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 $5 \times 10^9$ 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⁺CD23⁻sIgM⁻), (2) atypical CLL-like MBL (CD5⁻CD20⁺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 $0.5 \times 10^9$ 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 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
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 >4.0×10^9/L). In contrast to other studies that enrolled healthy individuals with normal blood (lymphocyte) count [5, 7], the present study enrolled individuals with lymphocytosis (>4.0×10^9/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-phycocerythrin (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×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
Table 1. Prevalence of MBL across geographical regions

| Country | Enrolled population | Flow cytometric analysis | Prevalence of MBL | References |
|---------|---------------------|--------------------------|-------------------|------------|
| Spain   | N = 608            | Eight-color flow cytometry (tube 1 and tube 2) | All MBL: 87 (14.3%) | Nieto et al. (2009) [5] |
|         | Age: > 40 years, Normal blood count | Tube 1: CD20, CD45, CD8+lambda, CD56+kappa, CD4, CD19, CD3, CD38 | - CLL-like MBL: 73 (12.0%) |          |
|         |                     | Tube 2: CD20, CD45, cytoplasmic Bcl2, CD23, CD19, CD10, CD5, CD38 | - CD5-MBL: 14 (2.3%) |          |
|         |                     | Six-color flow cytometry (tube 3) | All MBL: 87 |          |
|         |                     | Tube 3: CD20, kappa, lambda, CD19, CD10, CD5 | - CLL-like MBL: 14 (2.3%) |          |
|         |                     | Acquisition: 5 × 10^6 events/sample | - Atypical CLL-like MBL: 9 (2.5%) |          |
| USA     | N = 2,098          | Six-color flow cytometry: CD19, CD20, CD5, CD45, kappa, lambda | All MBL: 149 (7.1%) | Shim et al. (2014) [6] |
|         | Age: ≥ 45 years Blood donors | Acquisition: 5 × 10^6 events/sample | - CLL-like MBL: 101 (4.8%) |          |
|         |                     | All MBL: 149 | - Atypical CLL-like MBL: 23 (1.1%) |          |
|         |                     | - CD5-MBL: 21 (1.0%) |          |          |
| Uganda  | N = 302            | Eight-color flow cytometry: CD305, CD185, CD19, CD5, CD10, CD20, kappa, lambda | All MBL: 42 (13.9%) | Rawstron et al. (2017) [7] |
|         | Age: ≥ 45 years Normal blood count | Acquisition: 5 × 10^5 events/sample | - CLL-like MBL: 3/302 (1.0%) |          |
|         |                     | All MBL: 42 | - Atypical CLL-like MBL: 21/302 (13.5%) |          |
| Saudi Arabia | N = 365 | Eight-color flow cytometry: CD45, CD19, CD20, CD5, CD10, CD3, kappa, lambda | All MBL: 21 (5.7%) | Aljurf et al. (2017) [8] |
|         | Age: > 50 years Normal blood count | Acquisition: 1 × 10^6 events/sample | - CLL-like MBL: 10 (2.7%) |          |
|         |                     | All MBL: 21 | - Atypical CLL-like MBL: 2/365 (0.5%) |          |
| Korea   | N = 105            | Eight-color flow cytometry (tube 1) | All MBL: 3 (2.9%) | Present study |
|         | Age: ≥ 40 years Lymphocytosis (> 4,000 × 10^9/L) | CD45, CD19, CD20, CD5, CD10, CD23, kappa, lambda | - CLL-like MBL: 1 (0.95%) |          |
|         |                     | Six-color flow cytometry (tube 2) | All MBL: 3 |          |
|         |                     | Tube 2: CD45, CD19, CD5, CD38, CD79b, FMC7 | - Atypical CLL-like MBL: 1/105 (0.95%) |          |
|         |                     | Acquisition: 2 × 10^6 events/sample | - CD5-MBL: 1 (0.95%) |          |
|         |                     | B-cell compartment to total lymphocytes (%) | 59.4 |          |
|         |                     | Monoclonal B cell count (× 10^7/L) | 2.22 |          |
|         |                     | Acquisition: 2 × 10^6 events/sample | 2.22 |          |
|         |                     | B-cell compartment to total lymphocytes (%) | 0.46 |          |

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 (× 10^9/L)        | 205    | 314    | 173    |
| Leukocyte count (× 10^9/L)       | 8.30   | 10.34  | 10.23  |
| Lymphocyte count (× 10^9/L)      | 4.13   | 4.62   | 6.53   |
| B-cell count (× 10^9/L)          | 2.45   | 0.68   | 4.26   |
| B-cell compartment to total lymphocytes (%) | 59.4 | 12.3 | 65.2 |
| Monoclonal B cell count (× 10^7/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 |

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.

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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.

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ORCID
In Young Yoo https://orcid.org/0000-0003-1505-846X
Sung Hoan Bang https://orcid.org/0000-0003-0551-1398
Dae Jin Lim https://orcid.org/0000-0003-3079-5716
Seok Jin Kim https://orcid.org/0000-0002-2776-4401
Kyunga Kim https://orcid.org/0000-0002-0865-2236
Hee Jin Kim https://orcid.org/0000-0003-3741-4613
Sun-Hee Kim https://orcid.org/0000-0002-7542-5551
Duck Cho https://orcid.org/0000-0001-6861-3282

REFERENCES
1. Landgren O, Albitar M, Ma W, Abbasi F, Hayes RB, Ghia P, et al. B-cell clones as early markers for chronic lymphocytic leukemia. N Engl J Med 2009;360:659-67.
2. Shim YK, Middleton DC, Caporaso NE, Rachel JM, Landgren O, Abbasi F, et al. Prevalence of monoclonal B-cell lymphocytosis: a systematic review. Cytometry B Clin Cytom 2010;78(51):S10-8.
3. Criado I, Rodríguez-Caballero A, Gutiérrez ML, Pedreira CE, Alcoceba M, Nieto W, et al. Low-count monoclonal B-cell lymphocytosis persists after seven years of follow up and is associated with a poorer outcome. Haematologica 2018;103:1198-208.
4. Marti GE, Rawstron AC, Ghia P, Hillmen P, Houlston RS, Kay N, et al. Diagnostic criteria for monoclonal B-cell lymphocytosis. Br J Haematol 2005;130:325-32.
5. Nieto WG, Almeida J, Romero A, Teodosio C, López A, Henriques AF, et al. Increased frequency (12%) of circulating chronic lymphocytic leukemia-like B-cell clones in healthy subjects using a highly sensitive multicolor flow cytometry approach. Blood 2009;114:33-7.
6. Shim YK, Rachel JM, Ghia P, Boren J, Abbasi F, Dagkis A, et al. Monoclonal B-cell lymphocytosis in healthy blood donors: an unexpectedly common finding. Blood 2014;123:1319-26.
7. Rawstron AC, Ssemaganda A, de Tute R, Doughty C, Newton D, Vardi A, et al. Monoclonal B-cell lymphocytosis in a hospital-based UK population and a rural Ugandan population: a cross-sectional study. Lancet Haematol 2017;4:e334-40.
8. Aljurf M, Rawas F, Alnounou R, Bakshi N, Chaudhri N, Khalil S, et al. Prevalence and relative proportions of CLL and non-CLL monoclonal B-cell lymphocytosis phenotypes in the Middle Eastern population. Hematol Oncol Stem Cell Ther 2017;10:42-3.
9. Bajuk P, Furlan T, Cermelj P, Ceh M, Podgornik H. Monoclonal B-cell lymphocytosis in the population of Slovenian region of Lower Carniola.
10. Parikh SA, Chaffee KG, Larson MC, Hampel PJ, Call TG, Ding W, et al. Outcomes of a large cohort of individuals with clinically ascertained high-count monoclonal B-cell lymphocytosis. Haematologica 2018;103:e237-40.

11. Rossi D, Bruscaggin A, Spina V, Rasi S, Khiabanian H, Messina M, et al. Mutations of the SF3B1 splicing factor in chronic lymphocytic leukemia: association with progression and fludarabine-refractoriness. Blood 2011;118:6904-8.

12. Schnaiter A, Paschka P, Rossi M, Zenz T, Bühler A, Winkler D, et al. NOTCH1, SF3B1, and TP53 mutations in fludarabine-refractory CLL patients treated with alemtuzumab: results from the CLL2H trial of the GCLLSG. Blood 2013;122:1266-70.

13. Morabito F, Mosca L, Cutrona G, Agnelli L, Tuana G, Ferracin M, et al. Clinical monoclonal B lymphocytosis versus Rai 0 chronic lymphocytic leukemia: a comparison of cellular, cytogenetic, molecular, and clinical features. Clin Cancer Res 2013;19:5890-900.

14. Lanasa MC, Allgood SD, Slager SL, Dave SS, Love C, Marti GE, et al. Immunophenotypic and gene expression analysis of monoclonal B-cell lymphocytosis shows biologic characteristics associated with good prognosis CLL. Leukemia 2011;25:1459-66.

15. Jang MA, Yoo EH, Kim K, Kim WS, Jung CW, Kim SH, et al. Chronic lymphocytic leukemia in Korean patients: frequent atypical immunophenotype and relatively aggressive clinical behavior. Int J Hematol 2013;97:403-8.

16. Tomomatsu J, Isobe Y, Oshimi K, Tabe Y, Ishii K, Noguchi M, et al. Chronic lymphocytic leukemia in a Japanese population: varied immunophenotypic profile, distinctive usage of frequently mutated IGH gene, and indolent clinical behavior. Leuk Lymphoma 2010;51:2230-9.

17. Amato D, Oscier DG, Davis Z, Mould S, Zheng J, Kolomietz E, et al. Cytogenetic aberrations and immunoglobulin VH gene mutations in clinically benign CD5+ monoclonal B-cell lymphocytosis. Am J Clin Pathol 2007;128:333-8.

18. Wang C, Amato D, Fernandes B. CD5-negative phenotype of monoclonal B-lymphocytosis of undetermined significance (MLUS). Am J Hematol 2002;69:147-9.

19. Parker E, Macdonald JR, Wang C. Molecular characterization of a t(2;7) translocation linking CDK6 to the IGK locus in CD5(-) monoclonal B-cell lymphocytosis. Cancer Genet 2011;204:260-4.