

Accepted Manuscript

Low-grade B-cell lymphoma presenting primarily in the bone marrow

Kayoko Iwatani MD, Katsuyoshi Takata MD, PhD, Yasuharu Sato MD, PhD, Tomoko Miyata-Takata MD, PhD, Noriko Iwaki MD, Wei Cui PhD, Seiko Sawada-Kitamura MD, PhD, Hiroshi Sonobe MD, PhD, Maiko Tamura MD, PhD, Katsuhiko Saito MD, PhD, Katsuya Miyatani MD, PhD, Rie Yamasaki MD, PhD, Ichiro Yamadori MD, PhD, Nobuharu Fujii MD, PhD, Yasushi Terasaki MD, Yoshinobu Maeda MD, PhD, Mitsune Tanimoto MD PhD, Naoya Nakamura MD, PhD, Tadashi Yoshino MD, PhD

PII: S0046-8177(14)00089-6
DOI: doi: [10.1016/j.humpath.2014.02.010](https://doi.org/10.1016/j.humpath.2014.02.010)
Reference: YHUPA 3243

To appear in: *Human Pathology*

Received date: 12 October 2013
Revised date: 6 February 2014
Accepted date: 14 February 2014

Please cite this article as: Iwatani Kayoko, Takata Katsuyoshi, Sato Yasuharu, Miyata-Takata Tomoko, Iwaki Noriko, Cui Wei, Sawada-Kitamura Seiko, Sonobe Hiroshi, Tamura Maiko, Saito Katsuhiko, Miyatani Katsuya, Yamasaki Rie, Yamadori Ichiro, Fujii Nobuharu, Terasaki Yasushi, Maeda Yoshinobu, Tanimoto Mitsune, Nakamura Naoya, Yoshino Tadashi, Low-grade B-cell lymphoma presenting primarily in the bone marrow, *Human Pathology* (2014), doi: [10.1016/j.humpath.2014.02.010](https://doi.org/10.1016/j.humpath.2014.02.010)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



Low-grade B-cell lymphoma presenting primarily in the bone marrow

Kayoko Iwatani MD^a, Katsuyoshi Takata MD, PhD^{a,*}, Yasuharu Sato MD, PhD^a,
Tomoko Miyata-Takata MD, PhD^a, Noriko Iwaki MD^a, Wei Cui PhD^a, Seiko
Sawada-Kitamura MD, PhD^b, Hiroshi Sonobe MD, PhD^c, Maiko Tamura MD, PhD^d,
Katsuhiko Saito MD, PhD^e, Katsuya Miyatani MD, PhD^f, Rie Yamasaki MD, PhD^g,
Ichiro Yamadori MD, PhD^h, Nobuharu Fujii MD, PhDⁱ, Yasushi Terasaki MD^j,
Yoshinobu Maeda MD, PhDⁱ, Mitsune Tanimoto MD PhDⁱ, Naoya Nakamura MD,
PhD^k, Tadashi Yoshino MD, PhD^a

^aDepartment of Pathology, Okayama University Graduate School of Medicine, Dentistry,
and Pharmaceutical Sciences, Okayama 700-8558, Japan

^bDivision of Pathology, Kanazawa University Hospital, Kanazawa 920-8641, Japan

^cDivision of Pathology, Chugoku Central Hospital, Fukuyama 720-0001, Japan

^dDivision of Pathology, Hiroshima City Hospital, Hiroshima 730-8518, Japan

^eDepartment of Pathology, Toyama City Hospital, Toyama, 939-8511, Japan

^fDivision of Pathology, Mitoyo General Hospital, Kanonji 769-1695, Japan

^gDivision of Pathology, National Hospital Organization Iwakuni Clinical Center,

Iwakuni 740-8510, Japan

^hDivision of Pathology, National Hospital Organization Okayama Medical Center,
Okayama 701-1192, Japan

ⁱDepartment of Hematology and Oncology, Okayama University Graduate School of
Medicine, Dentistry, and Pharmaceutical Sciences, Okayama 700-8558, Japan

^jDivision of Hematology, Toyama City Hospital, Toyama 939-8511, Japan

^kDepartment of Pathology, Tokai University Institute of Medical Sciences, Isehara
259-1193, Japan

Key words: low-grade B cell lymphoma, bone marrow, LGBCL-NOS, MYD88

Running title: LGBCL presenting primarily in the BM

Disclosure of conflicts of interest: The authors declare no conflicts of interest.

Funding: This work was supported by a grant from the Japan Society for the Promotion
Science (JSPS no. 19590348 and 24790350)

***Corresponding author:** Katsuyoshi Takata, MD, PhD.

Department of Pathology,

Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical
Sciences

2-5-1 Shikata-cho Okayama 700-8558, Japan

E-mail address: katsuyoshi.t@h5.dion.ne.jp

Tel. +81 86 235 7150

Fax. +81 86 235 7156

ABSTRACT

Cases of low-grade B-cell lymphoma presenting primarily in the bone marrow are rare, and its clinicopathology remains unclear. We retrospectively examined patients with low-grade B-cell lymphoma presenting primarily in the bone marrow. Fourteen patients met the inclusion criteria, including 5 with lymphoplasmacytic lymphoma (LPL), 3 with chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), 2 with follicular lymphoma (FL), and 4 with low-grade B-cell lymphoma not otherwise specified (LGBCL-NOS). The median age was 69.5 years (range, 42–89 years), and a slight male predominance was noted (9 men and 5 women, 1.8: 1).

Immunohistochemically, all cases were positive for CD20. One case was positive for CD138. Both cases of FL were positive for CD10 and B-cell lymphoma 2 (BCL-2), and immunoglobulin heavy locus *IgH/BCL-2* rearrangement was observed by fluorescence *in situ* hybridization. The myeloid differentiation primary response gene (88) (*MYD88*) L265P mutation was observed in 3 of 5 LPL, 1 of 2 FL, and 2 of 4 LGBCL-NOS patients. Paraproteinemia was observed in 10 patients; immunoglobulin M and G paraproteinemia were observed in 6 and 3 patients, respectively. In this patient series, 3 patients had died at a median follow-up of 36.5 months; the cause of death of 1 LPL patient was malignant lymphoma itself. Thus, low-grade B-cell lymphoma presenting

primarily in the bone marrow has various subtypes, and approximately one-third of the patients had LGBCL-NOS. The immunophenotypic features and *MYD88* L265P mutation data of LGBCL-NOS suggested that some cases present with characteristics similar to those of LPL or marginal zone lymphoma.

INTRODUCTION

Low-grade B-cell lymphomas that are localized to certain organs have specific characteristics. For example, extranodal marginal zone lymphoma of the mucosa-associated lymphoid tissue (MALT) of the stomach is associated with chronic inflammation caused by *Helicobacter pylori*, gastrointestinal follicular lymphoma (FL) frequently occurs at the duodenum and spreads to the small intestine, and primary cutaneous follicle center lymphoma is localized to the skin and exhibits an indolent clinical course.

Low-grade B-cell lymphomas such as FL, lymphoplasmacytic lymphoma (LPL), and chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) frequently involve the bone marrow [1-5]. However, cases of low-grade B-cell lymphoma presenting primarily in the bone marrow are quite rare. Only one report [6] concerning primary bone marrow lymphoma has investigated this issue; however, the authors included aggressive B-cell lymphomas such as diffuse large B-cell lymphoma in the inclusion criteria. Therefore, the clinicopathological features and clinical behavior of low-grade B-cell lymphomas presenting primarily in the bone marrow are still unknown. In the present study, we retrospectively analyzed a series of 14 patients with low-grade B-cell lymphoma presenting primarily in the bone marrow and attempted to clarify the

clinicopathological and genotypic characteristics.

MATERIALS AND METHODS

Patient selection

The pathology database at our institution was searched for cases of low-grade B-cell lymphoma involving the bone marrow from January 2000 to December 2011. A total of 199 patient samples of bone marrow were retrieved. From them, cases of mantle cell lymphoma, multiple myeloma, aggressive B-cell lymphoma, or acute B-cell lymphoblastic leukemia as well as cases presenting with lymph node swelling, splenomegaly, hepatomegaly, any mass detectable by physical examination or computed tomography (CT), or bone changes detected by CT were excluded. Furthermore, among the patients who underwent ^{18}F fluorodeoxyglucose positron emission tomography/CT (^{18}F -FDG PET)/CT, those who exhibited a high standardized uptake value of ^{18}F -FDG outside the bone marrow were excluded. All patients in the present study underwent thoracic, abdominal, and pelvic CT scanning.

Diagnoses were made according to the current WHO 2008 classification. In the present study, cases of low-grade B-cell lymphoma that did not meet any classification

criteria according to the WHO classification were considered as low-grade B-cell lymphoma not otherwise specified (LGBCL-NOS). Multiple myeloma was ruled out by using the International Myeloma Working Group (IMWG) criteria [7]. CD20-positive multiple myeloma was also ruled out based on the presence of certain clinical features such as the absence of lytic bone lesions on CT, as well as CD19 positivity [8] and cyclin D1 negativity on immunohistochemistry [9]. B lymphoblastic leukemia/lymphoma were ruled out based on the findings of histopathology and immunostaining of TdT and MIC2. B-cell prolymphocytic leukemia was ruled out based on the findings of a peripheral blood smear. Hairy cell leukemia was ruled out based on the presence of Annexin A1 negativity by immunostaining and CD103 positivity by flow cytometry. Hairy cell leukemia-variant and splenic B-cell lymphoma/leukemia unclassifiable were ruled out based on the findings of flow cytometry and presence of splenomegaly.

A total of 14 patients met the inclusion criteria. Of the 14 patients, bone marrow biopsy findings were available in 7 patients and clot section findings were available in the other 7 patients. The major differential diagnostic points between CLL/SLL and LGBCL-NOS are described as follows. First, clinically, the patients who had elevated white blood cell counts generally had CLL/SLL; these cases were excluded for the

diagnosis of LGBCL-NOS. Second, histologically, the case that presented with monotonous cells with a condensed nucleus and partial presence of paraimmunoblasts was included among the CLL/SLL cases. Third, CD5- and CD23-positive cases, identified using immunohistochemical study or flow cytometry, were also included among the CLL/SLL cases and excluded for the diagnosis of LGBCL-NOS. The majority of patients with LPL had IgM paraproteinemia. Histologically, tumor cells in LPL are predominantly small lymphocytes admixed with plasma cells and plasmacytoid cells. Patients who presented with these clinical manifestations and histological characteristics were excluded for the diagnosis of LGBCL-NOS. We used the term “LGBCL-NOS” for cases that did not meet any WHO criteria, because they could not be further subtyped. Bone marrow aspirate cytology was available in 12 patients. Other clinical data were collected, including blood cell counts, serum biochemistry, including the levels of lactate dehydrogenase (LDH), soluble interleukin 2 receptor (sIL2-R), and M protein type. Survival outcomes were also examined. The invasion pattern of the bone marrow was evaluated according to the criteria proposed by Arber et al. [1]. The study protocol was approved by the Institutional Review Board of Okayama University, Okayama, Japan, and written informed consent was obtained from each patient. All study procedures were conducted in accordance with the guidelines of the Declaration

of Helsinki.

Flow cytometric analysis

Flow cytometric analysis was performed on 13 samples obtained from either bone marrow aspirates or peripheral blood using a single-tube, 2-color method as described previously [10]. Monoclonal antibodies against CD3, CD5, CD10, CD19, CD20, CD23, CD38, CD45, and Ig κ and Ig λ light chains were used. The data were acquired on a FACS Calibur flow cytometer (BD Biosciences, San Jose, CA, USA) and analyzed using CellQuest software (BD Biosciences, San Jose, CA, USA).

Immunohistochemistry and *in situ* hybridization

Immunohistochemical analysis was performed on 10% formalin-fixed, paraffin-embedded tissue sections. Automated immunostainers (Ventana Medical System, Tucson, AZ, USA; BOND-MAX, Leica Microsystems, Melbourne, Australia) [11] were used according to the manufacturers' instructions. The following primary antibodies were used (clone, dilution; source): CD20 (L26, 1:200; Dako Corporation, Carpinteria, CA, USA), CD3 (LN10, 1:200; Leica Microsystems), CD5 (4C7, 1:100;

Novocastra, Newcastle upon Tyne, UK), CD10 (56C6, 1:100; Novocastra), B-cell lymphoma 2 (BCL-2) (3.1, 1:400; Novocastra), CD21 (1F8, 1:20; Dako Corporation), CD23 (1B12, 1:100; Novocastra), Cyclin D1 (SP4, 1:50; Nichirei Biosciences, Tokyo, Japan), CD19 (LE-CD19, 1:50; Dako Corporation), CD138 (MI15, 1:100; Dako Corporation), B-cell lymphoma 6 (BCL-6) (PG-B6P, 1:100; Dako Corporation), multiple myeloma oncogene 1 (MUM1) (MUM1p, 1:50; Dako Corporation), AnnexinA1 (rabbit polyclonal to AnnexinA1, 1:100; Abcam, Cambridge, UK), and Ki-67 (MIB-1, 1: 10,000; Novocastra). After immunostaining, CD20, CD3, CD5, CD10, BCL-2, CD23, Cyclin D1, CD19, CD138, BCL-6, and AnnexinA1 were scored quantitatively as negative, partially positive (i.e., <30% positive tumor cells), or positive (i.e., >30% positive tumor cells). Cells were considered MUM1 positive if they exhibited >30% positivity for MUM1. Furthermore, the Ki-67 index was determined; it was considered low and high at <10% and >10%, respectively. Fluorescence *in situ* hybridization (FISH) analysis for immunoglobulin heavy locus (*IgH*)/*BCL-2* rearrangement t(14;18) was performed using a commercially available FISH probe set (Abbott Molecular Inc., Wiesbaden, Germany) [12] on paraffin blocks available from patients #9–14. FISH analysis for *API2/MALT1* t(11; 18)(q21; q21) was also performed for LPL and LGBCL-NOS patients using a commercially available FISH probe set

(Abbott Molecular Inc., Des Plaines, IL, USA) [13]. FISH analysis for *IRF4* gene translocation was also performed for immunohistochemically MUM1-positive samples using a commercially available FISH probe set (KREATECH, Amsterdam, The Netherlands) [14].

Screening for the myeloid differentiation primary response gene (88) (*MYD88*)

L265P mutations

DNA was isolated from formalin-fixed paraffin-embedded tissue using the QIAmp system (Qiagen). *MYD88* genes were amplified by polymerase chain reaction (PCR). The forward and reverse primers used for *MYD88* were 5'-GTTGAAGACTGGGCTTGTC-3' and 5'-GTGCAGGGGTTGGTGTAGTC-3', respectively. The purified PCR products were directly sequenced in both directions using the BigDye Terminator Cycle Sequencing Kit and analyzed on an automatic 3500 DNA sequencer (Applied Biosystems).

***IgVH* sequence analysis**

Immunoglobulin heavy chain gene rearrangement was detected by PCR. DNA was extracted from formalin-fixed paraffin-embedded tissue sections. Immunoglobulin

heavy chain genes were amplified by using semi-nested PCR, with the primers directed to the framework 2 region (FR2A: 5'-TGGRTCCGMCAGSCYYCNGG-3' for both the first and second PCR) and the joining region (LJH: 5'-TGAGGAGACGGTGACC-3' for the first PCR, and VLJH: 5'-GTGACCAGGGTNCCTTGGCCCCAG-3' for the second PCR). DNA samples were separately subjected to PCR with TAKARA Ex Taq (Takara Bio Inc., Otsu, Japan). The amplified products were electrophoresed on a 3% agarose gel. A product was determined to be "clonal" if a single or dominant discrete band was apparent. To sequence clonal bands, the PCR products were purified with ExoSAP-IT (USB, Affymetrix). Sequencing was performed using the BigDye Terminator version 3.1 Cycle Sequencing Kit (AB, Warrington, UK), and the reaction products were analyzed on an automatic 3130xl DNA sequencer (Applied Biosystems). The nucleotide sequences of VH regions were compared to deposited sequences using the IgBLAST database (<http://www.ncbi.nlm.nih.gov/igblast/>) in the PubMed Center.

RESULTS

Clinical manifestations and outcomes

The patients' clinical characteristics are summarized in Table 1. The median age was

69.5 years (range, 42–89 years), and a slight male predominance was noted (9 men and 5 women, 1.8:1). Compared to that noted among other subtypes, a female predominance (1 man and 3 women, 1:3) was observed in LGBCL-NOS. Four patients had tumor cells in the peripheral blood (#6, 9, 11, and 13), 3 patients presented with leukopenia (white blood cell [WBC] count $< 4.0 \times 10^9/L$), 7 patients presented with anemia (hemoglobin [Hb] concentration < 10.0 g/dL), and 5 patients presented with thrombocytopenia (platelets [Plt] $< 15 \times 10^9/L$). Four patients showed elevated LDH levels, and 6 patients showed high sIL2-R levels (sIL2-R $> 2.0 \times 10^3$ U/mL). With regard to M protein subtypes, all 5 LPL patients and 1 CLL/SLL patient had IgM, whereas 1 FL and 2 LGBCL-NOS patients had IgG.

Chemotherapy was performed in 9 of 14 patients. Among them, 7 patients received rituximab-containing therapy.

Three patients (#1, 2, and 8) died after 71, 83, and 62 months, respectively, with a median follow-up time of 36.5 months (range, 5–90 months): 2 patients had LPL (#1 and 2) and the other patient had CLL/SLL (#8). One LPL patient (#1) died because of intestinal hemorrhage, which was probably caused by thrombocytopenia. The other LPL patient (#2) died because of primary disease. One CLL/SLL patient (#8) died because of cerebral infarction.

Pathological findings and FISH results

Among 14 patients with low-grade B-cell lymphoma localized to the bone marrow, 5, 3, 2, and 4 patients had LPL, CLL/SLL, FL, and LGBCL-NOS, respectively. The histologic and immunophenotypic features are summarized in Table 2, Figure 1, and Figure 2.

In LPL patients, the tumor cells exhibited diffuse proliferation and included small- to medium-sized lymphocytes, plasmacytoid lymphocytes, and plasma cells (Figure 2A). The bone marrow invasion pattern was evaluated in 3 patients, which included mixed paratrabecular and interstitial, mixed nodular and interstitial, and interstitial patterns, respectively. With regard to immunohistochemistry, 5 samples were CD20 positive, CD19 positive, and CD138 negative, whereas 1 was CD5 positive. In CLL/SLL samples, the tumor cells consisted mainly of small monotonous lymphocytes with round nuclear contours; in addition, paraimmunoblast clusters were observed (Figure 2F).

Immunohistochemical analysis revealed that all 3 were CD20 positive. In addition, all samples were positive for CD5 and CD23, as determined by flow cytometric analysis and/or immunohistochemical analysis. In 2 FL patients, tumor cells showed a diffuse or mixed nodular and interstitial pattern of invasion, and tumor follicles were not observed

in either specimen. Tumor cells consisted of centrocytic cells admixed with some centroblastic cells (Figure 2K). Both samples were CD20, CD10 (#10 was partial), and BCL-2 positive according to immunohistochemical analysis. Follicular dendritic cells were not detected by CD21 immunostaining. The FISH study demonstrated *IGH-BCL2* translocations.

As a consequence of the exclusion criteria, 4 patients were classified as LGBCL-NOS. No patients had elevated WBC counts or IgM paraproteinemia. In all 4 bone marrow specimens, small- to medium-sized monotonous cells with condensed nuclear chromatin were observed without plasma cells, plasmacytoid lymphocytes, or paraimmunoblast clusters. In 2 patients, the pattern of bone marrow invasion was evaluated, which included mixed interstitial and diffuse, and diffuse patterns.

Immunohistochemical analysis showed that all 4 samples were CD20 positive. One patient (#14) was partially positive for CD10 and CD138. This patient's sample was positive for CD19 and negative for Cyclin D1. The samples of 2 of 4 LGBCL-NOS patients were positive for CD23.

There were no *IGH-BCL2* translocations in any of the 4 LGBCL-NOS patients according to the FISH study. Furthermore, the FISH study showed that there were no *API2-MALT1* translocations in LPL or LGBCL-NOS patients (data not shown). In

addition, there were no *IRF4* rearrangements in MUM1-positive patients (#8, #12, and #14; data not shown).

***MYD88* L265P mutation and IgVH gene usage analysis**

The *MYD88* L265P mutation was found in 6 of 14 patients including 3 of 5 LPL, 1 of 2 FL, and 2 of 4 LGBCL-NOS patients. The *MYD88* L265P mutation was not found in any of the CLL/SLL patients (Table 2 and Figure 3). VH usage was VH3-23, VH1-3, and VH4-34 in 3 CLL/SLL patients; VH3-7 and VH4-34 in 2 FL patients; and VH3-66 and VH4-59 in 2 LGBCL-NOS patients.

DISCUSSION

The present study analyzed different subtypes of low-grade B-cell lymphoma localized to the bone marrow, including LPL, CLL/SLL, FL, and LGBCL-NOS. Torlakovic et al. [15] reported that paratrabecular and mixed paratrabecular patterns are common in FL. Moreover, interstitial and mixed interstitial patterns are common in LPL [1]. However, bone marrow invasion patterns vary in the other types of low-grade B-cell lymphomas [1]. In the present study, paratrabecular, nodular, interstitial, and diffuse

patterns were all observed. However, the cases of low-grade B-cell lymphoma did not exhibit any specific pattern. Moreover, none of the samples exhibited an intra-sinusoidal pattern.

FL generally occurs in the lymph nodes and rarely in the gastrointestinal tract. After excluding other sites of involvement, only 2 patients met the diagnostic criteria for FL. Martinez et al. [6] described a series of 19 patients with primary bone marrow B-cell lymphoma, and only 4 patients had FL, including 3 patients who were positive for CD10 and BCL2. The findings of their patient series corroborate the present results regarding the rarity of primary bone marrow FL. Histologically, FL samples did not exhibit any tumor follicles. Only 4.6% of nodal FL cases exhibit a follicular pattern in the bone marrow [15]. Therefore, the 2 specimens in the present study might be the diffuse type of FL.

The tumor cells of patient #6 weakly expressed BCL6. Moreover, BCL6 was overexpressed in the tumor cells of patient #8. BCL6 is certainly a germinal center marker and is expressed in B-cell lymphoma of germinal center origin (i.e., FL, Burkitt's lymphoma, and some diffuse large B-cell lymphoma). CLL cells rarely express different levels of BCL6 regardless of mutational status, or the number of mutations or polymorphisms [16]. The CLL patients in the present study exhibited a high frequency

of BCL6 expression. However, only a few CLL/SLL patients were included in the case series, and hence, the high frequency of BCL6 expression may have been accidentally detected. Thus, further studies are required to completely understand these phenomena.

MYD88 is an adaptor molecule in Toll-like receptor and interleukin-1 receptor (IL-1R) signaling that leads to NF- κ B activation. Whole-genome sequencing in Waldenström macroglobulinemia identified a somatic variant (T→C) at position 38,182,641 in chromosome 3p22.2 that causes an amino acid change from leucine to proline (L265P) in the MYD88 protein [17]. The *MYD88* L265P mutation is found in 67–100% of LPL cases [17-22]. Moreover, the *MYD88* L265P mutation is occasionally found in non-germinal center diffuse large B-cell lymphoma (19%), but is rare or absent in germinal center diffuse large B-cell lymphoma (0%) [21]. Moreover, the *MYD88* L265P mutation is rarely found in MALT lymphoma (9%) [17, 18, 22], CLL/SLL (0–3%) [19, 20, 22], FL (4%) [20], or splenic marginal-zone lymphoma (4–21%) [17-19, 22].

In the present study, the *MYD88* L265P mutation was found in 3 of 5 LPL patients, 1 of 2 FL patients, and 2 of 4 LGBCL-NOS patients. Thus, the present results suggest that some LGBCL-NOS cases may share characteristics with LPL or marginal zone B-cell lymphoma. Nevertheless, it remains unclear whether LGBCL-NOS patients with the

MYD88 L265P mutation should be diagnosed with LPL. Therefore, further study is required to resolve this issue. However, patients with IgM paraproteinemia and LPL histology may be distinguished from those with LGBCL-NOS.

Among all patients analyzed, 3 died. One patient died of malignant lymphoma, whereas another patient died of intestinal hemorrhage, which was probably caused by severe thrombocytopenia. Thus, the cause of death was not only malignant lymphoma but also involved cytopenia or therapy. Therefore, the prognosis of low-grade B-cell lymphoma localized to the bone marrow should be studied further.

In conclusion, several subtypes of primary bone marrow lymphoma were identified in primary bone marrow lymphoma. Approximately one-third of patients with primary bone marrow lymphoma were classified as LGBCL-NOS, and shared clinicopathological and genotypic characteristics with LPL or marginal zone B-cell lymphoma. As the present study involved a relatively small sample size, additional studies with larger sample sizes are required to clarify the pathophysiology and prognosis of this disease.

ACKNOWLEDGEMENTS

The technical support of Ms. Okabe, Ms. Gion, and Ms. Yara Y. Kikuchi are greatly appreciated.

This work was supported by a grant from the Japan Society for the Promotion Science (JSPS no. 19590348 and 24790350)

REFERENCES

1. Arber DA, George TI. Bone marrow biopsy involvement by non-Hodgkin's lymphoma: frequency of lymphoma types, patterns, blood involvement, and discordance with other sites in 450 specimens. *Am J Surg Pathol* 2005; 29: 1549-1557.
2. Merli M, Arcaini L, Boveri E et al. Assessment of bone marrow involvement in non-Hodgkin's lymphomas: comparison between histology and flow cytometry. *Eur J Haematol.* 2010; 85: 405-415.
3. Arcaini L, Merli M, Passamonti F et al. Validation of follicular lymphoma international prognostic index 2 (FLIPI2) score in an independent series of follicular lymphoma patients. *Br J Haematol.* 2010: 455-457.
4. Morice WG, Chen D, Kurtin PJ, Hanson CA, McPhail ED. Novel immunophenotypic features of marrow lymphoplasmacytic lymphoma and correlation with Waldenstrom's macroglobulinemia. *Mod Pathol.* 2009; 22: 807-816.
5. Montillo M, Hamblin T, Hallek M, Montserrat E, Morra E. Chronic lymphocytic leukemia: novel prognostic factors and their relevance for risk-adapted therapeutic strategies. *Haematologica* 2005; 90: 391-399.

6. **Martinez A, Ponzoni M, Agostinelli C et al. International Extranodal Lymphoma Study G. Primary bone marrow lymphoma: an uncommon extranodal presentation of aggressive non-hodgkin lymphomas. Am J Surg Pathol 2012; 36: 296-304.**
7. **Dimopoulos M, Kyle R, Fermand JP et al. Consensus recommendations for standard investigative workup: report of the International Myeloma Workshop Consensus Panel 3. Blood 2011; 117: 4701-4705.**
8. **Seegmiller AC, Xu Y, McKenna RW, Karandikar NJ. Immunophenotypic differentiation between neoplastic plasma cells in mature B-cell lymphoma vs plasma cell myeloma. Am J Clin Pathol 2007; 127: 176-181.**
9. **Robillard N, Avet-Loiseau H, Garand R et al CD20 is associated with a small mature plasma cell morphology and t(11;14) in multiple myeloma. Blood 2003; 102: 1070-1071.**
10. **Kaleem Z, White G, Vollmer RT. Critical analysis and diagnostic usefulness of limited immunophenotyping of B-cell non-Hodgkin lymphomas by flow cytometry. Am J Clin Pathol 2001; 115: 136-142.**
11. **Loong F, Chan AC, Ho BC et al. Diffuse large B-cell lymphoma associated with chronic inflammation as an incidental finding and new clinical scenarios. Mod**

Pathol. 2010; 23: 493-501.

12. Einerson RR, Kurtin PJ, Dayharsh GA, Kimlinger TK, Remstein ED.

FISH is superior to PCR in detecting t(14;18)(q32;q21)-IgH/bcl-2 in follicular lymphoma using paraffin-embedded tissue samples. Am J Clin Pathol. 2005; 124: 421-429.

13. Toracchio S, Ota H, de Jong D et al. Translocation t(11;18)(q21;q21) in gastric B-cell lymphomas. Cancer Sci 2009; 100: 881-887.

14. Wada DA, Law ME, Hsi ED et al. Specificity of IRF4 translocations for primary cutaneous anaplastic large cell lymphoma: a multicenter study of 204 skin biopsies. Mod Pathol 2011; 24: 596-605.

15. Torlakovic E, Torlakovic G, Brunning RD. Follicular pattern of bone marrow involvement by follicular lymphoma. Am J Clin Pathol 2002; 118: 780-786.

16. Jantus LE, Reinoso MC, Garcia BC, Pendas J, Benet CC, Mayans FJR, Garcia CJ. BCL6: somatic mutations and expression in early-stage chronic lymphocytic leukemia. Leuk Lymphoma 2009; 50: 773-780.

17. Treon SP, Xu L, Yang G et al. MYD88 L265P somatic mutation in Waldenstrom's macroglobulinemia. N Engl J Med 2012; 367: 826-833.

18. Gachard N, Parrens M, Soubeyran I et al. IGHV gene features and MYD88 L265P mutation separate the three marginal zone. *Leukemia* 2013; 27: 183-189.
19. Jimenez C, Sebastian E, Del Carmen Chillón M et al. MYD88 L265P is a marker highly characteristic of, but not restricted to Waldenstrom's macroglobulinemia. *Leukemia* 2013; 27: 1722-1728.
20. Poulain S, Roumier C, Decambon A et al. MYD88 L265P mutation in Waldenstrom's macroglobulinemia. *Blood* 2013; 121: 4504-4511
21. Varettoni M, Arcaini L, Zibellini S et al. Prevalence and clinical significance of the MYD88 (L265P) somatic mutation in. *Blood* 2013; 121: 2522-2528.
22. Xu L, Hunter ZR, Yang G et al. MYD88 L265P in Waldenstrom macroglobulinemia, immunoglobulin M monoclonal. *Blood* 2013; 121: 2051-2058.

FIGURE LEGENDS

FIGURE 1. Histology of (A) LPL (#2), (B) CLL/SLL (#8), (C) FL (#10), and (D) LGBCL-NOS (#13) (hematoxylin and eosin [H&E], ×4). (A) LPL (#2) exhibits an interstitial pattern. (B) CLL/SLL (#8) exhibits aggregates of small lymphoid cells. (C) FL (#10) exhibits nodular (center) and interstitial (left) patterns. (D) LGBCL-NOS (#13) exhibits diffuse and interstitial patterns.

FIGURE 2. (A) In LPL (#2), the tumor cells exhibited diffuse proliferation and included small- to medium-sized lymphocytes, plasmacytoid lymphocytes, and plasma cells. Neoplastic cells in LPL are positive for (B) CD20, but negative for (C) CD10, (D) CD5, and (E) Cyclin D1. (F) In CLL/SLL samples (#6), the tumor cells consisted mainly of small monotonous lymphocytes with round nuclear contours; in addition, paraimmunoblast clusters were observed. Neoplastic cells in CLL/SLL lymphocytic lymphoma (CLL/SLL) (#8) are positive for (G) CD20, (H) CD5, and (I) CD23, but negative for (J) Cyclin D1. (K) In FL samples (#10), tumor cells consisted of centrocytic cells admixed with some centroblastic cells. Neoplastic cells in FL are positive for (L) CD20, (M) CD10, and (N) BCL-2, but negative for (O) CyclinD1. (P)

In LGBCL-NOS (#13), small- to medium-sized monotonous cells with condensed nuclear chromatin were observed without plasma cells, plasmacytoid lymphocytes, or paraimmunoblast clusters. Neoplastic cells in LGBCL-NOS are positive for (Q) CD20, but negative for (R) CD5, (S) CD10, and (T) CyclinD1.

FIGURE 3. The right sequence data shows a MYD88 L265P mutation-positive patient sample (A: #2, B: #11). The black arrow shows the mutation site.

Table 1. Clinical features and outcomes of low-grade B-cell lymphoma cases restricted to the bone marrow

No. #	Diagnosis	Age	Sex	PS	WBC Count ($\times 10^9$ /L)	Hb (g/dL)	Platelets ($\times 10^{10}$ /L)	sIL2-R (U/mL)	M protein	Genetic features	Treatment	Outcome	Cause of death	Follow-up time
1	LPL	67	M	0	3.3	5.6	15.0	1970	IgMk		Cyclosporine A	Dead	Intestinal hemorrhage	71
2	LPL	73	M	0	3.3	9.8	13.0	420	IgMk		R-CHOP	Dead	lymphoma	83
3	LPL	73	M	0	4.9	13.1	40.1	760	IgMk, BJk		NO	Alive		39
4	LPL	76	F	1	4.8	12.6	27.8	540	IgM λ	46, XX (20/20)	Cyclosporine A + prednisolone , Rituximab	Alive		34
5	LPL	67	M	1	4.5	13.2	20.9	272	IgMk	45, X, -Y (3/20), 47, XY, +18 (1/20), 46, XY (16/20)	IAPP	Alive		18
6	CLL/SLL	82	F	0	27.8	11.0	16.1	1390	—		NO	Lost to follow-up		88
7	CLL/SLL	89	M	4	4.5	11.1	21.0	7950	subtype unclear	46, XY (number data was not obtained)	NO	Lost to follow-up		5
8	CLL/SLL	64	M	0	6.8	7.7	46.7	1502	IgMk	45, X, -Y (5/20), 47, XY, +Y (1/20), 46, XY (14/20)	Rituximab, Fludarabine	Dead	Cerebral infarction	62
9	FL	62	M	0	8.5	6.0	11.9	2090	IgGk, BJk	46, XY, inv(9)(p12q13) (4/4)	R-CHOP	Alive		90
10	FL	72	M	0	7.0	7.3	6.5	8569	—	*	NO	Alive		10
11	LGBCL-NOS	65	M	0	21.9	13.8	21.0	12700	—	46, XY (20/20)	Cyclophosphamide	Alive		55
12	LGBCL-NOS	80	F	0	6.0	9.5	5.0	4830	—	46, XX (20/20)	R-THP-COP	Alive		32
13	LGBCL-NOS	42	F	0	2.8	8.3	10.0	5600	IgGk		Rituximab + Cladribine	Alive		18
14	LGBCL-NOS	47	F	0	5.9	10.7	40.2	2513	IgGk	46, XX (20/20)	NO	Alive		22

CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; F, failure; FL, follicular lymphoma; Hb, hemoglobin; IAPP, ifosfamide + adriamycin + cisplatin + peplomycin; LDH, lactate dehydrogenase; LGBCL-NOS, low-grade B-cell lymphoma not otherwise specified; LPL, lymphoplasmacytic lymphoma; PR, partial response; PS, performance status; sIL2-R, soluble interleukin-2 receptor; WBC, white blood cell, R-CHOP (rituximab+cyclophosphamide+ doxorubicin+vincristine+prednisolone), IAPP (ifosfamide+adriamycin+cisplatin+peplomycin), Rituximab, R-THP-COP (rituximab, cyclophosphamide, pirarubicin, vincristine and prednisolone), * 47, XY, add(1)(q21), t(14;18)(q32;q21), +ider(18)(q10)t(14;18) (3/20), 46, XY (17/20)

Table 2. Pathologic and immunophenotypic features of primary low-grade B-cell lymphoma patients

No.#	Diagnosis	Pattern	Cellularity	CD20	CD3	CD5	CD10	BCL2	CD23	CyclinD1	CD19	CD138	BCL-6	MUM1	AnnexinA1	MYD88		
																Ki-67 index	FISH t(14; 18)	L265P mutation
1	LPL	PT, INT	10%	+	—	—	—	ND	—	—	+	—	—	—	—	low	ND	+
2	LPL	NOD, INT	85%	+	—	—	—	ND	—	—	+	—	—	—	—	low	ND	+
3	LPL	ND	50%	+	—	—	—	ND	—	—	p+	—	—	—	—	low	ND	—
4	LPL	ND	10%	+	—	—	—	ND	—	—	p+	—	—	—	—	low	ND	+
5	LPL	INT	50%	+	—	+	—	ND	—	—	p+	—	—	—	—	low	ND	—
6	CLL/SLL	ND	85%	+	—	+	—	ND	+	—	+	—	+	—	—	low	ND	—
7	CLL/SLL	ND	80%	+	—	—*	—	ND	+	—	+	—	—	—	—	low	ND	—
8	CLL/SLL	ND	60%	+	—	+	—	ND	+	—	+	—	+	+	—	low	ND	—
9	FL	DIFF	100%	+	—	—	+	+	—	—	+	—	—	—	—	low	+	+
10	FL	NOD, INT	70%	+	—	—	+	+	—	—	+	—	+	—	—	low	+	—
11	LGBCL-NOS	ND	100%	+	—	—	—	ND	p+	—	+	—	—	—	—	low	UD	+
12	LGBCL-NOS	ND	80%	+	—	—	—	ND	—	—	+	—	—	+	—	low	—	—
13	LGBCL-NOS	INT, DIFF	90%	+	—	—	—	ND	—	—	UD	—	—	—	—	low	UD	—
14	LGBCL-NOS	DIFF	100%	+	—	—	p+	+	+	—	+	p+	—	+	—	low	—	+

*positive based on flow cytometric analysis.

CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; DIFF, diffuse; FL, follicular lymphoma; INT, interstitial; LGBCL-NOS, low-grade B-cell lymphoma not otherwise specified; LPL, lymphoplasmacytic lymphoma; ND, not done; NOD, nodular; p+, partially positive; PT, paratrabecular; UD, undetermined

FIGURE 1.

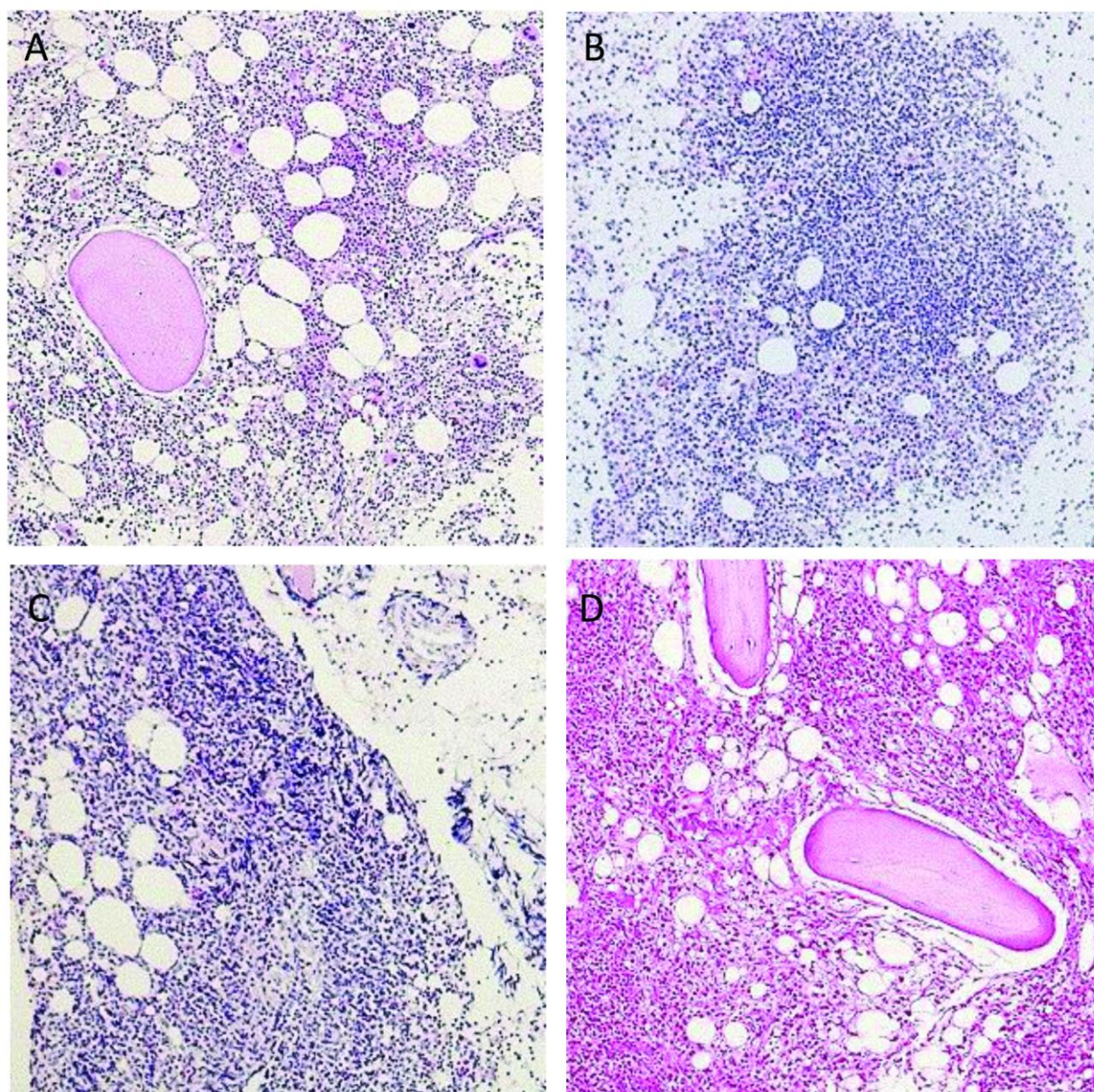
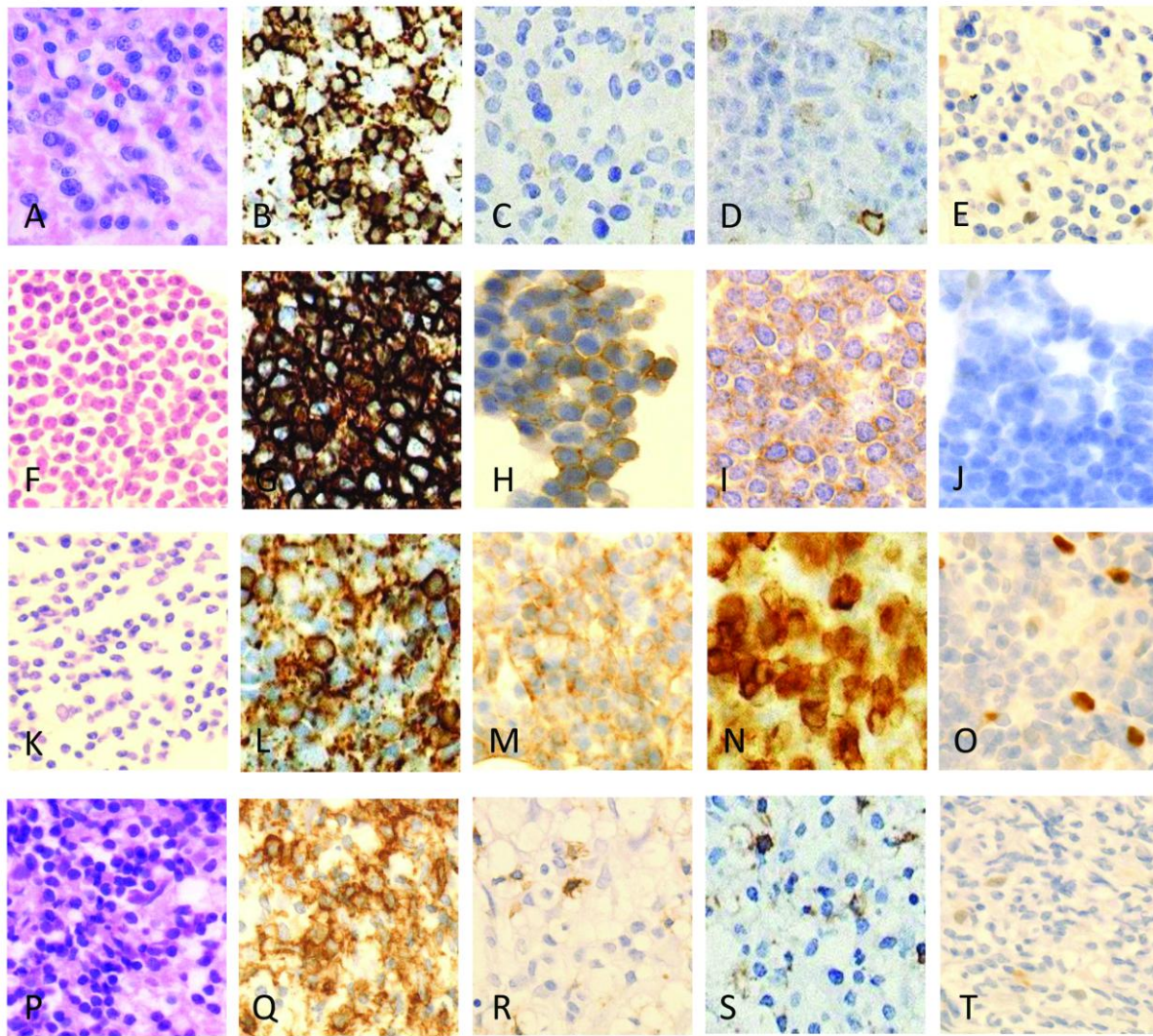
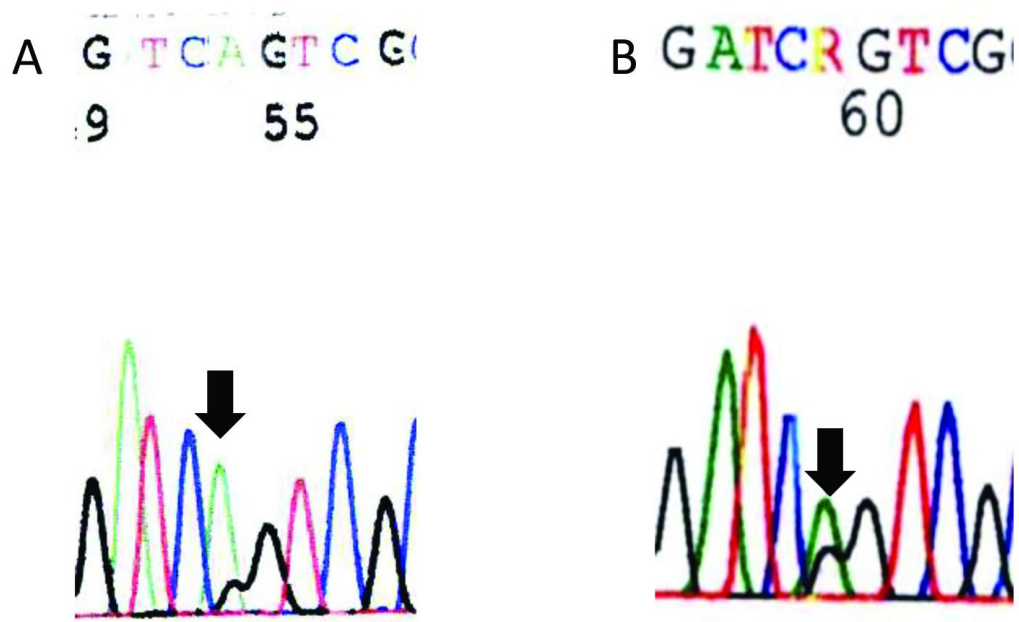


FIGURE 2.



ACCEPTED

FIGURE 3.



ACCEPTED MANUSCRIPT