count above 1900 cell/ μ l. In contrast, ~85% of MBL detected on population screening studies have a B-cell count below 500/ μ l, with 40% having fewer than 50 CLL phenotype cells/ μ l. Since several studies suggest that the number of B cells is a strong predictor of the clinical outcome of MBL patients,(18–20) it is reasonable to postulate that, from a biological point of view, MBL detected in clinical practice (“clinical MBL”) may be more similar to frank CLL than MBL detected on population screening (“population-screening MBL”).

Despite the similarities between CLL and clinical MBL reviewed above, several biologic differences between clinical MBL and population-screening MBL have recently been identified. First, while MBL was originally thought to represent exclusively monoclonal B lymphocytes, it has become evident that biclonal (14/73)(9), oligoclonal (4/6)(15), and polyclonal (6/89)(8) cases of MBL can be identified. This observation indicates that a CLL-like phenotype (CD5⁺, CD20^{dim}) is not necessarily related to the acquisition of monoclonality. These studies suggest the possibility that concomitant clones of CLL-like B-lymphocytes appear and persist in many otherwise healthy individuals where one clone may expand and, in a small subset of individuals, become predominant with time. Further investigation is now needed to elucidate the type of stimulation, antigen activation, or other events that induce some B lymphocytes to acquire the CLL phenotype.

While the studies are far from conclusive, differences in immunoglobulin repertoire have also been observed between individuals with clinical MBL and those with population-screening MBL. CLL is characterized by a biased usage of immunoglobulin heavy chain variable region (IGHV) genes with IGHV1-69, IGHV4-34, IGHV3-07 and IGHV3-23 the

most frequently used. The presence or absence of somatic mutations in the IGHV genes in patients with CLL correlates with clinical outcome where individuals with mutated IGHV genes having a longer time to first treatment and improved overall survival.(21, 22) Approximately 55% (range 47–58%) of CLL patients have mutated IGHV genes, although this frequency largely depends on the institution (primary vs tertiary referral centers). The IGHV 1-69 gene is the most frequently utilized gene in unmutated CLL cases, while the IGHV 4-34 gene is the most commonly used gene among patients with mutated CLL.(23) Patients with clinical MBL appear to be predominantly IGHV mutated (77–90%)(18, 19) and have an IGHV repertoire that closely resembles that of mutated CLL with IGHV3-07, IGHV3-23, and IGHV4-34 genes utilized in around half of the cases.(18) While some of these genes (i.e. IGHV 3-07, IGHV 3-23 and IGHV 4-34) have been also found to be expressed in familial population-screening MBL,(15) the overall IGHV repertoire of population-screening MBL appears strikingly different.(8, 9) Specifically, the absence of IGHV 1-69 and an under-representation of both IGHV 4-34 and IGHV 3-23 genes was apparent in individuals with population-screening MBL relative to those with CLL or clinical MBL.(8, 9) While a biased usage of the IGHV 4-59/61 gene in population-screening MBL has been suggested,(8) larger datasets are needed to determine whether or not a distinct restriction in IGHV repertoire occurs in patients with population-screening MBL and to define the random origin from the unselected repertoire expressed by the normal B lymphocyte pool.

Finally, 20–25% of CLL cases are characterized by the existence of stereotyped heavy chain complementarity determining region 3 (HCDR3) sequences within the functional heavy chain rearrangement which suggests the recognition of repetitive and shared antigenic epitopes in unrelated patients with CLL.(24) In contrast, stereotyped HCDR3 sequences are rarely present (<5%) among individuals with population-screening MBL, underscoring another biologic difference with frank CLL.(8)

NATURAL HISTORY AND RISK OF PROGRESSION

From MBL to CLL

A major clinical concern for persons diagnosed with MBL is whether or not they will develop CLL or another indolent NHL. Risk factors for progression are of key interest. At this time, only a few studies have addressed these questions.

In a prospective hospital-based cohort study from the U.K., Rawston et al.(18) monitored 185 subjects with CLL-phenotype MBL and lymphocytosis for a median of 6.7 years (range 0.2 to 11.8 years) and found progressive lymphocytosis in 51 (28%). Of these, 28 (15% of the entire cohort) progressed to a diagnosis CLL. In this study, the absolute B-cell count was the only independent prognostic factor associated with progressive lymphocytosis.(18) Only 13 (7%) individuals in this cohort required chemotherapy for treatment of CLL. During the period of follow-up, 62 (34%) subjects died, however only 4 of these deaths were due to CLL. Age at enrollment greater than 68 years and hemoglobin level below 12.5 g per deciliter were the only independent prognostic factors for death(18). Thus, based on 187 cases with a median follow-up of 6.7 years, this study reported that CLL requiring treatment develops in subjects with CLL-phenotype MBL and lymphocytosis at the rate of 1.1% per year.(18)

In a retrospective laboratory-based database study, Mulligan et al.(25) identified 414 MBL patients, 322 of whom had a CLL phenotype. Based on 220 patients with adequate longitudinal information (median follow-up 4.1 years, range 1.0–8.5 years), they found 76 (34.5%) patients progressed to CLL(26) including 28 (12.7%) patients who developed an absolute lymphocyte count (ALC) of more than $20 \times 10^9/L$ and 18 (8.2%) an ALC of more

than $30 \times 10^9/L$, the criterion for progression used by Rawstron et al.(18) (i.e., a rate of progression of approximately 2% per year). Further, they identified 5 MBL cases with an initial B-cell count of less than $1.9 \times 10^9/L$ who progressed to fulfill the current criteria for CLL: three cases progressed to have an ALC that only modestly exceeded the current CLL diagnostic criteria(26), and 2 cases had progressive lymphocytosis to $26.8 \times 10^9/L$ and $52.5 \times 10^9/L$ after 3.1 and 2.8 years, respectively.

In a retrospective hospital-based cohort study from the Mayo Clinic, Shanafelt et al.(19) explored the outcome of patients with CLL-like MBL relative to that of individuals with Rai stage 0 CLL. Using hematopathology records, the authors were able to identify 631 patients with newly diagnosed MBL or Rai stage 0 CLL. Within this cohort, 302 patients fulfilled the criteria for diagnosis of MBL (B-cell counts of 0.02 to $4.99 \times 10^9/L$); and 313 Rai stage 0 CLL (94 patients had Rai stage 0 CLL with an ALC $< 10 \times 10^9/L$; 219 patients had Rai stage 0 CLL with an ALC $> 10 \times 10^9/L$). Clinical information regarding date of diagnosis, physical examination, prognostic parameters, treatment history, and disease-related complications were abstracted from clinical records.

In this cohort, no differences in CD38 status, IGHV gene mutation status, ZAP-70 expression or cytogenetic abnormalities as detected by FISH were observed between individuals with MBL and Rai stage 0 CLL with an ALC $< 10 \times 10^9/L$. The percentage of MBL patients free of treatment at 1, 2, and 5 years was 99%, 98%, and 93%, respectively. (19) Among MBL patients, B-cell count as a continuous variable (hazard ratio [HR]=2.9, $P=0.04$) was predictive of time to treatment (TTT)(19). Notably, no difference in survival was observed based on the percentage of B-cells that had CLL-like phenotype among those individuals whose total B-cell count was less than $1.5 \times 10^9/L$ ($P = 0.71$) suggesting that having a high proportion of clonal B cells does not necessarily indicate an increased risk for progression among individuals with a total B-cell count $< 1.5 \times 10^9/L$. Among other demographic and prognostic parameters, CD38 status (HR=10.8; $P=0.006$) was associated with TTT, but age ($P=0.62$) and sex ($P=0.99$) were not(19). Because of an insufficient number of patients with ZAP-70, IGHV gene mutation status, or FISH analysis, the authors were unable to correlate these variables with TTT or OS. Taken together, the rate of progression from MBL to CLL requiring treatment during the first 5 years of follow-up was approximately 1.4% per year in this retrospective study of 302 patients. Primary indications for treatment for the MBL patients in this series were progressive lymphadenopathy ($n=2$), marrow failure/cytopenia ($n=3$), progressive lymphocytosis ($n=1$), and autoimmune hemolytic anemia ($n=1$).(19)

In a separate prospective, hospital-based cohort study from Italy, similar in design to the Mayo Clinic study(19), Rossi et al.(27) compared the outcome of patients with MBL to individuals with Rai stage 0 CLL. In a consecutive series of 460 newly diagnosed cases with B-cell lymphocytosis of CLL phenotype, the authors used the IWCLL 2008 guidelines(26) to classify individuals as having either MBL or CLL (B-cell count $< 5 \times 10^9/L$, respectively). The investigators identified 123 MBL cases and 337 CLL cases (Rai 0: 154; I-II: 129; III-IV: 54).

When compared to the 154 individuals with Rai 0 CLL, the 123 individuals with MBL had a lower percentage of bone marrow lymphocytes (median 20% vs. 40%, $P<0.001$), a lower prevalence of diffuse bone marrow involvement (2.4% vs. 10.2%, $P=0.034$), and lower beta-2-microglobulin (median 1.9 mg/l vs. 2.2 mg/l, $P=0.002$).(27) MBL cases were also found to have more preserved immune function than those with Rai 0 CLL. For example, MBL cases had higher immunoglobulin (Ig) levels: IgG (median 10.60 g/l vs. 9.74 g/l, $P=0.012$), IgA (median 1.55 g/l vs. 1.40 g/l, $P=0.038$), and IgM (median 0.74 g/l vs. 0.58 g/l, $P=0.003$) than those with Rai 0 CLL cases. Clinically, a lower infection risk was observed

in individuals with MBL compared to those with Rai 0 CLL.(27) Prior to initial chemotherapy treatment, the incidence of infection was 10.9 vs. 15.1 per 100 patient-years ($p=0.030$), for those with MBL and CLL respectively.(27)

Rossi and colleagues(27) found a lower prevalence of high-risk cytogenetic lesions in individuals with MBL compared to those with Rai 0 CLL. Specifically, MBL cases had a lower prevalence of $\text{del}(11\text{q}22)/\text{del}(17\text{p}13)$ (3.8% vs. 15.2%, $P=0.004$) and of *TP53* mutations (3.0% vs. 11.5%, $P=0.049$) compared to those with Rai 0 CLL.(27) In contrast, the percentage and prevalence of *IGHV* gene identity; distribution of *IGHV* genes; and prevalence of stereotyped HCDR3 did not differ significantly between MBL and Rai 0 CLL cases.(27) The expression patterns of CD38, ZAP70, and CD49d were not statistically different between the two groups.(27)

In the Italian study, 56 of the 123 MBL cases progressed to fulfill criteria for overt CLL ($n=37$) or SLL ($n=19$). The median time to developing CLL/SLL was 55.0 months. Intuitively, and consistent with the report by Rawstron *et al.* (18), MBL cases with higher B-cell counts at MBL diagnosis were more likely to subsequently develop a B-cell count above the $5 \times 10^9/\text{L}$ threshold currently used to make the diagnosis of CLL.(27) Overall, 19 of 123 MBL cases progressed to symptomatic CLL/SLL requiring treatment, accounting for a 10-year treatment-free survival of 68.7%. In univariate analysis, *IGHV* gene mutation status ($p<0.002$), CD38 expression ($p<0.001$), CD49d expression ($p=0.006$), and cytogenetic abnormalities on FISH testing ($p<0.001$) all predicted treatment-free survival(TFS) among individuals with MBL. On multivariable analysis the presence of +12 or $\text{del}(17\text{p}13)$ on FISH karyotype were the only independent predictors of TFS ($\text{HR}=5.39$, $P=0.001$). (27)

There remains great need for large, prospective MBL studies to better understand the natural history of MBL and define predictors of progression. Based on the similarities between 3(18, 19, 25) of the 4 large series(18, 19, 25, 27), our current estimate is that individuals with MBL have a 1–2% annual risk of requiring CLL-specific treatment. Future studies may identify the biologic characteristics associated with the risk of progression which in turn may allow physicians to better predict the risk of progression for a given individual. It is imperative that future prognostic studies in MBL focus not on the likelihood of the B-cell count rising above an arbitrary threshold (e.g. $5 \times 10^9/\text{L}$), but rather on the risk of developing clinically relevant outcomes such as disease related symptoms, need for treatment, or death.

Is CLL always preceded by MBL?

Another important question is whether all CLL cases are preceded by MBL, or if some (e.g. biologically aggressive) CLL cases develop *de novo*. This is of importance because if MBL consistently precedes CLL researchers could develop prospective studies to uncover the biologic mechanisms of CLL progression. To address this question, Landgren *et al.*(28) recently conducted a prospective cohort study based on 77,469 healthy adults who were enrolled in the nationwide, population-based, U.S. Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial.(28) The investigators identified 45 participants who were subsequently diagnosed with CLL during the period of longitudinal observation who had a pre-diagnostic peripheral blood sample available for analysis.

Using six-color flow cytometry and *IGHV* gene analysis by reverse-transcriptase-polymerase-chain-reaction (RT-PCR), the authors found evidence of MBL predating the CLL diagnosis in 44 patients (98%;).(28) Notably, MBL was present up to 6.4 years prior to CLL diagnosis in these individuals. In 41 patients (91%;), the clone was confirmed by both analysis methods.(28) The *IGHV* gene mutation status was determined in 35 of 45 MBL clones (78%). Of these, 16 (46%) were *IGHV3* subgroup genes (including 6 [17%]

IGHV3-23 genes) and 9 (26%) were *IGHV4* subgroup genes (including 4 [11%] *IGHV4-34* genes).(28). The distribution of mutated clones as compared with unmutated clones was similar regardless of the time at which the blood samples was obtained in relationship to subsequent CLL diagnosis. Although the number of *IGHV* unmutated samples was small, 3 of 8 *IGHV* unmutated clones were present more than 3 years before the CLL diagnosis, with 2 detectable 5 years before. Thus, this study suggests that virtually all cases of CLL, including both mutated and unmutated *IGHV* cases, are preceded by MBL which is often present for years prior to clinical CLL diagnosis.(28)

DISTINGUISHING MBL FROM CLL IN CLINICAL PRACTICE

Since the clonal B-cells of individuals with both CLL and CLL-like MBL share an identical immunophenotype, how to best differentiate MBL and Rai stage 0 CLL continues to be an area of controversy. From a historic perspective, the 1988(29) and 1996(30) diagnostic criteria for CLL classified individuals with a clonal population of characteristic immunophenotype and an ALC $>5 \times 10^9/L$ as having CLL. After recognition of MBL and publication of the 2005 MBL diagnostic criteria(6) which were based on B-cell count rather than ALC, there was initially overlap between the diagnostic criteria for CLL and MBL: individuals with an ALC $5 \times 10^9/L$ who had a B-cell $<5 \times 10^9/L$ fulfilled both the MBL and CLL diagnostic criteria. While this initially appeared to impact a small proportion of patients, subsequent studies indicated up to 40% of individuals with newly diagnosed Rai stage 0 CLL according to the 1988 and 1996 criteria fell in this area of overlap.(20, 31)

This important distinction between classifying a patient as having leukemia, as opposed to a pre-malignant condition should be based, at least in part, on the individual's risk of developing clinical complications and/or dying from the disease.(20, 31) In this regard, studies have now demonstrated that the ALC threshold used in the 1988 and 1996 CLL diagnostic criteria had no relationship to either TFS or OS while the B-cell threshold proposed in the 2005 MBL diagnostic criteria strongly relates to TFS.(19, 20) As previously discussed, the risk of progression to requiring CLL specific treatment among individuals with MBL is 1–2% per year(18, 19, 25) compared to 5–7% per year for individuals with Rai stage 0 CLL.(19, 27) While this difference appears subtle it equates to a 10 year risk of requiring treatment of 7–14% for individuals with MBL compared to 50–70% for patients with Rai stage 0 CLL. Consistent with these findings, the 2008 update to the CLL diagnostic criteria incorporated a B-cell threshold of $5 \times 10^9/L$ used in the 2005 MBL criteria.(26)

Although these changes have anchored the diagnosis of CLL to clinical outcome, further investigation is needed to determine how best to distinguish between CLL and MBL. First, B-cell count appears to relate to TFS and OS as a continuous variable(19, 20) and recent studies suggest higher B-cell thresholds (e.g. $11 \times 10^9/L$) may not only stratify TFS but also predict OS.(20) Second, while these studies have demonstrated the ability of the B-cell count to stratify survival in patients with a clonal population of CLL phenotype, they have not benchmarked the outcome of these individuals to the general population. Conceptually, the expected survival of individuals with a precursor state to malignancy (e.g. adenomatous colon polyps) should be the same as that of the general population unless they go on to develop the at-risk disease (e.g. colon cancer). While B-cell count is clearly established as a prognostic factor in patients with MBL, whether or not it can be used to identify those individuals with a circulating B-cell clone whose OS is shorter than unaffected individuals in the general population has not yet been determined. Third, while the diagnosis of CLL and MBL should be based in part on clinical outcomes, yet to be discovered biologic characteristics could be an equally important consideration. The 5% prevalence of MBL in the general population over age 60 coupled with the markedly lower prevalence of CLL is consistent with the multi-hit hypothesis of human malignancy and suggests additional

biologic events must occur for MBL to progress to CLL. Identification of the biologic events that related to progression could lead to incorporation of these characteristics in the classification system as well as the development of early intervention strategies.

CLINICAL MANAGEMENT OF MBL

Recommendations for Evaluation and Follow-up

There exists limited data on the clinical management of patients with MBL. The great majority of individuals with MBL (approximately 85%) are currently identified as part of the clinical evaluation of an abnormal blood count, most commonly lymphocytosis. These individuals with clinical MBL should undergo a complete evaluation by a hematologist/oncologist that includes a detailed family history (given the association between CLL and MBL), a review for B type symptoms, and a complete physical examination with comprehensive lymph node examination.

The most critical step in the clinical management of individuals with MBL is to insure the correct diagnosis. Individuals with MBL should be distinguished from patients with subtle manifestations of NHL. In the absence of constitutional symptoms (fevers, night sweats, fatigue, weight loss), individuals with CLL-like MBL can be distinguished from those with SLL based on the absence of palpable lymphadenopathy or organomegaly on physical exam (Table 3). Although marrow biopsy is not required, nearly all patients with clinically identified MBL will have some degree of bone marrow involvement if biopsy is performed. (27, 32) Although the median percent marrow involvement in clinical MBL appears to be between 10–20% more extensive involvement is observed in some individuals. (27, 32) In the series from Mayo Clinic, although 3/46 (6.5%) individuals with MBL had extensive marrow involvement on biopsy (70 to 80%) none of these 3 individuals had required treatment on up to 5 year follow-up. (32)

For individuals with an atypical CLL phenotype MBL or non-CLL phenotype MBL, a more thorough evaluation is required since standard staging for NHL requires CT scanning (chest, abdomen, pelvis) and bone marrow biopsy. (33) Individuals with radiographic evidence of adenopathy are best classified as having NHL with the sub-classification (e.g. marginal zone lymphoma, follicular lymphoma, mantle cell lymphoma) based on immunophenotyping and, where relevant, cytogenetic analysis (e.g FISH evaluating for t(11;14)) or immunohistochemistry (e.g. cyclin D1 staining). (33) In appropriate circumstances, serum/urine protein electrophoresis to evaluate for lymphoplasmacytic lymphoma should be pursued (Table 4).

As detailed, the average risk of progression requiring therapy among individuals with clinical CLL-like MBL appears to be about 1–2% per year. (18, 19, 25) Based on this data, we reassure individuals with CLL-like MBL identified in clinical practice that they are at low risk for developing CLL, counsel them regarding symptoms they should watch for (lymphadenopathy, fevers, night sweats, fatigue, weight loss), and recommend annual follow-up by a hematologist/oncologist with a complete blood count.

At this time we lack tools to predict which MBL cases will progress to CLL. In small series, surface expression of CD38 has been correlated with the future need for therapy in two (19, 27) of three studies. (18) Some (IGHV genes mutations, CD49d, FISH) but not all (ZAP-70, beta-2 microglobulin) prognostic parameters used to predict TFS in CLL were also reported to be useful prognostic parameter for clinical MBL patients in one small series. (27) Currently, there is insufficient evidence to routinely recommend such prognostic testing for individuals with MBL outside of investigational protocols.

In contrast to the measurable risk of progression in patients with clinical CLL-like MBL, progression among patients with population-screening CLL-like MBL is exceedingly rare in the authors' experience. As discussed in the prior sections, there is emerging data that the biology of population-screening MBL differs from that of CLL(8)(14) and clinical MBL and that population-screening MBL can be identified in up to 20% of the general population using highly sensitive assays.(9) Therefore, we believe that individuals with population-screening MBL likely *do not* have a risk of CLL that is substantially higher than that of the general population. Such patients do not require formal evaluation by a hematologist and surveillance beyond an annual examination by a primary care provider with complete blood count is not warranted.

The risk of progression among patients with atypical CLL phenotype MBL or non-CLL phenotype MBL is less well defined. For rare individuals with a phenotype and cytogenetic studies suggestive of mantle cell lymphoma or another aggressive NHL subtype but without adenopathy on CT scanning or substantial marrow involvement on marrow biopsy, we suggest clinical follow-up every 3–6 months with CT imaging at least every 6 months.(34) For those patients with atypical CLL phenotype MBL or non-CLL phenotype MBL whose immunophenotype is consistent with a more indolent NHL subtype (e.g. marginal zone lymphoma, follicular lymphoma), follow-up by a hematologist/oncologist every 6–12 months is recommended. The frequency of follow-up imaging requires clinical judgment and must be balanced with the risks associated with radiation exposure.(35)

Some have questioned whether or not it is ethical to inform individuals with population-screening MBL identified as part of research studies of their test results given the absence of evidence that population-screening MBL predicts a clinically important outcome (e.g. it lacks clinical validity as a predictor for developing CLL or other NHL subtypes) or that knowledge regarding population-screening MBL improves the subjects well-being (e.g. it lacks clinical utility).(36) It can, in fact, be argued that knowledge of low- count MBL may worsen an individual's well-being by causing undue anxiety and/or exposing them to possible discrimination by employers or life/health insurers.(36) While there is consensus that research subjects should be informed of new information gained from their participation,(37) whether the presence of population-screening MBL constitutes "meaningful information" can only be defined through ongoing and future studies.(36)

Other Clinical Issues

As flow cytometry is more widely utilized, more individuals with a mild absolute lymphocytosis will have a flow cytometric evaluation. In many laboratories, identification of a CLL-like population leads to a report stating "a clonal population consistent with a diagnosis of chronic lymphocytic leukemia or small lymphocytic lymphoma has been identified". This language may need to be revised to include the possibility the findings represent MBL. Due to recent updates to the diagnostic criteria for CLL(26, 30) many individuals fulfilling the criteria for a diagnosis of clinical MBL will have previously been given a diagnosis of CLL.(20, 31) Due to significant differences in risk and clinical outcome(20, 27, 31), it is important to inform such patient that the correct diagnosis is MBL, provide education that MBL is not a malignant diagnosis, and discuss the appropriate monitoring and follow-up. With time and counseling, most patients will understand that clinical MBL and CLL fall upon a continuum. The distinction that an MBL diagnosis indicates a higher lifetime risk of developing CLL, rather than being a diagnosis of leukemia, avoids the unnecessary distress associated with a leukemia diagnosis(38–40) and may aid the individual in discussions with their family. Similarly, MBL should not be referred to as "pre-leukemia" or "pre-CLL" as the majority of patients with clinical MBL will never develop symptoms or require CLL directed therapy.

The distinction between MBL and CLL also has several other practical ramifications. First, MBL does not currently have a specific ICD-9 code for billing purposes which forces clinicians to choose between coding individuals with MBL as lymphocytosis (ICD-9 code 288.8) or CLL (ICD-9 code 204.1) either of which creates potential problems. While the classification of “lymphocytosis” correctly identifies the non-malignant nature of MBL, it may create difficulties with insurance coverage for evaluation and care provided by a hematologist or diagnostic testing, particularly in some European countries. In contrast, in the U.S. health care system, a diagnosis of CLL could have implications for insurability as a pre-existing condition when individuals change their health care provider and may impact an individual’s ability to obtain life insurance. This distinction also has repercussions to insurance policies that provide specific benefits after a diagnosis of “cancer” causing some patients to lobby for the CLL code rather than the lymphocytosis code. These challenges illustrate some of the reasons why a new ICD-9 code specific to MBL is needed.

Another challenge encountered in clinical practice is the use of cellular products donated by individuals with MBL. Although one study found the frequency of MBL in blood donors to be quite low,(41) the study used a relatively insensitive screening assay that likely underestimated the true prevalence of MBL among blood donors. Further, there is no data regarding clinical outcomes from MBL-donor derived blood products to inform the decision of whether individuals with clinic MBL can safely serve as donors. Currently individuals with an underlying malignancy, including CLL, cannot donate blood, bone marrow, umbilical cord blood, or solid organs for transplantation. Since population-screening MBL can be identified in up to 20% of the adult population when evaluated using sensitive methods(9), this question could have profound affects on the supply of organ and blood products and warrants clinical studies. At present, it is premature to recommend screening blood or solid organ donors for MBL if the donor’s complete blood count (CBC) is normal.

A final challenge with regard to MBL patients providing cellular products pertains to stem cell transplant. Although the role of allogeneic stem cell transplant as a treatment for patients with CLL continues to be refined, it is a proven and routinely used therapy for selected CLL patients.(42–44) Since approximately 10% of CLL cases occur in a familial pattern(11) (10), and the prevalence of MBL is increased in such CLL families(12, 13), the question of whether relatives preparing to serve as a stem cell donor for CLL patients undergoing allogeneic transplant should undergo screening for MBL is a practical issue.(36) There is not currently consensus on whether or not matched relatives with MBL should be disqualified as donors. For many patients no alternative donor may be available and, even if available, the use of a matched unrelated donor could expose the patients to greater risk than transplant using a matched relative with MBL. Future studies are needed to clarify these issues.

SUMMARY AND CONCLUSIONS

Recent studies suggest that approximately 4% of the general population over age 40 harbor a population of clonal B-cells with the phenotype of either CLL or another low grade NHL. The biologic characteristics and clinical implications of MBL have begun to be defined and appear to differ based on whether it is identified during the diagnostic evaluation of lymphocytosis or incidentally discovered through screening studies using highly sensitive detection methods. Additional research into the epidemiology, biology, and natural history of MBL is needed (Table 5). The majority of individuals with MBL will not develop a hematologic malignancy.

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Table 1

Diagnostic Criteria and Sub-classification for MBL(adapted from (6))

DIAGNOSTIC CRITERIA
1. Documentation of clonal B-cell population ¹ by one or more of following:
a. light chain restriction:
• overall kappa:lamda ratio >3:1 or <0.3:1 or
• greater than 25% of B-cells lacking or expressing low level surface Immunoglobulin
b. heavy chain monoclonal IGHV rearrangements
2. Presence of a disease specific immunophenotype ²
3. Absolute B-cell Count <5 x 10 ⁹ cells/L
4. No other features of a lymphoproliferative disorder or autoimmune disease
a. normal physical exam (no lymphadenopathy or organomegaly)
b. absence of B-symptoms (e.g. fatigue, weight loss, night sweats) attributable to NHL
c. no autoimmune/infectious disease
SUBCLASSIFICATION:
A. CLL Like phenotype
- co-expression of CD5 with CD19; CD20 (dim); and CD23
- light chain restriction with dim surface immunoglobulin expression (very small MBL clones may be oligoclonal and thus not light chain restricted)
B. Atypical CLL phenotype
- co-expression of CD5 with CD19 but CD23 negative or CD20 (bright)
- light chain restriction with moderate to bright surface immunoglobulin expression
- exclude t(11;14) to rule out mantle cell lymphoma
C. Non-CLL phenotype
- CD5 negative
- Express CD20
- light chain restriction with moderate to bright surface immunoglobulin expression

¹Where possible, repeat assessment should demonstrate the monoclonal B-cell population is stable over 3-month period

²In the absence of a disease-specific immunophenotype, a highly skewed kappa:lamda can be the result of a reactive process.

Table 2

Prevalence of CLL-like MBL in population studies

The detected prevalence is highly dependent on the method of detection.

Study Group			Flow Cytometry				CLL-like MBL Prevalence	
Source	Median age (range)	n	N° of colors	CD19/CD5 + κ/λ	CD19/CD5 + CD20	Events (x10 ³)	All ages in study	>60yrs
U.S. residential population (3)	53 (40–78)	1926	2	No	No	N.S.	0.6%	>0.6% [*]
U.S. blood donors (41)	45 (18–79)	5141	2	No	No	N.S.	0.14%	0.9%
U.K. hospital outpatients (18)	57 (40–90)	910	4	Yes	Yes	200	3.5%	5.0%
Italy primary care(5)	74 (65–98)	500	4	Yes	Yes	200	5.5%	5.5% [‡]
U.K. hospital outpatients(18)	74 (60–80)	1520	4	Yes	No	200	5.1%	5.1% [‡]
Italy residential population(8)	55 (18–102)	1725	5	Yes	Yes	500	7.4%	8.9%
Spain primary care(9)	62 (40–97)	608	8	Yes	Yes	5000	12.0%	>20% [*]

* estimated from data

[‡] age above 65

[‡] age range 60–80.

Table 3

Distinguishing Between MBL, CLL, and SLL

	Clonal B-cells of CLL phenotype	Peripheral Blood B-cell Count $<5 \times 10^9/L$	Lymphadenopathy or hepatosplenomegaly	Bone Marrow Findings
MBL	yes	yes	no	marrow biopsy is <i>not</i> required as part of either the MBL or CLL diagnostic evaluation*
SLL	yes	yes	yes	
CLL	yes	no	yes or no	

* The limited available data suggests that nearly all patients with clinically identified MBL will have some degree of bone marrow involvement if marrow biopsy is performed with a median of 10–20% involvement.(27, 32) More extensive marrow involvement (70 to 80%) has been observed in some individuals with MBL but is not clearly related to risk of progression.(32)

Table 4

Recommendations For Evaluation and Follow-up In Routine Practice

RECOMMENDATIONS		Population-screening ¹ CLL-like MBL	Clinical ¹ CLL-like MBL	Atypical or Non-CLL like MBL identified in a clinical setting
DIAGNOSTIC EVALUATION	History ²	Yes	Yes	Yes
	Physical exam ³	Yes	Yes	Yes
	Immunophenotype of lymphocytes	Yes	Yes	Yes
	CBC with differential	Yes	Yes	Yes
	FISH testing with probe for t(11;14)	No	No	Yes ⁴
	CT scan chest/abd/pelvis	No	No	Yes
	Bone marrow biopsy	No	No	Yes
	CLL prognostic testing	No	No	No
COUNSELING & FOLLOW-UP	Patient counseling on symptoms to watch for ²	Yes	Yes	Yes
	Risk of progression requiring therapy	Low ⁵	1–2%/year	Undefined
	History ²	Routine medical care	annual	3–12 months ⁶
	Physical exam ³	Routine medical care	annual	3–12 months ⁶
	CBC with differential	annual	6–12 months	6–12 months ⁶
	CT scan chest/abd/pelvis	No	No	Clinical judgement ⁶

¹ CLL phenotype MBL identified as part of clinical evaluation of lymphocytosis is referred to as clinical CLL-like MBL while CLL phenotype MBL identified in research studies of patients with normal lymphocyte counts is referred to as population-screening CLL-like MBL

² Focus on constitutional symptoms (fevers, night sweats, weight loss, fatigue)

³ Focus on lymph node evaluation and assessment for hepatosplenomegaly

⁴ For patients with atypical phenotype CLL who are CD5+ but CD23– FISH testing with a probe to detect t(11;14) characteristic of mantle cell lymphoma should be performed.

⁵ Although limited data is available, progression among patients with population-screening CLL-like MBL is rare in the authors' experience. Because several series suggest that B-cell count is related to clinical outcome in individuals with MBL,(18–20) the risk of progression for individuals with population-screening CLL-like MBL is thought to be less than that of individuals with Clinical CLL-like MBL.

⁶ For the rare individuals fulfilling the criteria for MBL who have a phenotype and cytogenetic studies suggestive of mantle cell lymphoma or another aggressive NHL subtype, we suggest clinical follow-up every 3–6 months with CT imaging at least every 6 months. For those patients with atypical CLL phenotype MBL or non-CLL phenotype MBL whose immunophenotype is consistent with a more indolent NHL subtype, follow-up every 6–12 months is recommended and the frequency of follow-up imaging requires clinical judgment.

Table 5**Key Areas for Future MBL Research**

<p>I. Identification of epidemiologic factors associated with development of MBL</p> <ul style="list-style-type: none"> • genetics • health behaviors • exposures
<p>II. Clinical outcome of low count MBL</p> <ul style="list-style-type: none"> • CLL like MBL • atypical and non-CLL phenotype MBL • prognostic markers
<p>III. Identification of clinical and biologic parameters that allow optimal classification of patients with small B-cell clones (MBL vs. CLL vs. SLL)</p> <ul style="list-style-type: none"> • characteristics of the clone • characteristics of the host/environment
<p>IV. Identification of the biologic events related to progression of MBL to CLL or other NHL</p> <ul style="list-style-type: none"> • biologic events in the clonal B-cells • biologic events/interactions related to the host/environment • genetic characteristics of the clone • germline genetic characteristics of the host • behaviors • exposures
<p>V. Studies exploring a potential tissue equivalent of MBL</p>