Brief Communication
Diagnostic Hematology

Ann Lab Med 2020;40:409-413
https://doi.org/10.3343/alm.2020.40.5.409
ISSN 2234-3806 • eISSN 2234-3814

ANNALS OF LABORATORY MEDICINE

Prevalence and Immunophenotypic Characteristics of Monoclonal B-Cell Lymphocytosis in Healthy Korean Individuals With Lymphocytosis

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Epidemiological studies of monoclonal B-cell lymphocytosis (MBL) have been conducted in limited geographical regions. Little is known about the prevalence of MBL in Asia. We investigated the prevalence and immunophenotypic characteristics of MBL in Koreans who had idiopathic lymphocytosis (lymphocyte count $>4.0\times10^9$/L) and were ≥40 years of age. A total of 105 leftover peripheral blood samples met these criteria among those from 73,727 healthy individuals who visited the Health Promotion Center, Samsung Medical Center, Korea, from June 2018 to August 2019. The samples were analyzed using eight-color flow cytometry with the following monoclonal antibodies: CD45, CD5, CD10, CD19, CD20, CD23, and kappa and lambda light chains. The overall prevalence of MBL in the study population was 2.9% (3/105); there was one case of chronic lymphocytic leukemia (CLL)-like MBL ($CD5^+CD23^+$), one case of atypical CLL-like MBL ($CD5^+CD23^-$), and one case of $CD5^-$MBL with a lambda restriction pattern. This is the first study on the MBL prevalence in an East Asian population, and it reveals a relatively low prevalence of MBL in healthy Korean individuals with lymphocytosis.

Key Words: Chronic lymphocytic leukemia, Monoclonal B-cell lymphocytosis, Prevalence, Immunophenotype, Korea

Received: September 23, 2019
Revision received: November 13, 2019
Accepted: March 20, 2020

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Monoclonal B-cell lymphocytosis (MBL) is a precursor condition of various chronic lymphoproliferative disorders, mainly chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma [1]. MBL is defined by the presence of fewer than 5×10^9 monoclonal B cells per liter of peripheral blood in the absence of any clinical signs or symptoms of malignancy and infectious/autoimmune diseases [2]. MBL can be subdivided into three categories based on the immunophenotypic characteristics of the monoclonal B cells: (1) CLL-like MBL ($CD5^+CD20^{dim}CD23^+sIg^{low}$), (2) atypical CLL-like MBL ($CD5^+CD20^{bright+}CD23^-$), and (3) $CD5^-$MBL [2, 3]. Each type can be further subdivided into low-count MBL and high-count MBL, depending on whether there are fewer or more than 0.5×10^9/L monoclonal B cells, respectively. High-count MBL has been reported to progress to CLL at a rate of 1–2% cases per year [4]. However, most epidemiological studies of MBL have been conducted in limited geographical regions including Europe, the US, and the Middle East [3, 5–8], and little is known about the prevalence of MBL in Asia. Therefore, we investigated for the first time the prevalence of MBL and its immunophenotypic characteristics in a healthy Korean population ≥40 years and having idiopathic lymphocytosis.

This study was performed using 105 leftover EDTA-peripheral blood samples stored at 4°C up to seven days. They were collected from 105 healthy individuals (67 males and 38 females)

among the 73,727 healthy individuals who were ≥40 years old and visited the Health Promotion Center, Samsung Medical Center, Seoul, Korea, from June 2018 to August 2019. The inclusion criteria were as follows: (1) no history of malignancy, autoimmune disease or infectious disease and (2) the samples revealed idiopathic lymphocytosis (lymphocyte count $>4.0\times10^9$/L). In contrast to other studies that enrolled healthy individuals with normal blood (lymphocyte) count [5, 7], the present study enrolled individuals with lymphocytosis ($>4.0\times10^9$/L) because the results of our pilot study showed an extremely low prevalence of MBL in healthy Korean individuals older than 40 years and with normal blood counts (data not shown). We recorded the age, sex, and complete blood count (CBC) information, for the selected samples meeting the inclusion criteria. The CBC was determined on a Sysmex XN-9000 analyzer (Sysmex, Kobe, Japan) at the time of samples collection. The median age of the 105 healthy individuals was 56 years (range 40–81 years). The median absolute lymphocyte count was 4.3×10^9/L (range 4.0–6.7×10^9/L). The study was approved by the Institutional Review Board of Samsung Medical Center (IRB No. SMC 2018-05-083-001).

Identification and subtyping of MBL were performed by eight-color flow cytometry (FC) using the following monoclonal antibodies: CD5-fluorescein isothiocyanate (FITC) (Beckman Coulter Inc., Miami, FL, USA), kappa (κ) light chain-allophycocyanin (APC) (Becton Dickinson, San Jose, CA, USA), lambda (λ) light chain-phycoerythrin (PE) (Becton Dickinson), CD45-AmCyan (Becton Dickinson), CD19-peridinin-chlorophyll protein-Cyanine5.5 (Becton Dickinson), CD10-PE-Cyanine7 (Becton Dickinson), CD20-Pacific blue (Becton Dickinson), and CD23-APC-Cyanine7 (APC-Cy7; eBioscience, San Jose, CA, USA). The final concentration was adjusted to 100,000 cells per tube.

After incubation with the monoclonal antibodies for 15 minutes at room temperature (approximately 20 to 25°C), the samples were lysed by adding 2 mL of FACS Brand Lysing Solution (Becton Dickinson) and incubated for 10 minutes at room temperature. The samples were centrifuged at $540\times g$ for 5 minutes and washed twice with 2 mL of phosphate-buffered saline containing 0.5% bovine serum albumin (Sigma-Aldrich, St. Louis, MO, USA) and 0.09% sodium azide (Sigma-Aldrich). Data were acquired on a three-laser FACSCanto II flow cytometer (Becton Dickinson) and analyzed with Kaluza software (Becton Dickinson). The presence of a monoclonal B-cell subset was determined by a κ/λ ratio of >3:1 or κ/λ <0.3:1) or more than 25% of the B cells lacking surface immunoglobulin [5]. For suspected MBL cases with a monoclonal B cell subset, extended phenotyping was performed to determine the immunophenotypic characteristics of the clonal B cells using the following monoclonal antibodies: CD5-PE-Cyanine7 (Beckman Coulter), CD19-Pacific blue (eBioscience), CD38-APC-Cy7 (eBioscience), CD79b-APC (Becton Dickinson), FMC7-FITC (eBioscience), and CD45-AmCyan (Becton Dickinson). The prevalence of MBL was estimated using 95% confidence interval (CI) for the one-sample proportion. The estimation was performed with SPSS for Windows, version 11 (SPSS Inc., Chicago, IL, USA).

The overall prevalence of MBL was 2.9% (3/105, 95% CI, 0.9–8.1%) in healthy Koreans with idiopathic lymphocytosis. To the best of our knowledge, this is the first study on the MBL prevalence in an East Asian population, and the prevalence demonstrated here (3/105, 2.9%) is lower than that reported in other studies conducted using a similar sensitive method and age group (5.7–14.3%) [5-8]. Our calculations suggested that 105 samples would be required to obtain a 95% CI with a ±3% margin of error. A summary of the prevalence of MBL across various geographical regions is provided in Table 1.

For three MBL cases, detailed laboratory findings and immunophenotypic characteristics are shown in Table 2. Using the diagnostic criteria proposed by Marti, *et al.* [4], we classified Case 2 as atypical CLL-like MBL and Case 3 as CD5–MBL. Based on the cell count (cut-off value of 0.5×10^9/L), Case 1 and Case 3 can be categorized as high-count MBL (2.22×10^9/L and 4.26×10^9/L, respectively), which is also known as clinical MBL [9]. Parikh, *et al.* [10] conducted a large cohort study to assess the clinical progression of high-count MBL using the Mayo Clinic CLL database. They found that 7% of the patients in the cohort were treated for progression to CLL, and 0.4% required therapy for high-grade lymphoma. For Case 1, regular annual check-up showed monoclonal B cells with the same phenotype. However, since the absolute count of monoclonal B cells increased to 3.35×10^9/L, we recommended close follow-up to quickly identify progression to CLL and take adequate action as needed. In particular, genetic variants in *SF3B1* and *NOTCH* may be important for predicting the prognoses of CLL patients, because approximately 10–15% of CLL cases and approximately 1–3% of high-count MBL cases harbor these genetic variants [11-13].

We classified Case 2 as atypical CLL-like MBL based on the immunophenotyping results (expression of CD5 with strong expression of CD20), which revealed $CD5^+$ MBL featuring monoclonal B cells that were CD23-negative and CD79b-positive. However, FMC7, which is an important marker for distinguishing CLL and mantle cell lymphoma, shows an intermediate expression pattern in the majority of atypical CLL-like MBL and $CD5^-$MBL

https://doi.org/10.3343/alm.2020.40.5.409

Table 1. Prevalence of MBL across geographical regions

Country	Enrolled population	Flow cytometric analysis	Prevalence of MBL	References
Spain	N=608 Age: >40 years, Normal blood count	Eight-color flow cytometry (tube 1 and tube 2) Tube 1: CD20, CD45, CD8+lambda, CD56+kappa, CD4, CD19, CD3, CD38 Tube 2: CD20, CD45, cytoplasmic Bcl2, CD23, CD19, CD10, CD5, CD38 Six-color flow cytometry (tube 3) Tube 3: CD20, kappa, lambda, CD19, CD10, CD5 Acquisition: 5×10^6 events/sample	All MBL: 87 (14.3%) - CLL-like MBL: 73 (12.0%) - $CD5^-$MBL: 14 (2.3%)	Nieto *et al.* (2009) [5]
USA	N=2,098 Age: ≥45 years Blood donors	Six-color flow cytometry: CD19, CD20, CD5, CD45, kappa, lambda Acquisition: 5×10^5 events/sample	All MBL: 149 (7.1%) - CLL-like MBL: 101 (4.8%) - Atypical CLL-like MBL: 23 (1.1%) - $CD5^-$MBL: 21 (1.0%)	Shim *et al.* (2014) [6]
Uganda	N=302 Age: ≥45 years Normal blood count	Eight-color flow cytometry: CD305, CD185, CD19, CD5, CD10, CD20, kappa, lambda Acquisition: 5×10^5 events/sample	All MBL: 42 (13.9%) - CLL-like MBL: 3/302 (1.0%) - $CD5^-$MBL: 41/302 (13.6%)	Rawstron *et al.* (2017) [7]
Saudi Arabia	N=365 Age: >50 years Normal blood count	Eight-color flow cytometry: CD45, CD19, CD20, CD5, CD10, CD3, kappa, lambda Acquisition: 1×10^6 events/sample	All MBL: 21 (5.7%) - CLL-like MBL: 10 (2.7%) - Atypical CLL-like MBL: 9 (2.5%) - $CD5^-$MBL: 2 (0.5%)	Aljurf *et al.* (2017) [8]
Korea	N=105 Age: ≥40 years Lymphocytosis (> $4,000\times10^9$/L)	Eight-color flow cytometry (tube 1) CD45, CD19, CD20, CD5, CD10, CD23, kappa, lambda Six-color flow cytometry (tube 2) Tube 2: CD45, CD19, CD5, CD38, CD79b, FMC7 Acquisition: 2×10^5 events/sample	All MBL: 3 (2.9%) - CLL-like MBL: 1 (0.95%) - Atypical CLL-like MBL: 1/105 (0.95%) - $CD5^-$MBL: 1 (0.95%)	Present study

Abbreviations: MBL, monoclonal B cell lymphocytosis; CLL, chronic lymphocytic leukemia.

Table 2. Clinical and immunophenotypic characteristics of the three MBL cases

Characteristics	Case 1	Case 2	Case 3
Age (yr)	68	53	65
Sex	male	male	male
Hemoglobin (g/L)	154	175	157
Platelet count ($\times10^9$/L)	205	314	173
Leukocyte count ($\times10^9$/L)	8.30	10.34	10.23
Lymphocyte count ($\times10^9$/L)	4.13	4.62	6.53
B-cell count ($\times10^9$/L)	2.45	0.68	4.26
B-cell compartment to total lymphocytes (%)	59.4	12.3	65.2
Monoclonal B cell count ($\times10^9$/L)	2.22	0.46	4.26
Marker expression on monoclonal B cells			
CD5	Positive	Positive	Negative
CD10	Negative	Negative	Negative
CD19	Positive	Positive	Positive
CD20	Positive	Positive	Positive
CD23	Positive	Negative	Negative
CD38	Negative	Negative	Negative
CD79b	Dim positive	Positive	Positive
FMC7	Negative	Positive	Positive
Kappa/lambda	Lacked surface immunoglobulin	Lacked surface immunoglobulin	Lambda restriction

Abbreviation: MBL, monoclonal B cell lymphocytosis.

cases, in contrast to CLL-like MBL as in our Cases 2 and 3 [14]. In addition, several studies have reported that CLL cases with atypical features such as a positive reaction to FMC7 antibodies and/or a negative reaction to CD23 antibodies are more common in Asia [15, 16]. Although we did not perform molecular tests to confirm a translocation between 11q13 and 14q32, which is the hallmark of mantle cell lymphoma, Case 2 can be classified as atypical MBL given the lack of a specific finding during the regular check-up, including physical examination.

The phenotypic characteristics of $CD5^-$ MBL differ substantially from those of the other two MBL types. Unlike CLL-like MBL, the monoclonal B cells of $CD5^-$ MBL are characterized by expression of CD20 and CD79b, with strong surface expression of immunoglobulin and no expression of CD5 and CD23, as in Case 3 [17, 18]. Parker, *et al.* [19] reported that $CD5^-$MBL might be a precursor stage of splenic marginal zone lymphoma owing to a common genetic basis. With the exception of a cross-sectional study conducted in rural Uganda, which reported a high prevalence of $CD5^-$ MBL (13.6%), most population-based studies have found a relatively lower prevalence of $CD5^-$MBL (0.5–2.3%) compared with that of CLL-like MBL (Table 1) [3, 5-8].

The main limitation of this study is that it is a relatively small-scale, single-center study attempting to represent the Korean population, which did not account for the age and gender distribution among the MBL subtypes. Moreover, although several studies have shown that certain genetic variants are associated with CLL and high-count MBL outcomes, we could not confirm the presence of a chromosome abnormality or genetic variation in the three identified MBL cases. Therefore, a detailed follow-up study should be conducted to determine the potential for progression to CLL or other lymphoproliferative diseases in these MBL cases.

In conclusion, this is the first study on the MBL prevalence in an East Asian population and reveals a relatively low prevalence of MBL in healthy Korean individuals with lymphocytosis.

ACKNOWLEDGEMENTS

None declared.

AUTHOR CONTRIBUTIONS

IYY and DC designed the study, analyzed the data, and wrote the manuscript. DJL, HJK, SHK, and SJK collected the clinical samples, analyzed the data, and reviewed the manuscript. SHB participated in experiments. KK participated in statistical analysis. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors have no conflict of interest to declare.

RESEARCH FUNDING

None declared.

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