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RESEARCH ARTICLE

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How useful is bone marrow study as an initial investigative tool without lymph node biopsy in malignant lymphoma?: Eleven years of experience at a single institution

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Background: Bone marrow (BM) study plays an important role as initial investigation specimen of lymphoma as well as staging lymphoma. This study aimed to investigate the utility of BM studies for classification of lymphoma and evaluate features of BM involvement by lymphoma over a period of 11 years.

Methods: A total of 1162 cases of BM studies for lymphoma evaluation were reviewed for the incidence of lymphoma subtypes, the percentage of marrow involvement, the pattern of involvement and discordance with histopathologic diagnoses of lymph nodes and other tissues.

Results: A total of 255 of 1162 cases underwent BM study without pathologic information, and 108 cases show lymphoma involvement. Lymph node biopsy underwent in 66 cases, and 10 cases show discordant result between BM and lymph node biopsy. Seven discordant cases were due to insufficient further studies. Lymphoma was diagnosed only by BM study in 38 cases. Abnormal lymphocytes were found in BM aspiration in 34 cases. Also, abnormal clonal lymphocytes were detected by flow cytometry in 26 cases. Four cases showed disease-related chromosomal abnormalities. FISH analysis detected abnormal findings in two cases, however, discordant with other additional studies.

Conclusions: Discrepancies between the BM study and lymph node biopsy were due to insufficient further study and discordance of immunohistochemical stain result. BM study can be utilized as initial diagnosis of lymphoma by the combination of morphological feature, involvement pattern, and additional tests such as flow cytometry, chromosomal analysis, and FISH analysis. Thus, BM study with further analysis is an essential choice when lymph node biopsies are unavailable.

KEYWORDS

bone marrow involvement, bone marrow study, lymphoma diagnosis, lymphoma malignancies

1 | INTRODUCTION

Bone marrow (BM) study including aspiration and biopsy is a well-established diagnostic procedure and is regarded as the standard for staging lymphoma patients. In some various clinical situations, BM biopsy may be the least invasive method or the only means of obtaining samples for the classification of lymphoma. Differential diagnoses can be made by BM using the morphologic and immunohistochemical (IHC) criteria used for lymph nodes biopsies,[1] although other approaches of methods combined with BM biopsies,

TABLE 1 Incidences and bone marrow involvement frequencies of malignant lymphoma subtypes from 2006 to 2016

	No. (%)	'06	'07	'08	'09	'10	'11	'12	'13	'14	'15	'16	Spearman's correlation	Bone marrow involvement (% of incidence)
Total	1162 (100)	49	94	93	59	116	137	95	140	118	105	156		235 (20.2)
Mature B-cell neoplasms	883 (76)	36	59	76	42	93	108	82	105	80	80	122		180 (20.4)
Chronic lymphocytic lymphoma/ small lymphocytic lymphoma	19 (1.6)	–	1	2	–	1	3	–	2	4	2	4	**0.656/0.028**	16 (84.2)
Burkitt lymphoma	20 (1.7)	4	2	–	3	–	5	–	–	3	1	2	−0.164/0.631	14 (70)
Mantle cell lymphoma	41 (3.5)	–	2	2	2	5	9	–	8	4	4	5	0.531/0.093	20 (48.8)
Follicular lymphoma	36 (3.1)	–	2	–	4	2	1	4	4	5	4	10	**0.823/0.002**	17 (47.2)
Diffuse large B-cell lymphoma	558 (48)	24	33	54	23	54	75	50	65	42	57	81	**0.656/0.028**	82 (14.7)
External marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)	168 (14.5)	6	18	17	4	25	14	26	14	20	12	12	0.050/0.883	9 (5.4)
Waldenström macroglobulinemia	4 (0.3)	–	1	–	–	–	–	–	–	1	–	2	0.319/0.339	4 (100)
Lymphoplasmacytic lymphoma	9 (0.8)	–	–	–	–	3	–	–	3	1	–	2	0.459/0.155	3 (33.3)
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma	7 (0.6)	–	–	–	2	1	–	–	2	–	–	2	0.277/0.409	1 (14.3)
Splenic marginal zone B-cell lymphoma	2 (0.2)	–	–	–	–	–	–	–	2	–	–	–	0.200/0.555	2 (100)
Nodal marginal zone lymphoma	1 (0.1)	–	–	1	–	–	–	–	–	–	–	–	−0.300/0.370	1 (100)
Pediatric nodal marginal zone lymphoma	2 (0.2)	–	–	–	2	–	–	–	–	–	–	–	−0.200/0.555	0 (0)
Splenic B-cell lymphoma/leukemia, unclassifiable	1 (0.1)	–	–	–	–	–	–	–	1	–	–	–	0.200/0.555	1 (100)
Unclassified	15 (1.3)	2	–	–	2	2	1	2	4	–	–	2		10 (66.7)
Mature T and NK neoplasms	208 (17.9)	13	23	13	13	21	22	9	29	30	16	19		48 (23.1)
Peripheral T-cell lymphoma, NOS	57 (4.9)	4	7	4	1	10	2	2	6	12	4	5	0.170/0.617	20 (35.1)
Angioimmunoblastic T-cell lymphoma	47 (4)	2	8	2	2	–	8	2	13	2	6	2	0.084/0.805	12 (25.5)
Extranodal NK-/T-cell lymphoma, nasal type	59 (5.1)	0	4	4	8	10	10	4	4	8	3	4	0.014/0.966	5 (8.5)
Anaplastic large-cell lymphoma	19 (1.6)	2	2	2	2	1	–	–	2	4	–	4	0.058/0.866	6 (31.6)
Enteropathy-associated T-cell lymphoma	12 (1)	2	–	–	–	–	–	–	4	2	2	2	0.510/0.109	0 (0)
Mycosis fungoides	2 (0.2)	–	–	–	–	–	–	–	–	–	–	2	0.500/0.117	0 (0)
Subcutaneous panniculitis-like T-cell lymphoma	2 (0.2)	–	–	–	–	–	–	–	–	2	–	–	0.300/0.370	0 (0)
Hydroa vacciniforme-like lymphoproliferative disorder	1 (0.1)	–	–	1	–	–	–	–	–	–	–	–	−0.300/0.370	0 (0)
Aggressive NK-cell leukemia	1 (0.1)	1	–	–	–	–	–	–	–	–	–	–	−0.300/0.371	1 (100)
Unclassified	8 (0.7)	2	2	–	–	–	2	1	–	–	1	–		4 (50)
Hodgkin lymphoma	71 (6.1)	–	12	4	4	2	7	4	6	8	9	15	−0.300/0.370	7 (9.9)

Spearman's correlation coifficient with $P < .05$ is marked in bold.

TABLE 2 Bone marrow involvement patterns of malignant lymphoma by subtype

	Involvement patterns				
	Focal non-paratrabecular (% of total)	Focal paratrabecular (% of total)	Intra-sinusoidal (% of total)	Diffuse interstitial (% of total)	Diffuse solid (% of total)
Mature B-cell neoplasms					
Diffuse large B-cell lymphoma	11 (15.1)	7 (9.6)	8 (11)	29 (39.7)	18 (24.7)
Mantle cell lymphoma	5 (27.8)	2 (11.1)	1 (5.6)	5 (27.8)	5 (27.8)
Follicular lymphoma	–	5 (31.3)	2 (12.5)	4 (25)	5 (31.3)
Chronic lymphocytic lymphoma/small lymphocytic lymphoma	2 (13.3)	1 (6.7)	–	6 (40)	6 (40)
Burkitt lymphoma	–	–	–	4 (44.4)	5 (55.6)
Marginal zone B-cell lymphoma of MALT	2 (25)	2 (25)	–	3 (37.5)	1 (12.5)
Lymphoplasmacytic lymphoma	1 (33.3)	–	1 (33.3)	–	1 (33.3)
Nodal marginal zone lymphoma	–	–	–	–	1 (100)
Splenic B-cell lymphoma	–	–	1 (100)	–	–
Splenic B-cell lymphoma/leukemia, unclassifiable	–	–	–	1 (100)	–
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma	–	–	–	–	1 (100)
Waldenström macroglobulinemia	–	–	–	–	4 (100)
Mature T and NK neoplasms					
Peripheral T-cell lymphoma, NOS	2 (11.1)	5 (27.8)	–	6 (33.3)	5 (27.8)
Angioimmunoblastic T-cell lymphoma	4 (33.3)	2 (16.7)	–	4 (33.3)	2 (16.7)
Anaplastic large-cell lymphoma	2 (33.3)	–	–	1 (16.7)	3 (50)
Extranodal NK-/T-cell lymphoma, nasal type	2 (40)	–	–	1 (20)	2 (40)
Hodgkin lymphoma	–	1 (16.7)	–	–	5 (83.3)
Total	31	25	13	64	64

such as flow cytometry and IHC stain using lymph node infiltrates or extranodal specimens, have been used to define lymphoma subtypes. On the other hand, the diagnostic value of BM alone for the differential diagnosis of lymphoma has not been actively studied. In the present study, the utility of BM studies for the classification of lymphoma was investigated. Cases in which a BM was used for initial investigative specimens in lymphoma were analyzed, and cases in which a primary diagnosis of lymphoma made by BM study with or without following tissue biopsy were reviewed. BM lymphoma involvement ratios and the proportion of lymphomas staged by BM studies among all BM studies conducted throughout 11 years, and the incidence and BM involvement patterns of malignant lymphoma types were also reviewed.

2 | METHODS

A total of 1162 BM studies for lymphoma evaluation conducted at Gachon University Gil Medical Center (Republic of Korea) from January 2006 to December 2016 were retrospectively reviewed.

The BM biopsy involvement patterns of malignant lymphoma were classified into five types[2]: focal non-paratrabecular with a discrete aggregate random pattern in the non-paratrabecular area, focal paratrabecular, which molds against trabecular bone, intrasinusoidal which aligns inside identifiable sinuses, diffuse interstitial, which infiltrates among normal hematopoietic cells, and diffuse solid type, which causes complete effacement of marrow space between several trabecular bones.

Histopathologic diagnoses of lymph nodes and other tissues except BM were reviewed. All BM biopsy specimens were fixed with formalin and embedded in paraffin. BM biopsy sections were stained with hematoxylin and eosin (H&E). IHC staining for CD20, CD10, CD3, CD5, cyclin D1, BCL2, BCL6, CD15, CD30, Ki67, MUM1, and CD138 was reviewed for a diagnosis of BM involvement and involvement pattern. Two expert hematologists independently reviewed lymphoma BM involvement and involvement pattern for each case. Disagreements were resolved by exchange opinions of decisional evidence of a pattern and matched with BM biopsy slides and adopted an adequate involvement pattern. Lymphoma was diagnosed based on the WHO classification of hematopoietic tumors. Statistical analysis was performed using Spearman's correlation analysis to assess annual trends in lymphoma case numbers and the involvement frequencies of each lymphoma type.

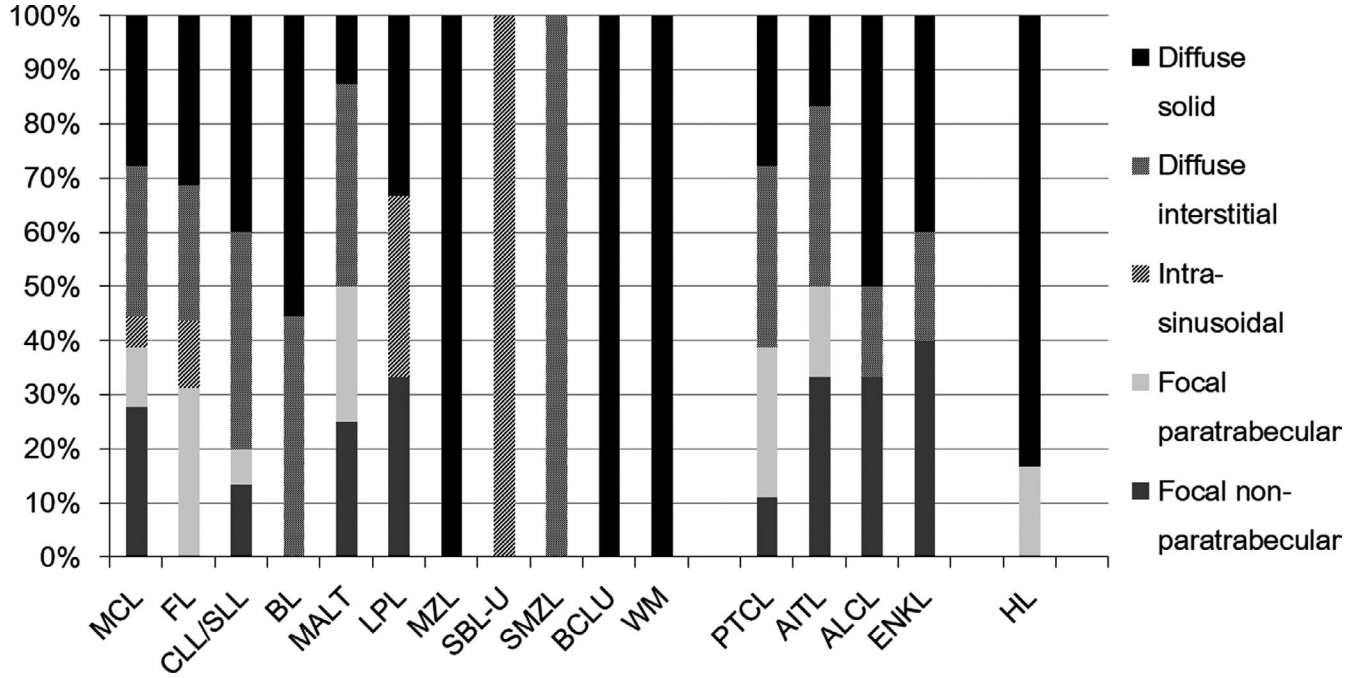

FIGURE 1 Proportion of involvement patterns by type of lymphoma

3 | RESULTS

3.1 | Bone marrow study for lymphoma evaluation of 11 years

A total of 6324 cases of BM studies were performed during the study period. A total of 1162 (18.4%) cases underwent BM study for lymphoma, and 255 (21.9%) cases of those were initially diagnosed lymphoma without any pathologic information obtained using some other specimen.

Of the 1162 malignant lymphoma cases, 1091 (93.9%) were of non-Hodgkin's lymphoma (NHL). The most common was B-cell lymphoma (BCL; 883 cases, 76.0%), followed by T-/NK-cell lymphoma (TCL; 208 cases, 17.9%) and Hodgkin lymphoma (HL; 71 cases, 6.1%). When malignant lymphoma annual incidences were compared, the proportions of diffuse large B-cell lymphoma, follicular lymphoma, and CLL/SLL showed significant increases among B-cell lymphomas (Table 1).

Bone marrow involvement was identified in 235 of 1162 cases underwent BM study for lymphoma. Of these 235 cases, 180 (20.4%) were of B-cell lymphoma, 48 (23.1%) were of T- and NK-cell lymphoma, and 7 cases (9.9%) were of Hodgkin's lymphoma. The lymphoma with the highest BM involvement rate was chronic lymphocytic lymphoma/small lymphocytic lymphoma (CLL/SLL), which was observed in 16 cases (84.2%), and involvement rates in Burkitt lymphoma (BL), mantle cell lymphoma (MCL), follicular lymphoma (FL), and diffuse large B-cell lymphoma (DLBCL) were 70% (14 cases), 48.8% (20 cases), 47.2% (17 cases), and 14.7% (82 cases), respectively. Among T and NK cells, the rates of lymphoma with marrow involvement involving ≥10 cases were 35.1% (20 cases) for peripheral T-cell lymphoma (PTCL) and 25.5% (12 cases) for angioimmunoblastic T-cell lymphoma (AITL).

Table 2 and Figure 1 summarize the involvement patterns of lymphoma types in each marrow involvement case. Of the B-cell lymphomas, the favored involvement patterns of DLBCL were a diffuse interstitial pattern (39.7%). In MCL, both focal non-paratrabecular (27.8%) and diffuse interstitial (27.8%) patterns were common. FL preferred focal paratrabecular (31.3%) and diffuse solid (31.3%) patterns. CLL exhibited both diffuse interstitial (40%) and diffuse solid (40%) patterns. BL predominantly adopted the diffuse solid pattern (55.6%). For T- and NK-cell lymphomas, PTCL mainly presented the diffuse interstitial pattern (33.3%), whereas AITL favored the focal non-paratrabecular (33.3%) and diffuse interstitial (33.3%) patterns.

3.2 | Initial evaluation of lymphoma by bone marrow study

Table 3 presents annual BM study numbers, proportions of lymphoma evaluations, and types of initial investigation specimens. BM cases increased during the study period (Spearman's correlation 0.836, P-value <0.05), but no significant change was observed in the annual proportion of lymphoma cases evaluated (P-value >0.05). However, BM study cases for initial evaluation of malignant lymphoma did eventually increase during the study period (P = 0.019), for example, nine cases (17.3%) in 2006 and 38 cases (24.4%) in 2016 (Table 1, Figure 2). In cases first evaluated for lymphoma by BM study, 108 (42.4%) of 255 cases showed lymphoma BM involvement. Sixty-six cases (61.1%) underwent tissue biopsy after BM study.

TABLE 3 Proportions and frequencies of initial investigation specimen in malignant lymphoma over the study period

	'06	'07	'08	'09	'10	'11	'12	'13	'14	'15	'16	Total	Spearman's correlation
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	
Total BM study	365 (100.0)	437 (100.0)	503 (100.0)	592 (100.0)	612 (100.0)	637 (100.0)	610 (100.0)	652 (100)	654 (100)	593 (100)	669 (100)	6324 (100)	**0.836/0.001**
Lymphoma evaluation	49 (13.4)	94 (21.5)	93 (18.5)	59 (10.0)	116 (19.0)	137 (21.5)	95 (15.6)	140 (21.5)	118 (18.0)	105 (17.7)	156 (23.3)	1162 (18.4)	0.291/0.385
Initial investigation													
Other tissue	43 (82.7)	87 (92.6)	84 (90.3)	47 (79.7)	96 (82.1)	95 (68.3)	71 (74.7)	103 (73.6)	86 (73.3)	80 (76.2)	118 (75.6)	909 (78.1)	**–0.691/0.019**
Bone marrow specimen	9 (17.3)	7 (7.4)	9 (9.7)	12 (20.3)	20 (17.9)	42 (31.7)	24 (25.3)	37 (26.4)	32 (26.7)	25 (23.8)	38 (24.4)	255 (21.9)	**0.691/0.019**

Spearman's correlation coifficient with $P < .05$ is marked in bold.

Types of lymphoma, concordance with lymph node biopsy, and BM involvement patterns are detailed in Table 4. Lymphoma cases with insufficient evidence for differential diagnosis in BM considered discordant with lymph node biopsy result. The chromosomal study was not done in two cases of AITL due to failing to aspirate BM sample. In those 108 cases, DLBCL occupied the most significant portion of B-cell neoplasm, and AITL was the most common T-cell neoplasm. Flow cytometric analysis was performed in 43 cases, and FISH analysis was performed to further evaluation of cytogenetic features in 10 cases.

Sixty-six (61.1%) of 108 primarily diagnosed by BM lymphoma cases underwent additional tissue biopsy after BM study. One DLBCL case, two mantle cell lymphoma cases, and four AITL cases were considered discordant with biopsy results due to an insufficient evaluation of the BM sample. Discrepancies in results between tissue biopsy and BM biopsy were confirmed for three patients (Table 5). One patient with monoclonal protein and enlarged lymph nodes diagnosed as mature B-cell neoplasm by BM study. Abnormal lymphocytosis in peripheral blood and marrow nucleated cells were almost replaced with abnormal lymphocytes. Flow cytometric analysis identified abnormal lymphocytes were clonal mature B cells with lambda restriction. Abnormal lymphocytes in marrow biopsy were negative in CD3, CD5, and CD79a IHC stain. However, no clonal B lymphocytes were found in IHC stain of excision lymph node biopsy. Abnormal lymphocytes in the lymph node were positive in CD3, CD4, and BCL-2 IHC stain. The final diagnosis of lymph node biopsy was AITL. Another patient diagnosed marginal zone lymphoma in BM study shows the diffuse interstitial involvement of CD5-negative, CD10-negative abnormal lymphocyte. Flow cytometric analysis was also consistent with BM study result. However, abnormal lymphocytes in the lymph node were focally positive in CD10 in IHC of additional lymph node biopsy, thus follicular lymphoma was diagnosed in lymph node biopsy. The third patient was diagnosed with diffuse large B-cell lymphoma/leukemia by BM study. Large abnormal lymphocytes replaced almost all nucleated cells in BM. They were identified as CD5-positive, CD10-negative mature B cell in flow cytometric analysis. Cyclin D1 IHC stain of abnormal lymphocytes was negative in BM biopsy section. Large-sized abnormal lymphocytes were also found in lymph node biopsy. Lymphoma cells in lymph nodes have mixed pathologic feature of both DLBCL and MCL. They were positive for cyclin D1, BCL-2, BCL-6, and MUM1 in IHC stain and large sized. Thus, this patient was finally diagnosed as pleomorphic mantle cell lymphoma/leukemia. The other 48 cases of lymphoma diagnosed by BM were concordant with lymph node biopsy findings.

In 38 (35.1%) of the 108 cases, lymphoma diagnosed mainly on BM examination without tissue biopsy (Table 6). In 34 cases (89.5%) of those 38 cases, abnormal lymphocytes were identified in BM aspiration. Abnormal lymphocyte proportion in aspiration were varied from 1% to 98%, and flow cytometric analysis was performed except for lymphoma with around 1% of abnormal lymphocyte in BM aspirate sample. Twenty-six (68.4%) of 38 cases of

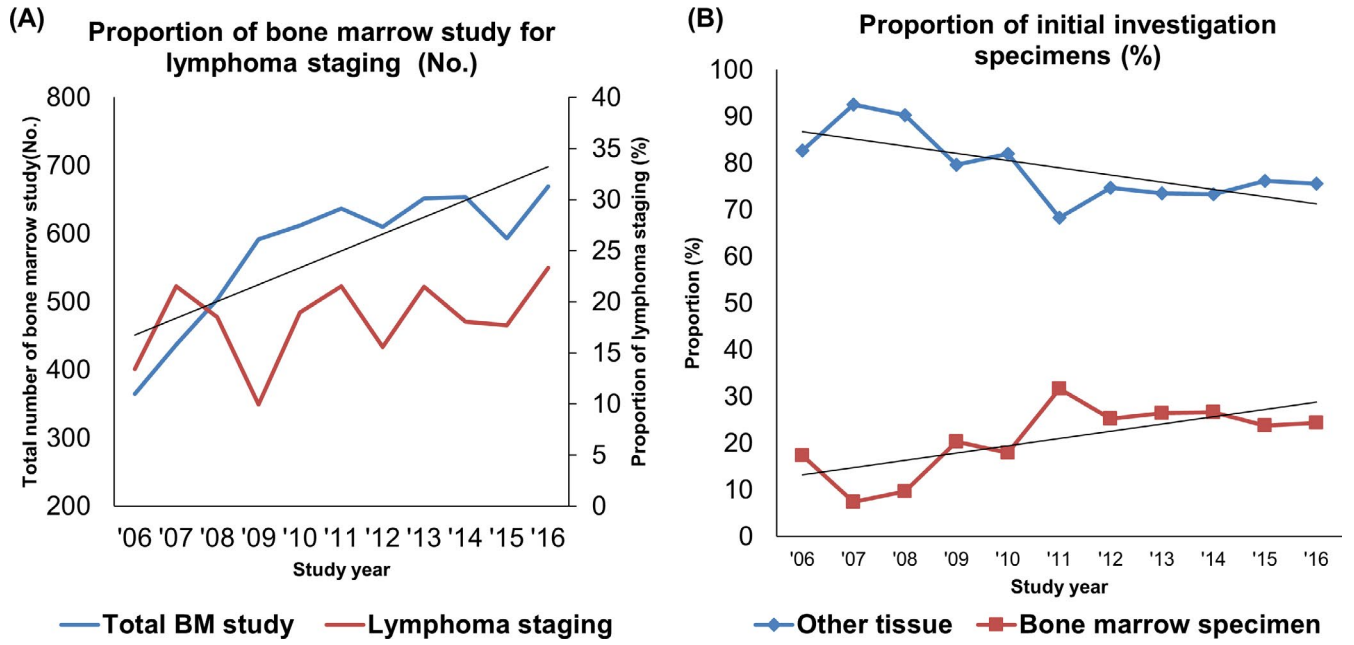

FIGURE 2 A, Proportion of bone marrow biopsies for lymphoma and (B) initial investigative specimens

lymphoma candidates exhibited clonality in the lymphocyte population and found to express specific CD antigens by flow cytometry. A chromosomal study was performed on all 38 cases. Complex karyotype was found in several cases. However, the relationship between lymphoma subtypes was not clarified. Disease-related chromosomal abnormalities were found in three Burkitt lymphoma cases. Additional FISH analysis for significant gene rearrangement in non-Hodgkin lymphoma was done in four cases and provided a suspicious IGH/CCND1 rearrangement in one case. However, morphologic features and CD5 negativity were not consentient with mantle cell lymphoma, thus diagnosed as marginal zone B-cell lymphoma. Twenty-nine (76.3%) of 38 lymphoma diagnosed cases were diagnosed as mature B-cell neoplasms and 8 (21.1%) cases as mature T and NK neoplasms.

4 | DISCUSSION

Malignant lymphoma is typically diagnosed and classified using tissue biopsies, such as lymph node biopsies. The incidence of lymphoma in the United States has steeply increased from the 1980 to 2000s.[3] Nowadays as increasing lymphoma cases, diagnosis of lymphoma is diagnosed using tissue and BM biopsies. In this study, we investigated the incidences of lymphomas classified in accord with the WHO classification scheme and marrow lymphoma involvement frequencies and patterns and also investigated the utility of BM study as an initial diagnostic specimen of malignant lymphoma.

The American Society of Clinical Oncology has made a recommendation regarding the initial evaluation and staging of lymphoma.[4] According to their recommendations, excisional biopsy is preferred to obtain adequate tissue for diagnosis, and that core-needle biopsy is considered when an excisional biopsy is not possible. Regarding lymphoma staging, BM biopsy is still regarded as the standard. However, many noninvasive procedures, such as radiologic examinations, have been studied for use in define BM involvement. In a recent study, it was suggested a BM study is not required for routine evaluations of patients with HL if PET-CT performed. Marrow involvement in the advanced-stage disease like DLBCL is usually sufficiently depicted by PET-CT, and BM biopsy provides little additional staging information. However, BM biopsy is indicated when a PET/CT scan is negative.[4,5] In another study, it was suggested BM biopsy could provide information in addition to BM involvement, such as information on the hematologic status of patients.[9] The present study shows a trend toward the initial diagnosis of lymphoma using BM specimens, which suggests BM biopsy is likely to remain an essential tool for the diagnosis and staging of lymphoma.

The total number of BM cases increased over the 11-year study period, but the proportion of BM study for lymphoma evaluation did not increase significantly. Nevertheless, the absolute number of lymphoma evaluation of BM study cases was increased as increasing total number of BM study. The purpose of BM study in lymphoma patients was disease staging in most of our cases.

Many studies have been conducted on the prevalence of malignant lymphoma types and rates of bone marrow involvement. In the present study, most lymphoma cases were NHL, and DLBCL was the most common lymphoma type of NHL, followed by MALT, MCL, and CLL. DLBCL was the most common lymphoma in another study conducted in a Spanish university hospital, an institute in Taiwan, and a report issued in North China. However, the second most common type differed among the studies.[10,11] The present study found BM involvement frequencies in some lymphoma types differ from previous reports. BL involvement had the second highest frequency

TABLE 4 Bone marrow and additional studies for malignant lymphoma without pathologic information

				Additional tests				
	No.	Bone marrow involvement	Inadequate biopsy	Additional biopsy/ concordance with BM	Chromosomal study	Chromosomal abnormality/ disease related	FISH analysis/ disease related	Flow cytometry/concordance with lymph node biopsy
Total	255	108 (42.4%)		66/56	253	22/8	10	43
Mature B-cell neoplasms								
Diffuse large B-cell lymphoma	110	28	4	18/16	110	8/2	–	5/5
Follicular lymphoma	13	11	–	11/10	13	3/–	–	8/8
Mantle cell lymphoma	13	9	–	8/6	13	1/–	3/2	5/4
Marginal zone B-cell lymphoma of MALT	8	2	–	1/1	8	–/–	2/1	2/2
Burkitt lymphoma	7	7	2	1/1	7	4/4	1/1	4/3
Chronic lymphocytic lymphoma/small lymphocytic lymphoma	2	2	–	2/2	2	–/–	–	2/2
Lymphoplasmacytic lymphoma	4	2	–	–	4	1/–	–	2/1
Waldenström macroglobulinemia	2	2	–	1/1	2	–/–	–	1/1
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma	2	0	–	–	2	–/–	–	–
Splenic marginal zone B-cell lymphoma	2	2	–	1/1	2	–/–	–	1/1
Nodal marginal zone lymphoma	1	1	–	1/1	1	–/–	–	–
Splenic B-cell lymphoma/leukemia, Unclassifiable	1	1	–	1/1	1	–/–	–	–
Unclassified	13	9	–	–	13	1/–	2/–	7
Mature T and NK neoplasms								
Angioimmunoblastic T-cell lymphoma	20	8	–	9/4	18	–/–	2/–	–
Peripheral T-cell lymphoma, NOS	11	7	–	5/5	11	–/–	–	–
Anaplastic large-cell lymphoma	11	6	–	3/3	11	3/2	–	4/–
Extranodal NK-/T-cell lymphoma, nasal type	5	1	–	–	5	–/–	–	1/1
Enteropathy-associated T-cell lymphoma	2	0	–	–	2	–/–	–	–
Aggressive NK-cell leukemia	1	1	–	–	1	1/–	–	–
Unclassified	6	4	2	–	6	–/–	–	–
Hodgkin lymphoma	21	5	–	4/4	21	–/–	–	1/–

TABLE 5 Cases with a discrepancy in results between bone marrow and lymph node biopsy

	Bone marrow	Lymph node biopsy
Case 1		
Flow cytometry	CD19(+), CD16(+) CD56(+), sIg lambda	–
IHC stain	CD3(–), CD5(–), CD79a(–)	CD3(+) CD4(+), BCL-2, kappa/lambda—polyclonal
Chromosome	45,X, –X[3]/46,XX[17]	–
Monoclonal protein	IgM kappa type	
Diagnosis	Mature B-cell lymphoma	Angioimmunoblastic T-cell lymphoma
Case 2		
Flow cytometry	CD19(+), CD20(+), lambda	–
IHC stain	CD79a(+), CD20(+), CD10(–),	CD20(+), Bcl-2(+), Bcl-6(+), focal CD10(+), cyclin D1(–)
Chromosome	47,XY,?add(1)(q42),+3,?i(8)(q10)[2] /46,XY[8]	–
Monoclonal protein	IgM, lambda type	
Diagnosis	Marginal zone lymphoma	Follicular lymphoma
Case 3		
Flow cytometry	CD45(+), HLA-DR(+), CD19(+), CD20(+), CD22(+), CD5(+)	–
IHC stain	CD20(+),CD5(+), CD10 weak(+), cyclin D1(–),	CD20(+), BCL2(+), BCL6(+), CD10 focal weak(+), CD5(+), cyclin D1(+), MUM1(+)
Chromosome	46,XY[20]	–
Diagnosis	Diffuse large B-cell lymphoma	Pleomorphic mantle cell lymphoma

(70%); however, involvement rates were much lower (1.8%-35%) in previous reports, and in case of T-cell lymphoma, the incidence of AITL is lower than other studies (>50%).[13,14] According to a study conducted by Stanford University, in which lymphoma incidence from 1992 to 2001 was reviewed, follicular lymphoma showed BM involvement most frequently followed by DLBCL. Study of The Korean Lymphoma Working Party from 1995 to 2006, a decade of research in a single Spanish institution, a single institution in Taiwan and North China found DLBCL showed the highest BM involvement incidence. In the present study, which was performed in our institution for over 11 years, DLBCL was the most common, followed by MALT, MCL, and CLL.

Lymphoma has characteristic bone marrow involvement patterns, and thus, hematologists can predict lymphoma types based on lymphoma cell morphology and these involvement patterns by H&E staining without IHC staining. Focal non-paratrabecular type in CLL/SLL, focal paratrabecular type in FL, intrasinusoidal type in SML, and diffuse interstitial type in BL are well-known favored involvement patterns of these lymphomas.[13,14] In the present study, involvement patterns well matched those described in previous studies, except for CLL/SLL. In previous studies, CLL/SLL favored a diffuse interstitial and focal non-paratrabecular pattern,[12,15,16] whereas in the present study, it favored diffuse interstitial and diffuse solid patterns.

The number of BM studies for lymphoma evaluations increased from 49 to 156 cases over the 11-year study period, and the proportion of BM studies as initial investigative specimens also increased. After lymphoma evaluation in BM study, about 61.1% of cases performed lymph node biopsies. When lymphoma was identifiable in BM study, overall concordance between BM and lymph node biopsy diagnosis was 84.85% (56 of 66 cases). B-cell lymphoma cases were more concordant between the two samples than T-cell lymphoma cases (88.89% vs 70.59%). Thomas Buhr et al[18] have previously reported a concordance rate of BM and lymph node biopsy in 124 patient was 91% in all types of lymphoma. Likewise in our study, the concordance rate of those was high (84.85%).

Among the 66 cases where additional tissue biopsy was performed after BM study, 3 cases show discrepancies in diagnosis between BM and lymph node specimen. Discrepancies in two cases were due to contradictory IHC results of disease-specific CD antigen between BM biopsy and tissue biopsy. In large B-cell lymphocyte case, which found in both BM sample and lymph node, the abnormal lymphocytes in the lymph node were positive for BCL-2, BCL-6, MUM1, and cyclin D1 stain. Moreover, considering morphologic features, DLBCL was provisionally diagnosed. After that, diagnosis changed after discussion in Hematopathology Study Group of Korean Society of Pathologists to pleomorphic mantle cell lymphoma (PMCL) with aberrant expression. FISH

TABLE 6 Malignant lymphoma cases diagnosed by bone marrow study

	Abnormal lymphocytes in aspiration, %	Bone marrow involvement pattern	Protein electrophoresis	Chromosomal study	FISH analysis	Flow cytometry
Diffuse large B-cell lymphoma						
Case 1	3.80	FP	No M-band	Complex	–	–
Case 2-3	–	DS	–	Complex	–	CD5(–), CD10(–) Mature B-cell neoplasm
Case 4-5	–	FP	IgG, Kappa	Normal	–	–
Case 6	47.20	DS	No M-band	Normal	–	–
Case 7	1.20	DI	–	Normal	–	–
Burkitt lymphoma						
Case 8	over 95	DS	–	Complex karyotypic clone with *t*(8;14)(q24;q32)	–	CD5(–), CD10(–) Mature B-cell neoplasm
Case 9-10	58	–	–	Complex karyotypic clone with *t*(8;22)(q24.1;q11.2)	–	CD5(–), CD10(+) Mature B-cell neoplasm
Case 11-13	1	DI, DS	–	Normal	–	–
Mature B-cell lymphoma						
Case 14	19	DI	No M-band	del(5q) clone	–	CD10(+) Mature B-cell neoplasm
Case 15	29.40	FP	–	Normal	Normal	CD5(–), CD10(–) Mature B-cell neoplasm
Case 16	63	DS	–	Normal	–	CD5(–), CD10(–) Mature B-cell neoplasm
Case 17	1	DS	–	Normal	–	–
Chronic lymphocytic lymphoma/small lymphocytic lymphoma						
Case 18-19	39.80	DI	–	Normal		CD5(–), CD10(–) Mature B-cell neoplasm
Lymphoplasmacytic lymphoma						
Case 20	16.40	IS	IgM, Kappa	Normal		CD5(–), CD10(–) Mature B-cell neoplasm
Case 21	23.40	DS	No M-band	Trisomy 8		
Marginal zone B-cell lymphoma						
Case 22	42.60	DI	No M-band	Normal	Suspicious IGH/CCND1 rearrangement	CD5(–), CD10(–) Mature B-cell neoplasm
Case 23	98.60	DS	–	Normal	–	CD5(–), CD10(–) Mature B-cell neoplasm

(Continues)

TABLE 6 *(Continued)*

	Abnormal lymphocytes in aspiration, %	Bone marrow involvement pattern	Protein electrophoresis	Chromosomal study	FISH analysis	Flow cytometry
Blastoid mantle cell lymphoma						
Case 24	81.40	DI	–	Normal (very few mitoses)	Normal	CD5(–), CD10(–) Mature B-cell neoplasm
Mantle cell lymphoma						
Case 25	8.60	IS	–	Normal	–	CD5(+), CD10(–), bright CD20(+), bright lambda(+) Mature B-cell neoplasm
Waldenström macroglobulinemia						
Case 26	24.20	DS	IgM, Kappa	Normal	–	CD5(–), CD10(–) Mature B-cell neoplasm.
R/O) CLL or follicular lymphoma						
Case 27	59.00	DI	–	Normal	–	CD5(–), CD10(+) Mature B-cell neoplasm
R/O) PLL or blastoid mantle						
Case 28	70.20	DI	–	Normal (Very few mitoses)	TP53 deletion	CD5(–), CD10(–) Mature B-cell neoplasm
Splenic marginal zone lymphoma						
Case 29	47.20	DS	–	Normal	–	CD5(–), CD10(–) Mature B-cell neoplasm
NK-cell lymphoma/leukemia						
Case 30	84	DS	–	Complex	–	CD16(+),CD56(+), CD5(–)
Case 31	14.80	DI	–	Normal	–	CD56(+), CD3(+), CD2(+), CD5(–)
Case 32-33	24	DS	–	Normal	–	CD16(+), CD56(+), CD5(–)
Anaplastic large T-cell lymphoma						
Case 34-35	43.80	DS	–	Complex hypotetraploid clone	–	CD4(–), CD8(–) Mature T-cell neoplasm
Peripheral T-cell lymphoma, NOS						
Case 36	1.20	FP		Normal	–	–
Mature T-cell lymphoma						
Case 37	9.00	–		Complex	–	CD2(+), sCD3(+), CD7(+), CD16(+), CD56(+)
Hodgkin lymphoma						
Case 38	3.60	DS		Normal	–	–

DI, diffuse interstitial; DS, diffuse solid; FP, focal paratrabecular; IS, intrasinusoidal.

panel study of frequent genetic abnormality in NHL was recommended for further research but not enforced. Large abnormal lymphocytes in BM were diagnosed as CD5(+), cyclin D1(−) DLBCL. IHC stain results of cyclin D1 were different in BM and LN, which seems to have led to different diagnoses. Pleomorphic features of PMCL resemble DLBCL. Expression of cyclin D1 supports distinguishes MCL from DLBCL. However, previous literature has reported about 2% of morphologic compatible DLBCL were PMCL, and 40% of them were cyclin D1-negative PMCL.[19] Thus, there was a limitation to determine which diagnosis is proper diagnosis without extra IHC stain, or gene mutation study. In one AITL case, abnormal lymphocytes of different lineage were found in BM biopsy and lymph node biopsy. A study found that the immunophenotype of AITL can vary among the site of involvement, and in rare, monotypic B cells concurrent in a different site. AITL found in bone marrow specimens frequently show altered CD3 expression with loss of CD3.[20] These characteristic phenotypic differences can affect our case. Thus, IHC screening with CD3 may not detect AITL cells due to complete loss of CD3. In three of the cases with the discrepancy, two cases could be considered as an effect of a rare variant. Thus, differences in diagnosis between BM and lymph node biopsy may exist in rare lymphoma variant. Although high concordance rates were previously reported between BM biopsy and lymph node biopsy for low-grade and high-grade B-cell lymphoma,[15,18] clinical features also have great clues. Thus communication among clinicians is necessary to reach a proper diagnosis.

Lymphoma diagnosed solely by BM study without tissue biopsy constituted 38 cases of BM studies conducted for initial lymphoma evaluation. Flow cytometric analysis, chromosomal study, FISH analysis, and protein electrophoresis were performed to reinforce the diagnostic power of BM studies. Flow cytometric analysis provides information about the presence of a clonal lymphoid cell population and the expression of particular CD antigen in these cells and also provides guidance regarding the selection of appropriate IHC stains for the differential diagnosis of lymphoma. In our study, flow cytometry was performed with the aspirate sample in 24 of 38 cases, and clonal lymphocyte was detected in all the 24 cases. Of the 24 cases, 19 were B-cell lymphomas. Flow cytometry confirmed the expression of CD10, CD5, and kappa or lambda restriction in every clonal B lymphocytes. Screening result of abnormal lymphocytes became the basis for appropriate application of IHC stain on BM biopsy section. Also, chromosomal studies provided disease-specific information. The chromosomal study detected disease-related abnormality in two Burkitt lymphoma cases. A FISH study was undergone in few cases, and in one case found lymphoma-related chromosomal abnormality. FISH analysis detected MCL-related gene rearrangement in one of two MZL cases; however, the result is discordant with other additional studies and morphologic features. Although additional analysis does not always provide useful results, these studies can provide critical information for the differential diagnosis of lymphoma. The preferred BM involvement patterns of different malignant lymphoma types are well known, and thus, BM involvement patterns can give diagnostic clues, as in tissue biopsies.

In summary, this study shows initial investigations of malignant lymphoma using BM specimens increased during the period 2006-2016, which demonstrates the growing importance of BM study as an investigative tool for malignant lymphoma. Although not all lymphoma involves BM, BM study which has high concordance rate with lymph node biopsy can be an acceptable choice for diagnosing lymphoma. Furthermore, combined with morphological feature and involvement pattern from BM biopsy and additional laboratory tests, such as flow cytometry, chromosomal study, FISH analysis, and clinical information, BM study offers a powerful means of diagnosing lymphoma.

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