CME/SAM

Needle-Core Biopsy in the Pathologic Diagnosis of Malignant Lymphoma Showing High Reproducibility Among Pathologists

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Key Words: Needle-core biopsy; Lymphoma; Pathologic diagnosis

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ABSTRACT

Objectives: *To evaluate the role of needle-core biopsy in the pathologic diagnosis of lymphoma.*

Methods: *One hundred and five cases with clinical suspicion for lymphoma were studied by 3 hematopathologists mimicking daily diagnostic service. The diagnostic result sheets were analyzed for diagnostic accuracy and reproducibility. The histologic pattern recognition by the 3 hematopathologists was also analyzed.*

Results: *The overall diagnostic accuracy, based on the consensus diagnosis, was 85% to 87%. High reproducibility of diagnosis in lymphoma was observed among pathologists. The tissue size was associated with the percentage of definitive diagnosis. Histologic patterns were well recognized on core tissues.*

Conclusions: *Needle-core biopsy is an effective technique for the diagnosis of lymphoma and should be considered the first-line procedure for cases with suspicion for lymphoma.*

Upon completion of this activity you will be able to:
- compare the efficacy of needle-core biopsies with excisional biopsies for diagnosis of malignant lymphoma.
- select adequate core needle size and evaluate adequacy of core tissue volume for diagnostic studies of lymphoma.
- select cases for which flow cytometry should be applied for accurate diagnosis and classification of malignant lymphoma.

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Questions appear on p 277. Exam is located at www.ascp.org/ajcpcme.

Malignant lymphoma is a group of neoplastic disorders arising from lymphoid tissues due to clonal expansion of B cells, T cells, or natural killer cells. The pathologic diagnosis of lymphoma is challenging. Excisional biopsy is considered the gold standard for the diagnosis and classification of lymphoma.[1,2] The role of needle-core biopsy in the diagnosis of lymphoma has been controversial. Most studies have been performed by interventional radiologists, who demonstrated that the diagnostic accuracy of needle-core biopsies was comparable with excisional biopsy. Needle-core biopsy for the diagnosis of lymphoma has shown to be less traumatic, more cost-effective, and well tolerated, especially for the patients who have deep-seated lesions or poor medical condition.[3-19] However, to our knowledge, reproducibility of diagnosis among pathologists, the histologic features of lymphoma on needle-core biopsy tissue, histologic pattern recognition, optimal size of the core needle and biopsy tissue, role of flow

cytometry, and other special studies have not been adequately addressed. Herein we report a retrospective study on 105 needle-core biopsy specimens from patients with a clinical suspicion for lymphoma. The study design was unique in that it mimics daily diagnostic service of pathology with sequential review of patient history and other clinical information, H&E-stained histology slides, immunohistochemical stains, flow cytometric data, and other special study results. We found that needle-core biopsy is an effective technique for the diagnosis of lymphoma.

Materials and Methods

Patients and Study Design

In total, 136 needle-core biopsy specimens from patients with a clinical suspicion for lymphoma were identified in the electronic medical record of the Creighton University Medical Center and Alegent Health between January 1, 2001, and July 30, 2008. In total, 105 cases qualified for the study; 31 cases were disqualified due to missing necessary diagnostic information. The cases of lymphocytic neoplasm were diagnosed and classified according to the World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues, published in 2001 and 2008.[20,23] Histologic slides, including slides with H&E and immunohistochemical stains, and other special studies were retrieved from the Creighton University Medical Center and Alegent Health files and then distributed to 3 hematopathologists (Q.H., H.N., and Q.X.). A diagnostic sheet was designed to mimic daily pathology diagnostic practice. Pathologists started with a review of the patients' medical history and clinical information and then studied the H&E slides. Initial differential diagnoses were recorded on the diagnostic result sheets with a confidence level from 1 to 3 (1, unknown; 2, suspicious; and 3, certain). A histologic pattern was also recorded, including nodular, diffuse, and mixed nodular and diffuse, with a confidence level recorded as mentioned above. After review of immunohistochemical stains, a diagnosis and histologic pattern were recorded with a confidence level as described above. Flow cytometric data and cytogenetic and molecular study results were then reviewed finally if available. A final diagnosis was made with a confidence level recorded the same way as above. Each of the 3 hematopathologists made a diagnosis independently without seeking a second opinion. The study protocol was approved by the institutional review boards of Creighton University Medical Center and Alegent Health.

Consensus Diagnosis

For each case, 4 diagnoses, including the initial diagnosis, were recorded for data analysis. Initial pathologic reports were issued by multiple pathologists and analyzed as the "initial diagnosis"; the remaining 3 diagnoses for each case were recorded by each hematopathologist. Consensus diagnosis was defined as 1 of the following 4 criteria: (1) a consensus diagnosis made by 3 or more pathologists (initial pathologist, Q.H., H.N., and Q.X.), with 6 inconclusive cases further reviewed by a fourth hematopathologist (K.F.); (2) a diagnosis confirmed by a follow-up excisional biopsy; (3) a diagnosis confirmed by molecular cytogenetics and/or molecular biology studies, including polymerase chain reaction (PCR) and/or fluorescence in situ hybridization (FISH) studies; and (4) a nonneoplastic diagnosis confirmed by follow-up for at least 3 years without signs and symptoms of neoplasia. The consensus diagnosis is considered the final diagnosis of each case. Diagnostic accuracy is defined as the percentage of correct diagnoses, given by each individual pathologist, compared with the consensus diagnosis.

Estimation of Biopsy Tissue Size

The size of the biopsy needles was identified from the clinical notes from the Department of Radiology. The size of the core tissues was determined according to the needle size and the total length of the core tissue measured on glass slides. The diameter of the core tissue was based on the inner diameter of the cutting needles. The volume of the tissue was estimated by the following formulation: volume = $(\text{diameter}/2)^2 \times \text{length} \times 3.14$.

Statistical Analysis

The χ^2 test was used to analyze the difference in diagnostic accuracy or reproducibility among varying biopsy needle sizes, tissue lengths, and volumes.

Results

General Information About the Patients and Biopsy Tissues

Of the 105 patients, 55 were male and 50 were female, for a male-to-female ratio of 1.1:1. Age of the patients ranged from 24 to 97 years (median, 67 years). In 84 (80%) of 105 cases, the diagnosis was made based on H&E and immunophenotyping, including immunohistochemical stains and/or flow cytometric analysis. Excisional biopsy was performed in 8 cases, 2 of which were for therapeutic purposes. Polymerase chain reaction for immunoglobulin heavy chain (IgH) gene and/or T-cell receptor (TCR) gene rearrangement was performed in 4 cases. A FISH study result was available in 11 cases. The size of core needles used ranged from 11 to 22 gauge. Mean length of core tissue was 23.4 mm. Most (68%) lesions were deep-seated **Table 1**. Complications occurred

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Table 1
General Information About Cases and Specimens

Characteristic	Value
Total No. of cases	105
Male/female ratio	1.1:1
Age, median (range), y	67 (24-97)
Definitive diagnosis without special studies[a] or excision, No. (%)	84 (80)
Excisional biopsy, No.	8
FISH, No.	11
Molecular biology study, No.	4
Flow cytometry study, No.	36
Needle size, gauge	11-22
Length of core tissue, mean (range), mm	23.4 (2-80)
Volume of core tissue, mean (range), mm^3	15.6 (0.54-147.7)
Lesion of body surface, No. (%)	34 (32)
Lesion of deep seated organs, No. (%)	71 (68)
Lymph node lesions, No. (%)	83 (79)
Extranodal lesions, No. (%)	22 (21)

FISH, fluorescence in situ hybridization.
[a] Special studies include molecular biology study or molecular cytogenetics study.

Table 2
Summary of the Consensus Diagnosis in 105 Cases

Lymphoma	Number	Others	Number
Follicular	15	Carcinoma	10
Diffuse large B cell	13	Thymoma	2
Small lymphocytic	8	Other neoplasm	4
Marginal zone	4	Reactive lymphoid hyperplasia	15
Mantle cell	2		
Burkitt	3	Granulomata	6
Peripheral T cell	2	Negative for malignancy/lymphoma	8
Hodgkin	7		
Other lymphoid neoplasm[a]	6	Subtotal	45
Subtotal	60		

[a] Other lymphocytic neoplasms include hairy cell leukemia, lymphoplasmacytic lymphoma, plasma cell myeloma, posttransplant lymphoproliferative disorder, and B-cell non-Hodgkin lymphoma unable to be further classified with needle-core biopsy.

in 8 (8%) of 105 cases, including 6 hemorrhages and 2 hematomas, which were all transient and well tolerated. No fatal complications were reported.

Diagnostic Accuracy and Reproducibility

Final diagnostic results for the 105 cases that qualified for this study are summarized in **Table 2**. Mature B-cell non-Hodgkin lymphomas, including follicular lymphoma, diffuse large B-cell lymphoma, and small lymphocytic lymphoma, were most common. Marginal zone B-cell lymphoma, mantle cell lymphoma, and Burkitt lymphoma also were seen. Two cases of peripheral T-cell lymphoma included 1 anaplastic large T-cell lymphoma involving subcutaneous tissue and skeletal muscle and 1 large granular lymphocyte leukemia involving the liver. Other types of lymphocytic neoplasm included hairy cell leukemia (n = 1); lymphoplasmacytic lymphoma (n = 1); extramedullary plasma cell myeloma (n = 1); posttransplant lymphoproliferative disorder (n = 1); B-cell non-Hodgkin lymphoma, not further classified (n = 1); and non-Hodgkin lymphoma, unclassifiable (n = 1). Seven cases of Hodgkin lymphoma were diagnosed, including 6 classic Hodgkin lymphomas and 1 nodular lymphocyte-predominant Hodgkin lymphoma. Ten cases of carcinoma included 4 metastatic adenocarcinomas involving the mediastinum, lung, and liver; 3 metastatic poorly differentiated carcinomas involving the neck, groin, and mediastinum; 2 small cell carcinomas of the lung involving the mediastinal lymph node; and 1 metastatic breast carcinoma involving the mediastinum. Two thymomas and 4 other tumors were identified, including 2 benign spindle cell neoplasms of the testes and kidney, 1 Warthin tumor of the parotid gland, and 1 myeloid sarcoma involving subcutaneous tissue.

Diagnostic accuracy and reproducibility for lymphoma were analyzed for the individual pathologist, with the results summarized in **Table 3**. Among the 105 cases, correct

Table 3
Diagnostic Accuracy and Reproducibility

		Diagnosis Made by Individual Pathologist, No.			
Characteristic	Consensus Diagnosis, No.	Initial	A	B	C
Lymphoma	60	57	57	56	58
Carcinoma	10	9	9	8	9
Other neoplasm	6	5	5	4	6
RH/GL/NEG	29	20	19	21	19
ALP/INAD	0	14	15	16	13
Total	105	105	105	105	105
Accuracy (n = 105), No. (%)[a]		91 (87)	90 (86)	89 (85)	91 (87)
Reproducibility of lymphoma diagnosis (n = 60), No. (%)		55 (92)	57 (95)	56 (93)	57 (95)
Reproducibility with lymphoma subclassification (n = 60), No. (%)		55 (92)	56 (93)	52 (87)	55 (92)

ALP, atypical lymphoid hyperplasia; GL, granuloma; INAD, inadequate for evaluation; NEG, negative for malignancy and lymphoma; RH, reactive hyperplasia.
[a] Compared with consensus diagnosis.

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diagnoses were made in 91 (87%) cases by the initial pathologists and similarly by all 3 pathologists (range, 89-91 cases). Among 60 cases with a consensus diagnosis of lymphoma, definitive correct diagnoses were made in 55 to 57 cases (92%-95%) among pathologists. Among the 14 cases without a definitive diagnosis initially, 1 case suspicious for thymoma and 1 case suspicious for Hodgkin lymphoma were confirmed by excisional biopsy. One case initially considered suspicious for classic Hodgkin lymphoma was diagnosed as classic Hodgkin lymphoma by 1 pathologist; it was called atypical lymphoid proliferation and granuloma by the other 2 pathologists, respectively. Two biopsy specimens were insufficient for diagnosis by the initial pathologist; 1 specimen showed granulomatous inflammation, and the other was metastatic poorly differentiated non–small cell carcinoma by excision. Among 8 cases initially called suspicious for lymphoma or atypical lymphoid hyperplasia by the initial pathologist, 1 was adenocarcinoma by the second needle-core biopsy, 1 was T-zone hyperplasia, 1 was Hashimoto thyroiditis, and 1 was nodular lymphocyte-predominant Hodgkin lymphoma by excision; 3 were reactive lymphoid hyperplasia by clinical follow-up; and 1 needle-core biopsy specimen of the groin lymph node, called granuloma with focal atypia by the initial pathologist, was called granuloma without atypia by the rest of the 3 pathologists.

Cases With Excision, Molecular Biology, and Molecular Cytogenetic Studies

Excision was performed in 8 cases, including 2 therapeutic excisions and 6 diagnostic excision biopsies, as shown in **Table 4**. Polymerase chain reaction for IgH or TCR gene rearrangement or cytogenetic studies were performed in 14 cases. Five cases were suspicious for follicular lymphoma based on their morphologic and immunophenotypic features. The FISH results for the t(14;18)/Bcl-2–IgH gene rearrangement confirmed the diagnoses. Polymerase chain reaction for TCR gene rearrangement was performed in 3 cases, including 2 cases of atypical T-cell proliferation and 1 case of marginal zone lymphoma. The results were negative for clonal TCR gene rearrangement and confirmed the morphologic diagnoses. In 3 cases, FISH for c-*myc* gene rearrangement was performed, of which 2 cases were suspicious for Burkitt lymphoma by morphology and were positive for c-*myc* gene rearrangement by FISH. One was diffuse large B-cell lymphoma and negative for c-*myc* gene rearrangement. One case with marginal zone lymphoma showed clonal IgH gene rearrangement by PCR. One small lymphocytic lymphoma was negative for t(11;14)/Bcl-1–IgH gene rearrangement. One myeloid sarcoma showed trisomy 21 by routine cytogenetic study.

Table 5
Changes in Diagnostic Confidence After Review of Flow Cytometry Results

Diagnosis	Total No. of Cases	Change From Uncertain to Confidence Certain, No. (%)[a]
Follicular lymphoma	18	5 (28)
Small lymphocytic lymphoma	15	6 (40)
Diffuse large B-cell lymphoma	8	0
Burkitt lymphoma	6	0
Other B-cell lymphoma	14	3 (21)
Reactive/granuloma	13	0
Percentage of change in total	74	14 (19)

[a] Uncertain in diagnosis includes level 1 (unknown diagnosis) and level 2 (suspicious for diagnosis). It is considered helpful in the diagnosis of lymphoma if diagnostic confidence level changes from "uncertain" to "certain" after review of flow cytometry results.

Role of Flow Cytometry in the Diagnosis With Needle-Core Biopsy Tissue

Flow cytometry study was performed in 36 (34%) of 105 cases. Seventy-four diagnostic result sheets with evaluation of flow cytometry were analyzed for its role in the diagnosis, with the results summarized in **Table 5**. Thirteen (72%) of 18 cases of follicular lymphoma were diagnosed with high confidence by H&E and immunohistochemical stains only.

Table 4
Summary of Cases With Surgical Excision

Case No.	Initial Diagnosis of Needle Biopsy Specimen	Diagnosis of Excision	Tissue	Purpose of Excision
1	Thymoma, suspicious	Thymoma, type B	Anterior mediastinal mass	Therapeutic
2	Benign spindle cell neoplasm, suspicious	Benign spindle cell neoplasm	Testicle	Therapeutic
3	Classic Hodgkin lymphoma, suspicious	Classic Hodgkin lymphoma	Neck mass	Diagnostic
4	MALT lymphoma, suspicious	MALT lymphoma	Lung mass	Diagnostic
5	Atypical CD4+ T-cell proliferation	Reactive T-zone hyperplasia	Axillary lymph node	Diagnostic
6	Atypical lymphoid hyperplasia	Nodular lymphocyte-predominant Hodgkin lymphoma	Mesentery lymph node	Diagnostic
7	Atypical lymphoid proliferation	Adenocarcinoma	Lung mass	Diagnostic
8	Inadequate for evaluation	Non–small cell carcinoma	Lung mass	Diagnostic

MALT, mucosa-associated lymphoid tissue.

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Flow cytometry was helpful in the diagnosis of 5 cases of follicular lymphoma. One diagnosis of follicular lymphoma was suspected (level 1, by H&E; level 2, by immunohistochemical stain and flow cytometric studies), and a definitive diagnosis of follicular lymphoma was reached with FISH study. A similar observation was noted in the diagnosis of small lymphocytic lymphoma cases, with flow cytometry study helpful for the diagnosis in 6 (40%) of 15 cases. Diagnostic confidence was high (level 3) in diffuse large B-cell lymphoma and Burkitt lymphoma with H&E and immunohistochemical stains, whereas flow cytometry study appeared to be noncontributory. Thirteen diagnoses of reactive lymphoid hyperplasia and granulomas were based on H&E and immunohistochemical stains with a high confidence level, with the flow cytometry result appearing noncontributory to the diagnosis. The flow cytometry study was helpful in varying degrees for other types of B-cell lymphoma, such as marginal zone lymphoma and hairy cell leukemia with lymph node involvement. Overall, flow cytometric study was considered helpful in 19% of the 74 diagnoses.

Association Between Needle and Tissue Sizes and Percentage of Definitive Diagnosis

Ninety-eight documented cases were divided into 3 groups according to needle size, tissue length, and tissue volume and analyzed for percentage of definitive diagnosis given by pathologists. The difference in percentage of definitive diagnosis among varying needle sizes, tissue lengths, and tissue volumes was analyzed by the χ^2 test. The percentage of definitive diagnosis of the group with the smallest tissue volume and tissue length was significantly lower than the rest of groups with a larger tissue volume and tissue length. The difference in the percentage of definitive diagnosis among cases with varying needle sizes was also noted, but it was not significant statistically ($P < .2$, χ^2 test) **Figure 1**.

Eight cases had procedure-related temporal hemorrhage or hematoma. The needle size for the procedure for these cases was 14 to 20 gauge, with a tissue volume from 3.5 to 80.4 mm^3 (mean, 20.6 mm^3). The tissue volume and needle sizes were not different from those cases without complications ($P > .05$).

Assessment of Histologic Patterns of Lymphoma on Needle-Core Biopsy Tissue

Histologic pattern recognition was assessed on needle-core biopsy tissue under a microscope at the beginning of the slide review for each case, and the histologic patterns were recorded as diffuse, nodular, and mixed with the following confidence levels: 1 (unknown), 2 (suspicious), and 3 (certain). In total, 156 diagnostic result sheets from the 3 hematopathologists qualified for analysis, with 109 (70%) of 156 diagnoses showing pattern recognition with certainty on H&E slides, which increased to 147 (94%) after reviewing the immunohistochemical stains **Figure 2**. The histologic patterns correlated well with the individual type of lymphoma, especially follicular lymphoma, diffuse large B-cell lymphoma, and Burkitt lymphoma.

Discussion

The pathologic diagnosis of lymphoma is challenging and based on clinical information, histologic features, immunophenotype, cytogenetics, and molecular studies.[20] Excisional

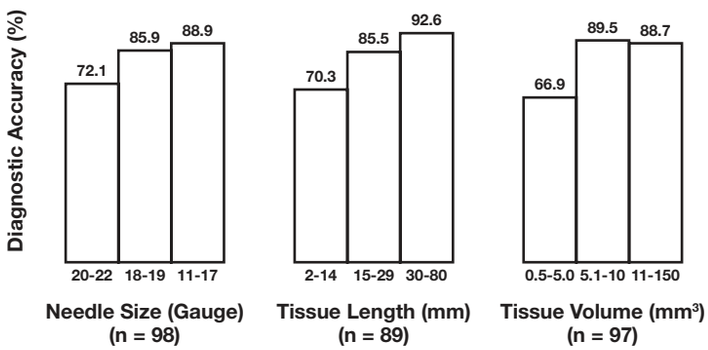

Figure 1 Total cases include cases with a definitive diagnosis and an uncertain diagnosis. Cases with an uncertain diagnosis include those without enough tissue, with atypical lymphoid proliferation, and suspicious for lymphoma. The diagnostic accuracy of the group (left bars) with the shortest tissue length (2-14 mm, n = 32) and smallest tissue volume (0.5-5.0 mm^3, n = 31) is significantly lower than the diagnostic accuracy of the other 2 groups (middle and right bars, $P < .03$ and $P = .05$, χ^2 test). The difference in diagnostic accuracy between the group with the lowest needle size (left bars, 20-22 gauge, n = 34) and the rest with a larger (middle bars) and the largest (right bars) needle size is not statistically significant ($P < .2$, χ^2 test).

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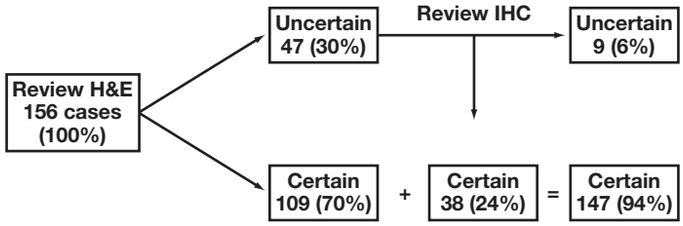

Figure 2 Histologic pattern recognition on needle-core biopsy tissue. IHC, immunohistochemical stain.

biopsy of a lymph node or tissue mass is considered a standard procedure for the diagnosis of lymphoma. However, excisional biopsy is more traumatic and expensive and less tolerable, especially for patients with deep-seated lesions and poor medical condition. With the advent of new technology, such as molecular biology and medical imaging, less aggressive procedures, such as imaging-guided needle-core biopsy and fine-needle aspirate cytology with flow cytometry, have been advocated for the diagnosis of lymphoma. Recent studies have shown that needle-core biopsy is a cost-effective procedure.[3] Needle-core biopsy has been used more often as a first-line diagnostic procedure for patients with clinical suspicion for lymphoma.[21] However, the diagnostic accuracy of needle-core biopsy in lymphoma has been controversial, and limited studies have been reported by pathologists.[3,4,21]

We studied needle-core biopsies of 105 cases performed at the Creighton University Medical Center and Alegent Health, which covers an academic medical center and several community hospitals based in Omaha, Nebraska, and western Iowa areas. The research was designed to mimic a prospective study and daily diagnostic work in pathology. High diagnostic accuracies ranging from 85% to 87% were observed in this study. The diagnostic reproducibility of the 60 cases of lymphoma ranged from 92% to 95% and, for lymphomas with subclassification, from 87% to 93%. False-positive diagnosis was not identified in this study. This result is close to that of excisional biopsy.[22] Only slight variations were observed among the 3 hematopathologists, and their results are close to those of the initial pathologist. No major discrepancies were identified among pathologists. Therefore, our study demonstrated that needle-core biopsy is a reliable and reproducible diagnostic procedure for diagnosing cases clinically suspicious for malignant lymphoma.

Multiple factors may affect the diagnostic accuracy and sensitivity. Sampling errors were identified in the study, as seen in the cases with neck lymphadenopathy and lung nodules. Therefore, in the cases with suspicious sampling error, a repeat of the biopsy or excision is recommended to avoid underdiagnosis due to sampling error. The diagnostic accuracy may vary with the different types of lymphoma. We reviewed previous reports of needle-core biopsy in the English literature with the subclassification of lymphoma, with the results of several reports published from 2000 to 2007 summed in **Table 6**.[4-8] Most of the studies were retrospective investigations, with diagnostic accuracy used to indicate the percentage of definitive diagnosis given by pathologists over total cases. A unique study on tissue microarray was reported by Farmer and colleagues.[4] Mimicking needle-core

Table 6
Review of the Diagnostic Accuracy of Different Types of Lymphoma[a]

Lymphoma	Hu et al[b] (Current)	de Larrinoa et al[5] (2007)	Farmer et al[4] (2007)	Sklair-Levy et al[6] (2000)	Balestreri et al[7] (2005)	Li et al[8] (2005)	Average
DLBL	12/13 (92)	34/37 (92)	29/32 (90)			32/36 (89)	107/118 (91)
FL	15/15 (100)	27/28 (96)	20/40 (50)			1/2 (50)	63/85 (74)
SLL	8/8 (100)	3/3 (100)	24/28 (86)	7/7 (100)		2/2 (100)	44/48 (92)
MCL	2/2 (100)		21/24 (88)			3/3 (100)	26/29 (90)
MZBL	4/4 (100)	1/1 (100)	16/40 (40)			6/7 (86)	27/52 (52)
Pre T			12/12 (100)				12/12 (100)
PTCL/NK	2/2 (100)	5/6 (83)	24/32 (75)			15/22 (68)	46/62 (74)
NHL/others	8/9 (89)	12/12 (100)		64/69 (93)	101/113 (89)	0/1	185/204 (91)
HL	4/7 (57)	11/15 (73)	50/60 (83)	33/38 (87)	18/24 (75)	2/7 (29)	118/151 (78)
Overall accuracy	55/60 (92)	93/102 (91)	196/268 (73)	104/114 (91)	119/137 (87)	61/80 (76)	628/761 (83)

DLBL, diffuse large B-cell lymphoma; FL, follicular lymphoma; HL, Hodgkin lymphoma; MCL, mantle cell lymphoma; MZBL, marginal zone B-cell lymphoma; NHL, non-Hodgkin lymphoma; Pre T: precursor T-cell neoplasm; PTCL/NK, peripheral T-cell lymphoma/natural killer cell neoplasm; SLL, small lymphocytic lymphoma.
[a] Values are presented as number/total number (%).
[b] Data from the results of the initial pathologist of the current study.

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biopsy, the diagnoses on tissue microarray were compared with original excision biopsy specimens. As shown in Table 6, lower diagnostic accuracy was noted in marginal zone B-cell lymphoma (52%). However, the diagnosis of 4 cases of marginal zone lymphoma in our study was definitive, with high consistency among 4 pathologists. A conclusion could not be reached for marginal zone lymphoma due to a limited case number. In the review of 3 major types of lymphoma in our study, including 144 diagnoses made by the 4 pathologists (36 cases of diffuse large B-cell lymphoma, follicular lymphoma, and small lymphocytic lymphoma), the overall diagnostic accuracy reached 97% with a high confidence level. The paucity of Hodgkin cells in the biopsy tissue may have affected the diagnostic accuracy of Hodgkin lymphoma. The combined diagnostic accuracy of 4 pathologists for 7 cases of Hodgkin lymphoma was 64% in our study. However, 2 cases showed extremely small core tissue, with a tissue length of 2 mm and 3 mm, respectively. Therefore, the size of the needle-core biopsy tissue is an important factor that potentially affects the diagnostic accuracy.

The size of the tissue is related to the size of the biopsy needle, tissue length and number or passes of biopsy tissue, and tissue volume. Analysis of tissue length and volume indicated that the diagnostic accuracy increased with the increase of biopsied tissue length and volume. Analysis of needle size also showed that biopsy with large needles (19 gauge or larger) appeared to increase the diagnostic accuracy of lymphoma compared with smaller needles (smaller than 19 gauge), although the difference lacked statistical significance. This result was likely affected by other cofactors, such as tissue length. Therefore, we recommend that the size of the biopsy needle should be 19 gauge or larger, obtaining at least 3 to 4 cores with each 5 to 10 mm in length (total length, 15-30 mm or longer) for histologic diagnosis. With the tissue for histology, about 20 to 30 tissue sections may be made for routine H&E and potential immunohistochemical stains if each section is cut no more than 3 to 4 μm in thickness. Needle sizes larger than 19 gauge or longer core tissues may slightly increase the diagnostic accuracy and are recommended for surface lesions if clinically indicated.

The current WHO classification for lymphoid neoplasms is based on clinical information; cytologic, histologic, and immunophenotypic features; and other special study results.[20,23] In this study, 13% of the cases required FISH and/or molecular studies to reach a final diagnosis and subclassification. The role of flow cytometry may have been overemphasized in the lymphoma diagnosis in previous studies. In the majority (66%) of the cases in this study, no flow cytometry study was done. In the cases with flow cytometry, it was helpful in 19%, mainly follicular lymphoma and small lymphocytic lymphoma. In the other types of B-cell lymphomas, such as diffuse large B-cell lymphoma and Burkitt lymphoma, flow cytometry showed a limited role if needle-core biopsy tissue was enough for H&E and immunohistochemical stains, as shown in Table 5. However, this observation is limited in 6-color flow cytometry. The role of 10-color flow cytometry in the diagnosis of lymphoma with needle-core biopsy tissue has not been elucidated.

It is well known that histologic features are essential in the diagnosis of lymphoma. Histologic patterns, such as diffuse, nodular, and mixed nodular and diffuse, were used for the classification of lymphoma previously, as in the Rapport classification and the International Working Formation classification.[24,25] It has been argued that the lack of histologic patterns is the disadvantage of needle-core biopsy tissue. However, our study demonstrated that histologic patterns were well recognized in the needle-core biopsy tissue in 94% of the cases, which correlated well with the final diagnosis and the type of lymphoma. Immunohistochemical staining significantly improved the pattern recognition (Figure 2). Reactive lymphoid follicles showed a characteristic zonal pattern on the CD79a stain, delineating polarization with the strongly positive mantle zone and weakly stained germinal center. This feature is only rarely seen in follicular lymphoma and therefore is helpful in the differential diagnosis **Image 1** and **Image 2**. Staining for CD21 is helpful in identifying residual lymphoid follicles in small core tissue, especially when a definitive histologic pattern is uncertain on H&E sections. Overfixation may be encountered for a small needle-core biopsy tissue, and it may be challenging to appreciate the cytologic features due to shrinking cell size, such as Reed-Sternberg cells. Immunohistochemical staining for MUM-1, CD30, and CD15 may be helpful in these cases. This issue is also commonly encountered in large cell lymphoma and the grading of follicular lymphoma. Staining for Ki67 is helpful and may highlight large proliferating centroblasts and facilitate the grading.[26]

In summary, needle-core biopsy is a reliable and cost-effective diagnostic procedure. Histologic patterns of lymphoma are recognizable in core biopsy tissue and are useful in the pathologic diagnosis of lymphoma. Flow cytometry has showed a limited role in the diagnosis of lymphoma, and its use should be selected with more caution. Biopsy needles of 19 gauge or larger are recommended, and at least 3 to 4 tissue cores, with each 5 to 10 mm in length (total length, 15-30 mm or longer), should be taken. Needle-core biopsy is less traumatic and well tolerated by patients. It should be recommended in cases suspicious for lymphoma as a first-line procedure, especially for the deep-seated thoracic and abdominal lesions and for patients with poor medical condition.

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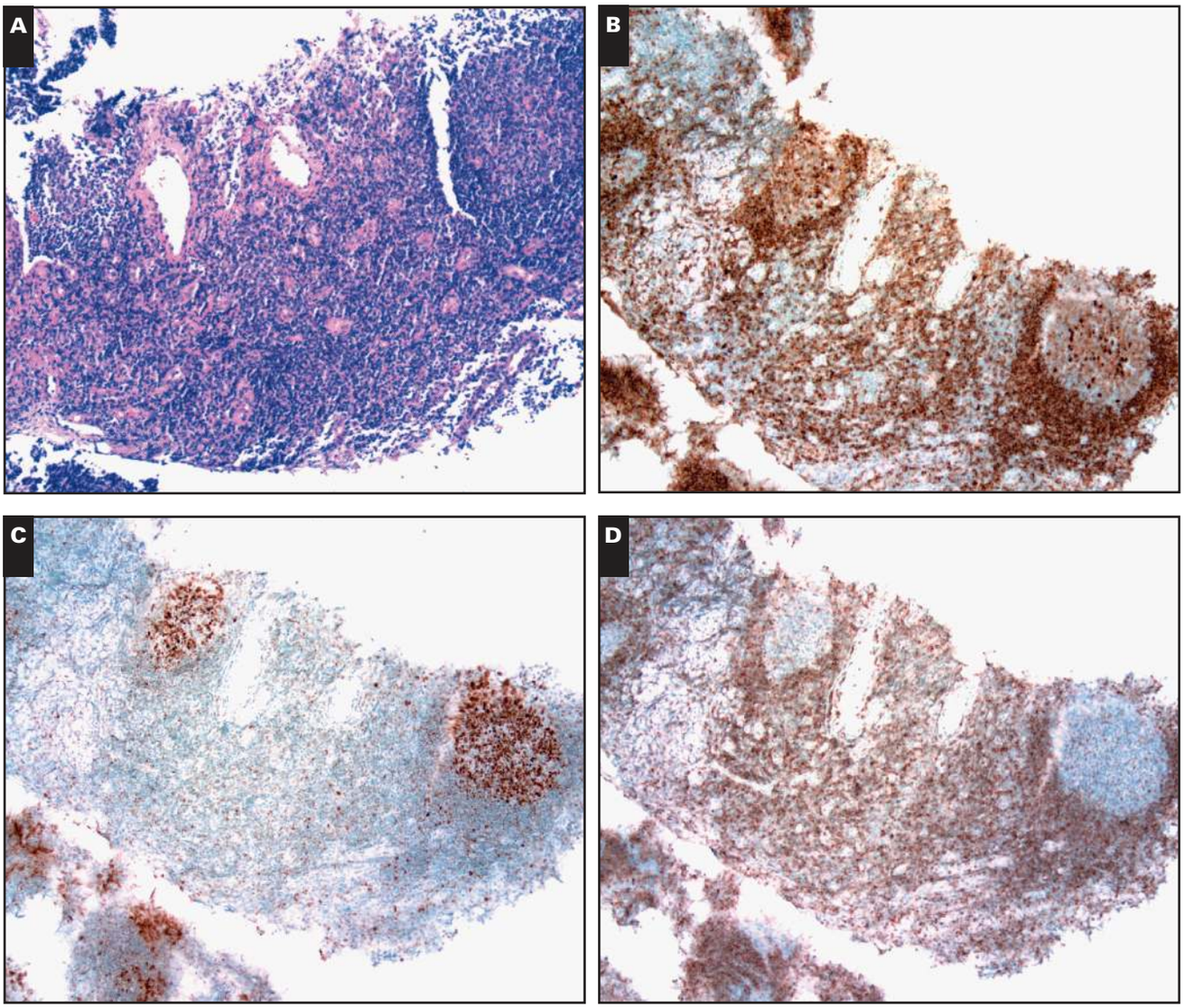

Image 1 Sections of a lymph node needle-core biopsy specimen show a nodular pattern with follicular hyperplasia on H&E (**A**, ×200). Staining for CD79a (**B**, ×200) shows a "zonal pattern" with weakly positive germinal centers and strongly positive mantle zones, characteristic of benign hyperplastic follicles. Staining for Bcl-6 and Bcl-2 (**C** and **D**, ×200) shows germinal centers positive for Bcl-6 but negative for Bcl-2.

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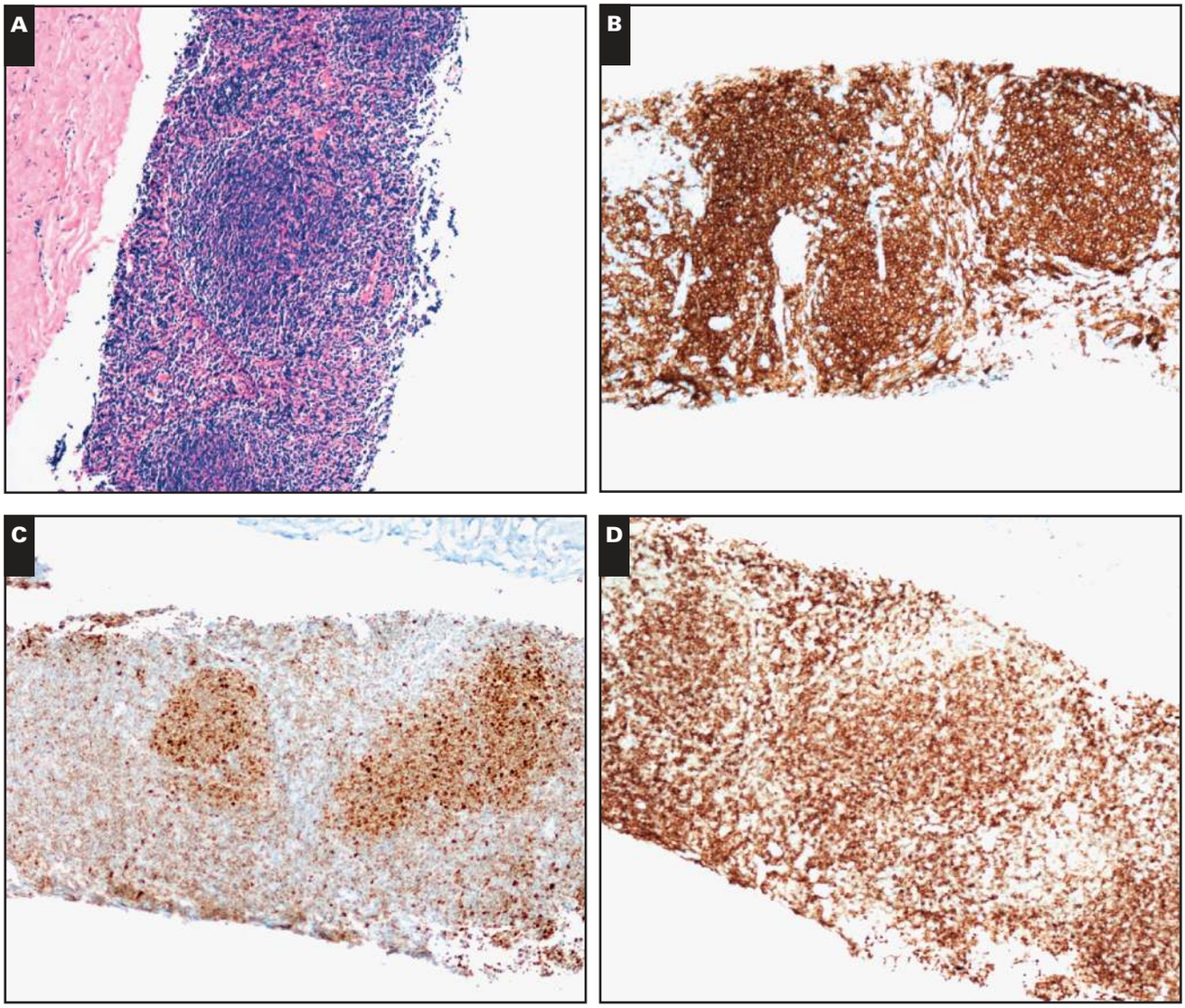

Image 2 Sections of a lymph node needle-core biopsy specimen show a nodular pattern of follicular lymphoma on H&E (**A**, ×200). Staining for CD79a (**B**, ×200) shows positive follicles without a "zonal pattern." Staining for Bcl-6 and Bcl-2 (**C** and **D**, ×200) reveals germinal centers positive for both Bcl-6 and Bcl-2.

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