원저
Lab Med Online
Vol. 13, No. 3: 172-181, July 2023
https://doi.org/10.47429/lmo.2023.13.3.172

진단혈액학 LMO

다양한 림프종 등급에 따른 B세포계열림프종 골수침범의 진단에 사용되는 검사들의 일치율 분석

Concordance of Current Tests Used for the Diagnosis of Bone Marrow Involvement in B-Lineage Lymphoma with Respect to Various Lymphoma Grade

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Background: We evaluated the concordance of bone marrow (BM) aspirates, biopsy, immunohistochemical (IHC) staining, fluorescence in situ hybridization (FISH), and karyotyping used in diagnosing BM involvement in B-lineage lymphoma and assessed the concordance characteristics for lymphoma grade.
Methods: Total 127 B-lineage lymphoma patients (92 high grade [HG], 35 low grade [LG]) diagnosed during recent 54 months and who underwent BM study were prospectively enrolled. BM aspiration/biopsy/CD3 and CD20 IHC staining/FISH/karyotyping were performed in each case, and results were compared.
Results: Discrepancy rates (DR) between BM aspirates/biopsy and IHC staining were 14.2% and 6.3%, respectively, and IHC staining detected additional 13.4% and 6.5% BM-involved cases in BM aspirates-normal/biopsy-normal cases. DR between integrated BM involvement interpretation (IBMII, defined as abnormality in at least one morphologic evaluation) and karyotyping/FISH results was 34.6% and 22.0%, respectively, and FISH-abnormal/karyotyping-normal cases were more frequent than FISH-normal/karyotyping-abnormal cases. DR among IBMII, karyotyping, and FISH in LG lymphoma was significantly higher than in HG lymphoma (71.4% vs. 23.9%; $P<0.001$), and the proportion of cases with IBMII-involved but karyotyping/FISH-normal was significantly higher in LG lymphoma than HG lymphoma (45.7% vs. 8.7%; $P<0.001$). With FISH analysis, an additional 6.5% of HG and 25.7% of LG lymphoma cases showed abnormal results concordant with IBMII.
Conclusions: IHC staining would be useful in the sensitive detection of BM involvement. FISH analysis can reduce DR and more sensitively detect BM involved cases compared to karyotyping. DR among IBMII, karyotyping, and FISH is higher in LG lymphoma than in HG lymphoma.

Key Words: B-lineage lymphoma, Bone marrow study, Concordance, Fluorescence in situ hybridization, Karyotyping, Lymphoma grade

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Received: March 21, 2023
Revision received: April 20, 2023
Accepted: May 8, 2023

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INTRODUCTION

The bone marrow (BM) study is an essential staging workup process in patients with malignant lymphoma. The diagnosis of BM involvement in malignant lymphoma suggests that systemic involvement of the lesion, thus, assisting clinicians to administer systemic treatments, such as chemotherapy [1, 2]. Therefore, accurately assessing BM involvement status in malignant lymphoma is critical in determining treatment modality. The local treatment strategy is beneficial, especially in follicular lymphoma (FL) and mucosa-associated lymphoid tissue (MALT) lymphoma, and the accurate evaluation of BM involvement status can be emphasized

in these lymphomas [3, 4]. Low- and high-grade B-lineage lymphomas can be classified by their BM involvement frequency and pattern. Higher frequency of BM involvement in low grade lymphomas, such as chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL, >75%), FL (50–60%), mantle cell lymphoma (MCL, 55–90%), lymphoplasmacytic lymphoma (LPL, >80%), marginal zone lymphoma (MZL, 30–100%) was reported when compared to high grade lymphomas, such as diffuse large B cell lymphoma (DLBCL, 20–30%) and Burkitt lymphoma (BL, >40%) [5, 6].

The BM involvement of malignant lymphoma was confirmed when the neoplastic lymphoid cells were found in the bilateral BM aspiration and biopsy slides. Although five major patterns of BM infiltrations were previously mentioned [7], the morphologic features and involvement patterns of neoplastic lymphoid cells found in BM involved cases show significant heterogeneity. In addition, it is challenging to confirm BM involvement if neoplastic lymphoid cells are infiltrated individually in a dispersed pattern. The immunohistochemical (IHC) staining using an antibody specific to the cluster of differentiation (CD) antigen present on the surface of neoplastic lymphoid cells are currently employed as a further approach [8-13], and CD3 and CD20 are widely used for T and B cells, respectively. Besides morphologic evaluations, karyotype analyses are frequently requested for the sensitive detection of cytogenetic abnormalities found in neoplastic lymphoid cells in BM aspirates. However, fluorescence in situ hybridization (FISH) is also occasionally performed to detect neoplastic lymphoid cells with higher sensitivity compared to morphologic and karyotype analyses. Currently, karyotype and FISH analyses are used to detect minimal residual diseases in malignant lymphoma patients with genetic abnormalities detected at diagnosis.

To date, analyses of the incidences and histologic patterns of BM involvement, the discrepancy between histology and flow cytometry, and the morphologic discordance between lymph nodes and BM have been performed against non-Hodgkin's lymphoma [14-17]. However, a comprehensive study focusing on the concordance rates and discrepancies between each study result, including BM examination (BM aspiration/biopsy/IHC results), and additionally requested tests, such as karyotype and FISH analyses, has not been performed. Since the division capacity of each lymphoma cell is different, differences in the detection sensitivity of karyotype/FISH analysis with respect to lymphoma grade status (low grade vs. high grade) is expected. Discrepant results can also be found between morphologic evaluations and karyotype/FISH analysis because of the differences in the specimens used by the two types of tests. To address these issues, we performed a prospective analysis of the concordance of five tests used in diagnosing BM involvement in B-lineage lymphoma and evaluated their characteristics for different lymphoma grades.

MATERIALS AND METHODS

1. Sample collection

Between April 2018 and October 2022, a total of 127 patients who were diagnosed with B-lineage lymphoma in the Pusan National University Hospital were prospectively enrolled in this study. Among them, 92 patients had high grade lymphoma (86 patients with DLBCL, 3 patients with BL, and 3 patients with high grade B cell lymphoma), while 35 patients had low grade lymphoma (20 patients with FL, 5 patients with MCL, 4 patients with MZL, 2 patients with MBL, 2 patients with LPL, and 2 patients with hairy cell leukemia [HCL]).

A BM aspiration and bilateral BM biopsy were performed in each patient for staging workup. One peripheral blood EDTA, 2 BM aspirate EDTAs, 1 heparin tube for cytogenetic tests, and a BM biopsy (plain tube with neutral buffered formalin) were collected from each patient. Clinical data of patients was obtained by reviewing their electronic medical records. This study was approved by the Institutional Review Board of the Pusan National University Hospital (Approval number: 1804-027-066), and was performed following the Helsinki Declaration principles.

2. Morphologic evaluation of BM slides

Each patient's sample was used to prepare peripheral blood smear slides (Wright-Giemsa stain), BM aspirate slides (Wright-Giemsa stain), BM biopsy slides (hematoxylin and eosin stain), and CD3/CD20 IHC staining slides. These slides were reviewed by a hematopathologist with more than 10 years of experience in laboratory hematology. The BM involvement of B-lineage lymphoma was considered when the neoplastic lymphoid cell infiltrations [7] were present in at least one of three tests, such as BM aspirates, biopsy, or IHC stains (defined as integrated BM involvement interpretation, IBMII).

3. Conventional karyotyping

The BM aspirate samples in the heparin tube were used for chromosome analysis. Both cultivation and harvesting were carried out following standard techniques [18]. After performing the G-banding as described previously [19], a metafer™ image analysis system (MetaSystems, Althlussheim, Germany) was employed for further analysis. When possible, 20 metaphase cells were karyotyped, and at least two metaphase cells were analyzed. The karyotyping was interpreted according to the most recent version of the ISCN 2020 (International System for Human Cytogenomic Nomenclature 2020) guidelines [20].

4. Interphase FISH analysis

The FISH lymphoma panel comprises DNA probes, which include locus specific identifier (LSI) MYC (8q24) dual-color break-apart rearrangement probe, LSI IGH/CCND1 t(11;14) (q13;q32.3) dual-color probe, XL t(14;18) IGH/BCL2 dual fusion probe, and BCL6 (3q27) break-apart probe. FISH analyses were performed on cell suspensions prepared from BM aspirates. A total of 300 interphase cells were analyzed, and the ratio of cells with abnormal FISH patterns among the 300 nucleated cells was expressed as a percentage. The FISH results were interpreted according to the most recent version of the ISCN 2020 guidelines [20].

5. Comparison of BM aspiration, biopsy, and IHC staining results

The results of 127 patients' BM aspiration, biopsy, and IHC staining regarding the involvement of BM were compared between two subgroups (BM aspiration vs. biopsy, BM aspiration vs. IHC, and BM biopsy vs. IHC), and the discrepancy rates (DR) between two subgroups (BM aspiration vs. biopsy, BM aspiration vs. IHC, and BM biopsy vs. IHC), were also estimated and compared.

6. BM aspiration, biopsy, and IHC staining results with respect to lymphoma grade

The BM aspiration, biopsy, and IHC stain results about BM involvement status obtained from 127 patients were categorized into six subtypes according to the consistency among the three results, and these results were compared between patients with high grade lymphoma and those with low grade lymphoma. DR among the three results were also calculated and compared.

7. Comparison of IBMII, karyotype, and FISH results

The IBMII was selected as a final decision of BM morphology results. The IBMII, karyotype, and FISH analysis results about BM involvement status obtained from 127 patients were compared between two subgroups (IBMII vs. karyotype, IBMII vs. FISH, and karyotype vs. FISH), and DR between two subgroups were also calculated and compared.

8. IBMII, karyotype, and FISH results with respect to lymphoma grade

The IBMII, karyotype, and FISH analysis results about BM involvement status obtained from 127 patients were categorized into seven subtypes conferring the consistency among three results, and these results were compared between patients of high grade lymphoma and those with low grade lymphoma. DR among the three results were also analyzed and compared. DR among IBMII, karyotype, and FISH, and the proportion of IBMII-involved but karyotype/FISH-normal cases in high grade and low grade lymphoma were compared using the Chi-square test, and P values <0.05 were considered statistically significant.

9. Patients with morphologic BM involvement at diagnosis and follow-up examinations

We analyzed the data of eleven patients who experienced morphologic BM involvement at diagnosis and follow-up examinations to evaluate each test for minimal residual disease monitoring. BM aspiration, biopsy, IHC stain, karyotype, and FISH performed at follow-up were analyzed and result distributions were compared. In the comparison of DR among IBMII, karyotype, and FISH in high grade and low grade lymphoma, the Fisher's exact test was used and P values <0.05 were considered statistically significant.

RESULTS

1. Comparison of BM aspiration, biopsy, and IHC stain results

Among the total 127 cases, discrepancies between BM aspiration and biopsy were 14 (discrepancy rates [DR] of 11.0%), out of which 11 (8.7%) cases were BM aspiration-not involved but BM biopsy-involved (Table 1). In the comparison between BM aspirates/biopsy and IHC stain results, discrepancies were 18 (DR of 14.2%)

https://doi.org/10.47429/lmo.2023.13.3.172

Table 1. Comparison of bone marrow aspiration, biopsy, and immunohistochemical staining results for 127 B-lineage lymphoma patients enrolled in this study

BM aspiration	BM biopsy		Discrepancy rates (%)	Total
	No involvement	Involvement		
No involvement	77 (60.6%)	11 (8.7%)	14/127 (11.0)	88
Involvement	3 (2.4%)	36 (28.3%)		39
Total	80	47		127 (100.0%)
BM aspiration	IHC		Discrepancy rates (%)	Total
	No involvement	Involvement		
No involvement	71 (55.9%)	17 (13.4%)	18/127 (14.2)	88
Involvement	1 (0.8%)	38 (29.9%)		39
Total	72	55		127 (100.0%)
BM biopsy	IHC		Discrepancy rates (%)	Total
	No involvement	Involvement		
No involvement	72 (57.7%)	8 (6.5%)	8/127 (6.3)	80
Involvement	0 (0.0%)	47 (35.8%)		47
Total	72	55		127 (100.0%)

Abbreviations: IHC, immunohistochemical staining; BM, bone marrow.

Table 2. Result distributions of bone marrow aspiration, biopsy, and immunohistochemical staining with respect to lymphoma grade

BM aspiration/BM biopsy/IHC	N of cases (%)	Discrepant cases (%)
(-)/(-)/(-)	71 (55.9)	20/127 (15.8)
(-)/(-)/(+)	6 (4.7)	
(+)/(-)/(-)	1 (0.8)	
(+)/(-)(+)	2 (1.6)	
(-)/(+)/(+)	11 (8.7)	
(+)/(+)/(+)	36 (28.3)	
High grade* (N=92)	N of cases (%)	Discrepant cases (%)
(-)/(-)/(-)	63 (68.5)	14/92 (15.3)
(-)/(-)/(+)	3 (3.3)	
(+)/(-)/(+)	2 (2.2)	
(-)/(+)(+)	9 (9.8)	
(+)/(+)/(+)	15 (16.2)	
Low grade† (N=35)	N of cases (%)	Discrepant cases (%)
(-)/(-)/(-)	8 (22.9)	6/35 (17.1)
(-)/(-)/(+)	3 (8.6)	
(+)/(-)/(-)	1 (2.9)	
(-)/(+)(+)	2 (5.6)	
(+)/(+)/(+)	21 (60.0)	

*Diffuse large B cell lymphoma, high grade B cell lymphoma, and Burkitt lymphoma were included in the high grade lymphoma category.; †Mature B cell lymphoma, mantle cell lymphoma, follicular lymphoma, marginal zone lymphoma, hairy cell leukemia, and lymphoplasmacytic lymphoma were included in low grade lymphoma category.
Abbreviations: BM, bone marrow; IHC, immunohistochemical staining.

Table 3. Comparison of IBMII results, karyotype, and fluorescence in situ hybridization results for 127 patients enrolled in this study

IBMII*	Karyotype		Discrepancy rates (%)	Total
	Normal	Abnormal		
No involvement	66 (52.0%)	5 (3.9%)	44/127 (34.6)	71
Involvement	39 (30.7%)	17 (13.4%)		56
Total	105	22		127 (100.0%)
IBMII*	FISH		Discrepancy rates (%)	Total
	Normal	Abnormal		
No involvement	70 (55.1%)	1 (0.8%)	28/127 (22.0)	71
Involvement	27 (21.3%)	29 (22.8%)		56
Total	97	30		127 (100.0%)
Karyotype	FISH		Discrepancy rates (%)	Total
	Normal	Abnormal		
Normal	90 (70.9%)	15 (11.8%)	22/127 (17.3)	105
Abnormal	7 (5.5%)	15 (11.8%)		22
Total	97	30		127 (100.0%)

*Involvement in IBMII results was defined as a case that showed positivity in at least one of three morphologic evaluations, such as BM aspiration, biopsy, and IHC.
Abbreviations: IBMII, integrated bone marrow involvement interpretation; BM, bone marrow; FISH, fluorescence in situ hybridization.

and 8 (DR of 6.3%), respectively, and almost all cases were IHC-involved but BM aspiration/biopsy-not involved, except for one. IHC staining could detect an additional 17 (13.4%) and 8 (6.5%) cases with BM involvement without definite evidence of BM involvement in BM aspiration or biopsy, respectively.

2. BM aspiration, biopsy, and IHC staining results with respect to lymphoma grade

Out of the total 127 cases, discrepancies among BM aspiration, biopsy, and IHC stain results were 20 (DR of 15.8%), and among them, 17 (13.4%) cases were BM aspiration-not involved but IHC-involved regardless of BM biopsy status (Table 2). In 92 cases with

high grade lymphoma, discrepancies among the three test results were 14 (DR of 15.3%) and among them, 12 cases were BM aspiration-not involved but IHC-involved regardless of BM biopsy status. In 35 cases with low grade lymphoma, discrepancies among three test results were 6 (DR of 17.1%), and among them, 5 cases were BM aspiration-not involved but IHC-involved regardless of BM biopsy status. DR among the three test results were not significantly different with respect to lymphoma grade, and their characteristics were negligible.

3. Comparison of IBMII, karyotype, and FISH results

Among the total 127 cases, discrepancies between IBMII and karyotype/FISH results were 44 (DR of 34.6%) and 28 (DR of 22.0%), respectively (Table 3). Most of the discrepant cases were IBMII-involved but karyotype-normal (39 cases, 30.7%) and FISH-normal (27 cases, 21.3%) cases. FISH results could reduce DR from 34.6% to 22.0% compared to karyotype results. When karyotype and FISH results were compared, discrepancy cases were 22 (DR of 17.3%), and FISH-abnormal but karyotype-normal cases (15 cases) were more frequent than FISH-normal but karyotype-abnormal cases (7 cases), which indicates FISH analysis can detect abnormal cases with a higher sensitivity than karyotype analysis.

4. IBMII, karyotype, and FISH results with respect to lymphoma grade

Among the total 127 cases, discrepant cases among IBMII, karyotype, and FISH results were 47 (DR of 31.5%), and among them, 24 (18.9%) cases were IBMII-involved but karyotype-normal/FISH-normal, and 15 (DR of 11.8%) cases were IBMII-involved and FISH-abnormal but karyotype-normal (Table 4). In 92 cases with high grade lymphoma, discrepancies among the three test results were 22 (DR of 23.9%), and among them, 8 (DR of 8.7%) cases were IBMII-involved but karyotype-normal/FISH-normal, and 6 (DR of 6.5%) cases were IBMII-involved and FISH-abnormal but karyotype-normal. In 35 cases with low grade lymphoma, discrepancies among the three test results were 25 (DR of 71.4%) and among them 16 (DR of 45.7%) cases were IBMII-involved but karyotype-normal/FISH-normal, and 9 (DR of 25.7%) cases with IBMII-involved and FISH-abnormal but karyotype-normal.

DR among the three test results were significantly different with respect to lymphoma grade. DR was significantly higher in low grade lymphoma than in high grade lymphoma (71.4% vs. 23.9%; $P<0.001$). The proportion of IBMII-involved but karyotype/FISH-normal cases was also significantly higher in low grade lymphoma than in high grade lymphoma (45.7% vs. 8.7%; $P<0.001$). With the application of FISH analysis, an additional 6.5% of high grade and 25.7% of low grade lymphoma cases can yield positive results, which is concordant with morphologic evaluations.

Table 4. Result distributions of IBMII, karyotype, and fluorescence in situ hybridization with respect to lymphoma grade

IBMII*/Karyotype/FISH	N of cases (%)	Discrepant cases (%)
(-)/(-)/(-)	66 (52.0)	47/127 (31.5)
(-)/(+)/(+)	1 (0.8)	
(+)/(-)/(-)	24 (18.9)	
(-)/(+)(-)	4 (3.1)	
(+)/(+)/(-)	3 (2.4)	
(+)/(-)/(+)	15 (11.8)	
(+)/(+)/(+)	14 (11.0)	
High grade† (N=92)	N of cases (%)	Discrepant cases (%)
(-)/(-)/(-)	58 (63.1)	22/92 (23.9)§
(-)/(+)/(+)	1 (1.1)	
(+)/(-)/(-)	8 (8.7)	
(-)/(+)(-)	4 (4.3)	
(+)/(+)/(-)	3 (3.3)	
(+)/(-)/(+)	6 (6.5)	
(+)/(+)/(+)	12 (13.0)	
Low grade‡ (N=35)	N of cases (%)	Discrepant cases (%)
(-)/(-)/(-)	8 (22.9)	25/35 (71.4)§
(-)/(+)/(+)	0 (0.0)	
(+)/(-)/(-)	16 (45.7)	
(-)/(+)(-)	0 (0.0)	
(+)/(+)/(-)	0 (0.0)	
(+)/(-)/(+)	9 (25.7)	
(+)/(+)/(+)	2 (5.7)	

*Involvement in IBMII results was defined as a case that showed positivity in at least one of three morphologic evaluations such as BM aspiration, biopsy, and IHC.; †Diffuse large B cell lymphoma, high grade B cell lymphoma, and Burkitt lymphoma were included in the high grade lymphoma category.; ‡Mature B cell lymphoma, mantle cell lymphoma, follicular lymphoma, marginal zone lymphoma, hairy cell leukemia, and lymphoplasmacytic lymphoma were included in the low grade lymphoma category.; §Discrepancy rate was significantly higher in low grade lymphoma than in high grade lymphoma (71.4% vs. 23.9%; $P<0.001$).
Abbreviations: BM, bone marrow; FISH, fluorescence in situ hybridization.

5. Patients with morphologic BM involvement at diagnosis and follow-up examinations

A total of 11 patients (seven with high grade and four with low grade lymphomas) with morphologic BM involvement at diagnosis and follow-up are summarized in Table 5. Among the 30 follow-up events (numbers) including those at diagnosis, discrepancies among IBMII, karyotype, and FISH results were 10 (DR of

https://doi.org/10.47429/lmo.2023.13.3.172

https://doi.org/10.47429/lmo.2023.13.3.172

Table 5. Summary of bone marrow aspiration, biopsy, immunohistochemical staining, karyotype, and fluorescence in situ hybridization results obtained from eleven patients showing morphologic bone marrow involvement at diagnosis and follow-up examinations

Case No. (Dx)	F/U No.	Evidence of BM involvement			FISH results		Karyotype
		Asp.	Bx.	IHC	Probe	Pattern	
1 DLBCL	1	+	+	+	MYC	nuc ish(MYCx2)(5'LSIMYC sep 3'LSIMYC)x1[179/300]	48~51,XY,+der(1;13)(q10;q10),del(2)(p11.1),+del(3)(p25).+4,add(6)(p25),del(7)(p15),-8,del(8)(q24.1),+del(9)(p13),+?10,dup(11)(q13q21),add(14)(q32),del(17)(p11.2),+add(18)(p11.2),+add(18)(p11.2)x2,+20,add(21)(p13)[cp20]
					IGH/CCND1	Not done	
					IGH/BCL2	nuc ish(IGHx2~5,BCL2x3~5)[158/300]	
						nuc ish(IGHx2,BCL2x3)[33/300]/(IGHx3,BCL2x3)[101/300]/(IGHx4,BCL2x4)[16/300]/(IGHx5,BCL2x5)[8/300]	
	2	-	-	-	MYC	nuc ish(MYCx2)[300]	46,XY[20]
	3	+	+	+	MYC	nuc ish(MYCx2)(5'LSIMYC sep 3'LSIMYCx1)[6/300]	48,XY,del(8)(q24.1),-14,+3mar[1]/46,XY[19]
2 DLBCL	1	-	+	+	MYC	nuc ish(MYCx2)(5'LSIMYC sep 3'LSIMYCx1)[22/300]	50,XY,+add(X)(p11.4),add(1)(p36.1),inv ins(1;1)(q12;q32q21),+2,t(2;8)(p12;q24.1),+7,del(9)(q13q32),+12,t(14;18)(q32;q21.3)[7]/46,XY[13]
					IGH/CCND1	nuc ish(CCND1x2,IGHx3)[22/300]	
					IGH/BCL2	nuc ish(IGH,BCL2)x3(IGH con BCL2x2)[11/300]/(IGH,BCL2)x2(IGH con BCL2x1)[14/300]	
	2	-	-	-	MYC	nuc ish(MYCx2)(5'LSIMYC sep 3'LSIMYCx1)[6/300]	46,XY[20]
					IGH/BCL2	nuc ish(IGH,BCL2)x2(IGH con BCL2x1)[7/300]	
	3	-	-	-	MYC	nuc ish(MYCx2)[300]	46,XY[20]
					IGH/BCL2	nuc ish(IGH,BCL2)X2[300]	
3 DLBCL	1	+	+	+	MYC	nuc ish(5'MYCx2,3'MYCx3)(5'MYC SEP 3'MYCx1)[248/300]	52,XX,+5,+del(6)(q12q23),+del(7)(p22),t(8;14)(q24;q32),+del(10)(p13),+12,der(14)ins(14;?)(q24;?)t(14;18)(q32;q21),+21[14]/46,XX[3]
					IGH/CCND1	nuc ish(CCND1x2,IGHx3)[224/300]	
					IGH/BCL2	nuc ish(IGH,BCL2)x3(IGH con BCL2x2)[224/300]	
	2	-	-	-	MYC	nuc ish(MYCx2)[300]	46,XX[20]
					IGH/BCL2	nuc ish(IGH,BCL2)X2[300]	
	3	-	-	-	MYC	nuc ish(MYCx2)(5'MYC sep 3'MYCx1)[18/300]	51,XX,+5,+del(6)(q12q23),+del(7)(p22),t(8;14)(q24;q32),+12,+21[2]/46,XX[18]
					IGH/BCL2	nuc ish(IGH,BCL2)x2(IGH con BCL2x1)[24/300]	
4 DLBCL	1	-	+	+	MYC	nuc ish(MYCx2)[300]	50,XX,add(2)(p13),-3,del(4)(q31),+add(7)(q11.2),?t(3;9)(p13;p24),+add(9)(q13),+add(12)(q24.1),13cenh+x2,+13,+add(15)(q24),-16,add(17)(q25),der(19)t(1;19)(q23;p13.3)[6]/46,XX[4]
					IGH/CCND1	nuc ish(CCND1,IGH)x2[300]	
					IGH/BCL2	nuc ish(IGHx2,BCL2x3)[45/300]	
	2	-	-	-	IGH/BCL2	nuc ish(IGH,BCL2)X2[300]	46,XX[20]
5 DLBCL	1	+	+	+	MYC	nuc ish(MYCx3)[104/300]	47,XX,add(1)(?q21),add(12)(p11.2),-18,+3mar[cp4]/46,XX[7]
					IGH/CCND1	nuc ish(CCND1,IGH)x2[300]	
					IGH/BCL2	nuc ish(IGH,BCL2)X2[300]	
	2	-	-	-	MYC	nuc ish(MYCx2)[300]	46,XX[20]
	3	-	-	-	MYC	nuc ish(MYCx2)[300]	46,XX[20]
	4	-	-	-	MYC	nuc ish(MYCx2)[300]	46,XX[20]

(*Continued to the next page*)

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Table 5. Continued

Case No. (Dx)	F/U No.	Evidence of BM involvement			FISH results		Karyotype
		Asp.	Bx.	IHC	Probe	Pattern	
6 DLBCL	1	+	+	+	MYC	nuc ish(MYCx4)[256/300]/(MYCx5)[10/300]	71~78<3n>,XXY,-Y,+del(2)(p13)x2,+del(3)(p21),del(3)(q21),del(3)(q25),+del(5)(q36),-6,+7,+8,+8,+10,+10,-11,+12,-13,add(14)(q32),-17,-18,+2~7mar[cp19]/46,XY[1]
					IGH/CCND1	nuc ish(CCND1x2,IGHx5)[150/300]/(CCND1x2,IGHx6)[135/300]	
					IGH/BCL2	nuc ish(IGH,BCL2)X5[30/300]/(IGH,BCL2)X5(IGH con BCL2x3)[126/300]/(IGH,BCL2)X5(IGH con BCL2x2)[135/300]	
	2	-	-	-	MYC	nuc ish(MYCx2)[300]	46,XY[20]
					IGH/CCND1	nuc ish(CCND1,IGH)x2[300]	
					IGH/BCL2	nuc ish(IGH,BCL2)X2[300]	
	3	-	-	-	MYC	nuc ish(MYCx2)[300]	46,XY[20]
					IGH/CCND1	nuc ish(CCND1,IGH)x2[300]	
					IGH/BCL2	nuc ish(IGH,BCL2)X2[300]	
7 HCL	1	+	+	+	MYC	nuc ish(MYCx3)[131/300]	46,XY[20]
					IGH/CCND1	nuc ish(CCND1,IGH)x2[300]	
					IGH/BCL2	nuc ish(IGH,BCL2)X2[300]	
	2	+	+	+	MYC	nuc ish(MYCx2)(5'MYC sep 3'MYCx1)[8/300]/(MYCx3)[148/300]	46,XY[11]
	3	+	+	+	MYC	nuc ish(MYCx3)[255/300]	46,XY[1]
8 FL	1	+	+	+	MYC	nuc ish(MYCx2)(5'MYC sep 3'MYCx1)[18/300]	46,XY[12]
					IGH/CCND1	nuc ish(CCND1x2,IGHx3)[203/300]	
					IGH/BCL2	nuc ish(IGH,BCL2)x3(IGH con BCL2x2)[219/300]	
	2	+	+	+	IGH/BCL2	nuc ish(IGH,BCL2)x3(IGH con BCL2x2)[6/300]	46,XY[17]
9 DLBCL	1	-	-	+	MYC	nuc ish(MYCx2)[300]	46,XY[14]
					IGH/CCND1	nuc ish(CCND1,IGH)x2(CCND1 con IGHx1)[12/300]	
					IGH/BCL2	nuc ish(IGH,BCL2)X2[300]	
	2	-	-	-	IGH/CCND1	nuc ish(CCND1,IGH)x2[300]	46,XY[20]
	3	-	-	-	IGH/CCND1	nuc ish(CCND1,IGH)x2[300]	46,XY[20]
10 FL	1	+	+	+	MYC	nuc ish(MYCx3)[20/300]	46,XX[10]
					IGH/CCND1	nuc ish(CCND1,IGH)x2[300]	
					IGH/BCL2	nuc ish(IGH,BCL2)X2[300]	
	2	-	-	-	MYC	nuc ish(MYCx2)[300]	46,XX[11]
11 FL	1	+	+	+	MYC	nuc ish(MYCx2)[300]	46,XY[20]
					IGH/CCND1	nuc ish(CCND1,IGH)x2[300]	
					IGH/BCL2	nuc ish(IGH,BCL2)x3(IGH con BCL2x2)[6/300]	
	2	-	-	-	IGH/BCL2	nuc ish(IGH,BCL2)X2[300]	46,XY[20]

Abbreviations: Dx, diagnosis; F/U, follow-up; Asp., aspiration; Bx., biopsy; IHC, immunohistochemical staining; FISH, fluorescence in situ hybridization; BM, bone marrow; DLBCL, diffuse large B cell lymphoma; FL, follicular lymphoma; HCL, hariy cell leukemia.

https://doi.org/10.47429/lmo.2023.13.3.172

33.3%). Follow-up events with high grade lymphoma were 21, and among them, discrepancies among the three test results were three (DR of 14.3%).

In three discrepant cases in high grade lymphoma, the first case (case number 2) showed BM involvement in all three tests at diagnosis and at the first follow-up during treatment, both IBMII and karyotype converted to normal but still showed abnormal results in FISH with the frequency of 2.0–2.3%, and finally at the second follow-up during treatment, all three tests showed normal results. The second case (case number 3) exhibited BM involvement in all three tests at diagnosis and at the first follow-up during treatment, all three tests converted to normal but at the second follow-up during treatment, IBMII remained normal, however, FISH analysis showed abnormal results with the frequency of 6.0–8.0% and karyotype analysis also showed abnormal results with the frequency of 10.0%. The third case (case number 9) showed IBMII-involved and FISH-abnormal but karyotype-normal results at diagnosis.

Among 9 follow-up events from 4 patients with low grade lymphoma, discrepancies among three test results were 7 (DR of 77.8%), indicating a significantly higher DR than those with high grade lymphoma (DR of 14.3%, $P=0.002$), and all of them had IBMII-involved and FISH-abnormal but karyotype-normal.

DISCUSSION

The present study revealed that DR between BM aspirates/biopsy and IHC results are 6.3–14.2%, and almost all were BM aspiration/biopsy-not involved but IHC-involved cases, and IHC staining could detect an additional 6.5–13.4% of cases with BM involvement without definite morphologic evidence of involvement in BM aspiration or biopsy. This result suggests that IHC staining would be a useful and sensitive detection method for BM involvement in B-lineage lymphoma. When the three test results were considered together, DR among the three test results were 15.8%, and most of the discrepant cases were BM aspiration-not involved but IHC-involved cases regardless of BM biopsy status. This result may indicate that the improved sensitivity of IHC staining in detecting BM involvement can be maximized in cases without definite morphologic evidence of neoplastic lymphoid cell infiltrations in BM aspirates. When lymphoma grade was considered, we found that the DR among the three test results were not significantly different with respect to lymphoma grade (high grade 15.3% and low grade 17.1%), and their characteristics were also not significantly different. This result suggests that the lymphoma grade does not affect the efficacy of IHC staining in detecting BM involvement.

Our study found that DR between IBMII and karyotype/FISH results were 22.0–34.6%, and most of the discrepant cases were IBMII-involved but karyotype-normal/FISH-normal cases. When compared with karyotype results, we found that FISH results can reduce DR from 34.6% to 22.0%, and cases with FISH-abnormal but karyotype-normal were more frequent than those with FISH-normal but karyotype-abnormal. These results suggest that FISH analysis can detect BM associated cases with higher sensitivity than karyotype analysis.

Interestingly, we demonstrated that the incidences and characteristics of discrepancies were significantly different with respect to lymphoma grade. The DR found in low grade lymphoma were significantly higher than those found in high grade lymphoma (71.4% vs. 23.9%; $P<0.001$), and the proportion of cases with IBMII-involved but karyotype/FISH-normal was significantly higher in low grade lymphoma, compared to high grade lymphoma (45.7% vs. 8.7%; $P<0.001$). Discrepancies found in high grade lymphoma showed heterogeneity, but in low grade lymphoma there was significant skewness toward IBMII-involved but karyotype-normal cases. Also, it was found that with the application of FISH analysis, an additional 6.5% of high grade lymphoma cases and 25.7% of low grade lymphoma cases might show abnormal results, which is concordant with the IBMII. All these results indicate that the sensitivity gain from performing FISH would be greater in patients with low-grade lymphoma compared to high-grade lymphoma. Subsequently, performing a follow-up study also showed concordant results, and these results indicate that our suggestions can be applied to patients during follow-up as well as those at diagnosis. Our results support the speculation that the mitotic activity in neoplastic lymphoid cells in low grade lymphoma is low and that the decrease in division capacity of neoplastic lymphoid cells observed in patients with low grade lymphoma contributes to the low sensitivity of detecting the presence of neoplastic lymphoid cells in karyotype analysis. In such cases, FISH analysis can be a useful alternative test. Additionally, the observations that FISH analysis can detect minimal residual diseases and early relapse during treatment in some patients with high grade lymphoma suggest that FISH analysis would also be helpful in patients with high grade

lymphoma.

In a previous study [21], the detection rates of BM involvement in 17 FL patients from BM aspirates, BM biopsy, karyotyping, and IGH (immunoglobulin heavy chain) FISH were 35.3%, 35.3%, 0.0%, and 41.1%, respectively. Researchers also reported that FISH could detect BM involvement in all cases that were positive by BM biopsy and could additionally detect BM involvement in one patient who was defined as negative by BM biopsy. Our present study showed that the detection rates of BM involvement by IBMII are 44.0%, and can be increased to 48.0% when karyotyping and FISH analysis are added, which can partly support the previous study results [21]. However, since this study included only patients with FL, direct comparison between this study results and our present study results would be limited.

Another study [22] used four FISH panels (1q, BCL6, IGH, and p16) for the detection of BM involvement in 150 non-Hodgkin lymphoma patients; the detection rates of BM involvement by morphologic evaluation, karyotyping, and FISH analysis were reported as 19.3%, 2.0%, and 5.3%, respectively, which was lower than our present study. DR between morphologic evaluation and FISH analysis were reported as 15.3%, which was also lower than our present study results (22.0%). Technological advances in karyotyping and differences in FISH panel composition may have contributed to the discrepancy in our results.

Since our present study did not evaluate more advanced molecular technologies, such as next-generation sequencing for detecting minimal residual diseases in B-lineage lymphoma, an integrated study including these tests would be required to provide more comprehensive results.

In summary, our present study showed that IHC staining would be useful in sensitively detecting BM involvement in B-lineage lymphoma. When morphology, FISH, and karyotype analyses are considered together, FISH analysis can reduce DR from 34.6% to 22.0%, and detect BM-involved cases with higher sensitivity than karyotype analysis. Patients with low grade lymphoma show higher DR, a more skewed discrepancy pattern in IBMII-involved but karyotype/FISH-normal cases, and can induce higher concordance with abnormal morphologic findings with the application of FISH compared with high grade lymphoma. Follow-up evaluations also showed similar results, and it can be expected that the increase in detection sensitivity obtained by performing FISH analysis would be greater in low grade lymphoma.

요 약

배경: B세포계열림프종의 골수 침범 진단에 사용되는 골수흡인, 생검, 면역조직화학(IHC) 염색, fluorescence in situ hybridization (FISH) 및 핵형 분석의 일치도를 평가하고 림프종 등급에 따른 일치도 특성을 평가하였다.

방법: 최근 54개월 동안 림프종의 진단 및 골수검사를 시행받은 127명의 B세포계열림프종 환자[고등급(HG) 92명, 저등급(LG) 35명]가 본 연구에 전향적으로 포함되었다. 각 사례에 대해 골수흡인/생검/CD3 및 CD20 면역조직화학염색/FISH/핵형분석이 각각 시행되었고, 결과를 비교하였다.

결과: 골수흡인/생검과 면역조직화학염색 결과 간의 불일치율은 각각 14.2%/6.3%였으며, 면역조직화학염색의 시행은 골수흡인-정상/생검-정상 결과를 보인 사례 중 각각 13.4%/6.5%에서 골수침범 결과를 추가로 도출할 수 있었다. 통합된 골수 형태(integrated bone marrow involvement interpretation [IBMII], 세 가지 중 최소 한 가지 이상의 형태학적 평가에서 골수침범으로 확인된 경우로 정의)와 핵형분석/FISH 결과 사이의 불일치율은 각각 34.6%/22.0%였으며, FISH-비정상/핵형분석-정상인 경우가 FISH-정상/핵형분석-비정상인 경우보다 더 많았다. 저등급 림프종에서 IBMII, 핵형분석, FISH 중 불일치를 보인 경우가 고등급 림프종보다 유의하게 높았고(71.4% vs. 23.9%; $P<0.001$), IBMII에서 골수침범을 보였으나 핵형분석/FISH 결과가 정상인 경우의 비율이 저등급 림프종에서 고등급 림프종에 비해 유의하게 높았다(45.7% vs. 8.7%; $P<0.001$). 추가적으로, FISH 분석을 통해 6.5%의 고등급 림프종과 25.7%의 저등급 림프종 사례에서 IBMII와 일치하는 비정상적인 결과를 도출할 수 있었다.

결론: 면역조직화학염색은 B세포계열림프종의 골수침범을 민감하게 진단하는 데 유용할 것이다. FISH 분석은 핵형 분석에 비해 IBMII 결과와의 불일치율을 줄이고 골수침범 증례를 더 민감하게 진단할 수 있다. 저등급 림프종은 고등급 림프종에 비해 더 높은 불일치율을 보인다.

Conflicts of Interest

None declared.

Acknowledgements

This study was funded by Biomedical Research Institute Grant (2018B009), Pusan National University Hospital.

https://doi.org/10.47429/lmo.2023.13.3.172

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