Seminars in Oncology 43 (2016) 201–208

Contents lists available at ScienceDirect

Seminars in Oncology

journal homepage: www.elsevier.com/locate/ysonc

# What does it mean I have a monoclonal B-cell lymphocytosis?: Recent insights and new challenges

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ARTICLE INFO

*Keywords:*
Monoclonal B-cell lymphocytosis
Chronic lymphocytic leukemia
Immunoglobulin genes
Cytogenetic
Next-generation sequencing

ABSTRACT

Monoclonal B-cell lymphocytosis (MBL) is defined as a laboratory abnormality where small ( $< 5 \times 10^9$/L) clonal B-cell populations are detected in the peripheral blood of otherwise healthy subjects. According to the immunophenotype, MBL is labeled as chronic lymphocytic leukemia (CLL)-like (75% of cases), atypical CLL, and CD5-negative. Concentration of clonal B cells differentiates low- (LC) and high-count (HC)-MBL ( $<$ or $\geq$ $0.5 \times 10^9$/L, respectively). Thanks to technical improvements, we are able to identify CLL-like clonal B-cell populations at increased frequency with age, but we are still far from understanding its relationship with clinically overt CLL. LC-MBL, requiring high-throughput screening technique to be identified in population studies, seems to be a bird of a different feather and several hints suggest that LC-MBL is related to aging and/or chronic antigenic stimulation. Immunogenetic, cytogenetic and genetic data support the notion that HC-MBL, usually identified in the clinical setting, is a premalignant condition and, based on biological parameters, it is frequently difficult to differentiate it from early stage CLL. The rapid improvement and widespread availability of cutting-edge technology, in particular next-generation sequencing (NGS), raises hope that we are getting closer to unveiling the fundamental nature of MBL and CLL and how they are related to each other.



## 1. Introduction

The defined syndrome of monoclonal B-cell lymphocytosis (MBL) recently celebrated its tenth birthday in 2015, as the first consensus panel guidelines adopting the current diagnostic criteria were published in 2005 [1]. Though being recognized as a distinct entity, MBL remains heterogeneous and complex in its essence and significance. That notwithstanding, the huge progress made in the past 10 years in defining and understanding its pathogenesis should not be underestimated, thanks to widespread availability and use of multiparameter flow cytometry, the technical improvement of genome sequencing and the information derived from mouse models. As "with great power comes great responsibility", we have now the chance to clarify the essence of MBL as well as its potential relationship with chronic lymphocytic leukemia (CLL). In this review we thoroughly review our current knowledge about MBL focusing our attention on the most relevant open issues and unanswered questions.

## 2. What we know

### 2.1. Outlining the roots: history and definitions

Monoclonal B-cell lymphocytosis is defined as a laboratory abnormality where *small* clonal B-cell populations are detected in the peripheral blood of otherwise *healthy* subjects. In this setting *small* was considered below $5 \times 10^9$ clonal B cells per liter and *healthy* means that no signs or symptoms of lymphoproliferative disorders or autoimmune diseases are reported [1,2]. Based on the name of this entity, a usual misconception is that people with MBL should also have an abnormality in terms of increased number of lymphocytes at a routine white blood cell count. This is definitely not the rule and indeed the term *lymphocytosis* should be referred to the B lymphocytes only and in particular it indicates that the diagnosis should be suspected when there is an increase of "*monoclonal B lymphocytes*", virtually absent in healthy individuals. The criteria for MBL diagnosis are intertwined with the changes in CLL diagnostic criteria, as the 2005 consensus guidelines [1] have been subsequently adopted by the World Health Organization (WHO) [3] and the International Workshop on Chronic Lymphocytic Leukemia (IWCLL) [4]. MBL is further classified according to two different parameters: the immunophenotypic profile and the size of the B-cell clone [1,2].

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http://dx.doi.org/10.1053/j.seminoncol.2016.02.013
0093-7754/

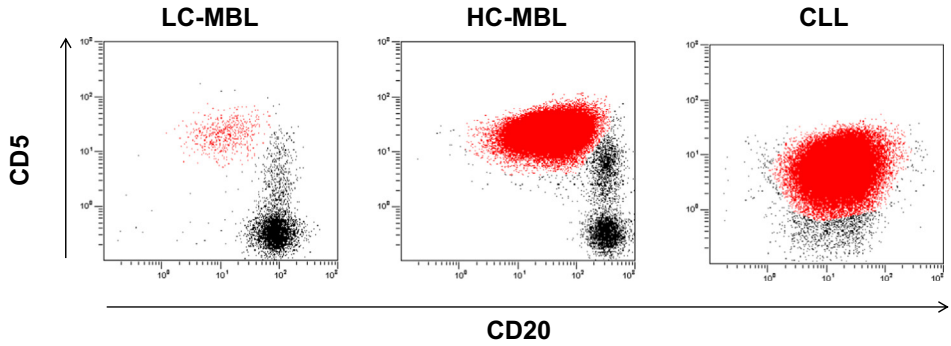


**Fig. 1.** Identical immunophenotypic profile but different biological significance in LC-MBL, HC-MBL, and CLL. From the right to the left, dot plots representing CD20 and CD5 expression on $CD19^+$ B cells from a LC-MBL, HC-MBL, and CLL subject are depicted. All three conditions are characterized by CD5 expression along with $CD20^{dim}$ and are virtually indistinguishable except for the clone size and the proportion of normal residual B cells.

(A) According to the immunophenotype the following categories were identified:

- CLL-like (representing 70%–75% of MBL): $CD19^+$ B cells belonging to this group are positive for CD5 and CD23, express low levels of CD20 ($CD20^{dim}$) and of surface light and heavy chain (kappa or $lambda^{dim}$, apparently negative, and mainly IgM and/or $IgD^{dim}$), as typically found in CLL cases (Fig. 1);
- Atypical CLL: $CD19^+$ B cells are positive for CD5, but do not express (or express at very low density and in low percentage) CD23 and/or show high levels of CD20 expression (CD20 bright). The absence of t(11;14) must be confirmed in order to exclude mantle cell lymphoma (MCL) diagnosis;
- CD5-negative: $CD19^+$ B cells are negative for CD5 expression, do not express any other distinctive flow cytometry markers (eg, CD103 or CD10), thereby being potentially related to B cells of marginal-zone or lymphoplasmacytic lymphomas.

Being less frequent than CLL-like MBL, the two latter categories are ill-defined entities and only limited information is currently available, while the vast majority of clinical series and population studies were focused on CLL-like cases.

(B) Based on the concentration of clonal B cells, MBL can be further classified (Fig. 1) as:

- Low-count (LC): originally known as “population screening” or “general population”, as it is usually detected in the setting of population-based studies in subjects without lymphocytosis through high-sensitivity flow cytometry techniques;
- High-count (HC): usually associated with an increase in absolute lymphocyte count, being recognized in the clinical setting and therefore previously defined as “clinical” or “with lymphocytosis”.

Though being still debated, the most widely applied cut-off to differentiate these two conditions is 0.5 x $10^9$ clonal B cells/L. The MBL existence was initially recognized thanks to population surveys. Initial studies were performed in the setting of environmental health investigations in the late 1990s where the increase of $CD19^+$ $CD5^+$ B cells was evaluated as potential biomarker of hazardous waste exposure [5,6]. The prevalence of MBL was negligible (only 0.6%) and was later explained by the low sensitivity of the flow cytometry technique available at that time (two-color panel, no clonality assessment) but drew the attention of the scientific community to this condition, that turned out to be of particular interest for CLL experts.

The subsequent technological improvement gave the chance to perform systematic population studies that shed light on the real prevalence of MBL. Applying more sensitive flow cytometry techniques, different groups worldwide were able to identify tiny B-cell clonal populations in the peripheral blood of normal individuals [7–11]. Thanks to four-, five-, and, more recently, eight-color panels, it became evident that MBL was a much more frequent phenomenon in the general population (3.5%, 6.7%, and 12%, respectively) [7,10,11] than previously thought. Population studies highlighted that MBL is more common in elderly people (usually older than 65 years) and in male and that the frequency of CLL-like MBL is particularly high in first-degree relatives of CLL patients (reaching up to 18% in high-risk families) [12]. That notwithstanding, MBL is not limited to the aged population as it can be detected also among blood donors older than 45 years and even at younger age (18–40 years) in relatives of CLL patients [13]. Because initially the MBL prevalence appeared to increase with the use of progressively more sensitive techniques of detection, it was postulated that virtually all individuals could be carrying such cells circulating in the blood. This assumption turned out not to be true, considering that a cutting-edge flow cytometry approach ten times more sensitive than the previous ones was able to detect only a two-fold increase in MBL frequency in the individuals studied [14]. Recently, a mathematical algorithm has been proposed suggesting that this could be a matter of age and a CLL-like clone would be detected in the vast majority of tested population if subjects were old enough and larger blood volumes had been screened, with MBL frequency raising up to 100% in healthy subjects older than 70 years [15].

After the revision of CLL diagnostic criteria in 2008, the use of MBL definition became popular also in the clinical setting, being recognized with increased frequency. Thanks to the widespread technological improvement, nowadays in up to 70% of cases CLL diagnosis is made in asymptomatic individuals due to detection of lymphocytosis at routine blood tests. It has been determined that about 40-50% of cases previously classified as Rai stage 0 CLL (ie, with no other signs or symptoms of CLL beside a variable level of lymphocytosis) have been re-categorized as HC-MBL [16]. This term, though being more reassuring for the patients, avoiding the ominous word “leukemia”, does not affect the pre-neoplastic essence of this condition and still requires life-long follow up. Overall, this change in terminology reduced the incidence of CLL and altered the distribution of initial Rai stage at diagnosis, thus modifying the overall clinical outcome of the remaining CLL, with a shortened median time to treatment [17].

### *2.2. The role of the absolute lymphocyte count: drawing the line between “individuals” and “patients”*

As reported, the most widely accepted cut-off value for differentiating HC- and LC-MBL is 0.5 x 109 clonal B cells/L. This value first was proposed in a comprehensive meta-analysis of MBL