CME/SAM

Combined Core Needle Biopsy and Fine-Needle Aspiration With Ancillary Studies Correlate Highly With Traditional Techniques in the Diagnosis of Nodal-Based Lymphoma

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Upon completion of this activity you will be able to:
- list the limitations of core needle biopsies and fine-needle aspirations with ancillary studies in the diagnosis of lymphoma.
- outline the strategy for optimal core biopsy sampling and diagnostic testing to diagnose lymphoma.
- predict what implications the diagnosis of lymphoma made on a core needle biopsy has on the management of patients.

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Abstract

Core needle biopsy (CNB) and fine-needle aspiration (FNA) are increasingly replacing excisional lymph node biopsy in the diagnosis of lymphomas. However, evaluation of CNB and FNA remains challenging owing to limited architectural information and the more detailed subclassification of lymphomas required by the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. *Our study is the largest study to assess diagnostic accuracy of CNB and FNA in conjunction with ancillary studies. We analyzed 263 cases and a diagnosis was established in 237, of which 193 were completely subclassified. In cases in which excisional biopsy was available as a reference for comparison, CNB and FNA had a sensitivity of 96.5%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 90%. CNB and FNA with ancillary studies represent a viable alternative in the diagnosis of lymphoma, as long as the number and size of cores for morphologic studies are not compromised.*

For many years, excisional biopsy of the lymph node was considered the "gold standard" for evaluation of lymphoproliferative disorders. Older lymphoma classification schemes, such as the Working Formulation, depended on the histologic architecture for accurate diagnosis, which was mainly based on morphologic and immunohistochemical studies. In recent years, genetics and immunophenotypic analysis by flow cytometry have become pivotal in the accurate classification of lymphoproliferative disorders. The *WHO* [World Health Organization] *Classification of Tumours of Haematopoietic and Lymphoid Tissues* has considerably expanded its classification of lymphomas based on molecular and cytogenetic profiling and immunophenotyping.[1] In particular, genetic studies, such as fluorescence in situ hybridization (FISH) for common lymphoma translocations and T-cell receptor *(TCR)* and immunoglobulin heavy chain *(IGH)* gene rearrangement studies on paraffin-embedded tissue samples, have enabled accurate diagnosis on small amounts of tissue without the need for excisional biopsy. Core needle biopsy (CNB) and fine-needle aspiration (FNA) are associated with fewer complications and lower cost than excisional biopsy, especially when deeply seated lymph nodes are involved.[2-8] A potentially decisive diagnosis of lymphoma or benignity by CNB and FNA is particularly important for patients whose condition may be too unstable for undergoing general anesthesia and open surgical biopsy.

Diagnostic accuracy of lymphoma diagnosis using CNB and/or FNA have largely been reported for deep-sited lymph nodes, mostly by European groups, and the rate of diagnostic accuracy varies between 70% and 98%, depending on the type of care facility performing these procedures. Limitations are mostly related to the lack of sufficient tissue for diagnosis.[2-4,7,9]

Because in our institution (Washington University Medical Center, St Louis, MO) we observed the trend that CNB and FNA are no longer preferably used for deep-seated lymph nodes, but seem to have become the standard procedure for obtaining lymph node tissue in general, we wanted to analyze the diagnostic accuracy of these procedures in a large case study and investigate whether ancillary studies, such as flow cytometry, immunohistochemical analysis, and genetics, can compensate for the limitations in morphologic analysis in the diagnosis of lymphoproliferative disorders.

Materials and Methods

Case Selection

The study protocol was reviewed and approved by the Washington University Institutional Review Board. For this retrospective cohort study, a search of the database of the Division of Anatomic and Molecular Pathology, Washington University School of Medicine, St Louis, was conducted for patients who underwent a CNB of a lymph node for a suspected diagnosis of lymphoma in a 7-year period from 2003 to 2009. This search generated a list of 263 cases. Clinical and pathology records were retrospectively reviewed to obtain data on patient age, biopsy location, core needle size and number of passes, final pathologic diagnosis, and ancillary studies (flow cytometry, immunohistochemical analysis, *IGH* or *TCR* gene rearrangement studies, FISH for common lymphoma-related translocations, or excisional biopsy) performed to aid in final diagnosis. Diagnosis was made following the guidelines of the *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*.[1] In cases of an incomplete diagnosis, information about the subsequent clinical management was obtained from patients' medical records.

Specimen Analysis

CNB and FNA specimens were obtained in 1 session, almost exclusively by radiologists. Generally, FNA specimens for cytology and/or flow cytometry were generated first, followed by core material that was sent directly to the Hematopathology Service.

All 263 CNB specimens were signed out by trained hematopathologists with at least 1 year of hematopathology experience (A.H., J.L.F., R.B., T.T.N., and F.K.). A varied selection of immunohistochemical studies for further lineage differentiation (CD3, CD5, CD10, CD15, CD20, CD30, CD45, BCL-2, BCL-6, CD79a, Mum-1, Ki-67, MelanA, anaplastic lymphoma kinase-1 [ALK-1], cyclin D1, cytokeratin AE1-3, and terminal deoxynucleotidyl transferase) and/or in situ hybridization for κ and λ messenger RNA was performed on 176 cases and interpreted by the pathologist who signed out the case. Flow cytometry was performed on 11 CNB specimens, 7 of which had adequate and sufficient material for analysis. The panel of CD3, CD19, and surface κ and λ was used to screen for a possible B-cell lymphoma. If a clonal B-cell population was detected, CD5, CD10, CD23, and CD20 were added to the panel. In cases with a marked predominance of CD3+ T lymphocytes, additional T-cell analysis was expanded to include CD1, CD2, CD4, CD5, CD7, CD8, CD30, CD56/CD16, TCRα/β, and TCRγ/δ. Flow cytometric findings were signed out by the primary hematopathologist responsible for the case.

FNA was performed in 192 of 263 cases, mainly to retrieve a specimen for flow cytometric analysis. In 170 cases flow cytometry was done, of which 130 revealed ample cell suspension for analysis. The decision to perform flow cytometry on an FNA specimen was made by the clinician who strongly suspected a lymphoma diagnosis or the cytopathologist who triaged the rapid Romanowsky– and Papanicolaou-stained smears at the bedside. While the cytopathologist signed out the FNA report, the primary hematopathologist signing out the CNB report interpreted the flow cytometric results of the FNA, as well as of a cytocentrifuged specimen obtained from the FNA for cytologic features. The same flow cytometric procedures were applied to the FNA samples as described for CNB. By adding the 7 cases of adequate flow cytometry samples from CNB, a total of 137 adequate flow cytometry specimens were included in this study.

For our study, cases were designated "incomplete diagnosis" if a hematopathologist "favored" or "was suspicious of" the lymphomatous or benign nature of the lymph node. A diagnosis of B- or T-cell lymphoma without further subclassification was also included in the "incomplete diagnosis" category. The "decisive diagnosis" category included all unequivocal diagnoses of benign lymph nodes or lymphomas with full classification. If a diagnosis could not be established because the specimens were too small for meaningful analysis or because findings were equivocal to confirm or rule out lymphoma, it was categorized into the "nondiagnostic" category.

In 54 cases, diagnoses from a subsequent excisional biopsy were available for comparison.

Statistical Analysis

The χ^2 was used to compare rates between categorical variables. Sensitivity, specificity, positive predictive value, and negative predictive value of needle biopsy were calculated using standard 2 × 2 tables. Statistical significance was set at $P < .05$. Statistical analysis was performed using SPSS for Windows, version 10 (SPSS, Chicago, IL).

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Results

Lymph Node Location, Needle Gauge, Number of Passes, and Ancillary Studies

The number of lymph node CNBs increased with time, from 14 cases in 2003 to a stable number of approximately 45 or 50 cases per year since 2006. It has become customary at Washington University Medical Center for adult patients to be scheduled for a CNB as a first approach of tissue retrieval because the waiting time for an appointment is much shorter than for an excisional biopsy. Pediatric patients usually undergo an excisional lymph node biopsy. While the number of excisional biopsies and CNBs increased steadily over the years, the percentage of CNBs of overall node biopsies increased from about 28% in 2003 to 70% in 2007 and 61% in 2009. The anatomic locations of biopsied lymph nodes are summarized in **Table 1**. The most common sites included the cervical/clavicular area (28.1%), axilla (19.4%), and groin (18.6%). The majority of CNBs and FNAs were performed by radiologists (~95%) with the remaining 5% performed by surgeons or endoscopists. An 18-gauge needle was used in 62.1% of cases to obtain tissue, followed by a 20-gauge needle in 27.9% of cases. The greatest number of passes obtained per case was 3 (31.5%), followed by 4 passes (23.5%) and 2 passes (21.3%). In only 41.7% of cases were 4 or more passes performed. A portion of these cores obtained by CNB was sent to flow cytometry in 11 of the 263 cases, of which only 7 were adequate.

Table 1
Location of 263 Biopsied Lymph Nodes

Anatomic Location	No. (%)
Superficial lymph nodes	
Cervical and clavicular region	74 (28.1)
Axilla	51 (19.4)
Groin	49 (18.6)
Deep-seated lymph nodes	
Thorax	10 (3.8)
Abdomen	32 (12.2)
Retroperitoneum	34 (12.9)
Pelvis	13 (4.9)

Table 2
Decisive Diagnoses of 193 Core-Needle Biopsies

Conclusive Diagnosis	No. (%)	No. (%) of New Cases*
Non-Hodgkin B-cell	111 (57.5)	76 (68.5)
DLBCL	53 (27.5)	42 (79)
Follicular	36 (18.7)	19 (53)
SLL/CLL	10 (5.2)	5 (50)
Burkitt	6 (3.1)	5 (83)
Marginal	3 (1.6)	2 (67)
Mantle	3 (1.6)	3 (100)
Benign	62 (32.1)	62 (100)
Hodgkin	16 (8.3)	9 (56)
T-cell lymphoma	4 (2.1)	1 (25)

DLBCL, diffuse large B-cell lymphoma; SLL/CLL, small lymphocytic lymphoma/chronic lymphocytic leukemia.
* Percentages are based on the number of cases in the preceding column. Of the 131 decisive lymphoma diagnoses, 86 represented a new diagnosis and 45 demonstrated a recurrence.

CNB Diagnoses With or Without Ancillary Studies

Overall, a diagnosis was made in 237 cases (90.1%). In 193 (81.4%) of these 237 cases, the diagnosis was decisive and was incomplete in 44. Cases with decisive diagnoses were benign in 62 cases (32.1%) and included reactive hyperplasia or various types of lymphadenitis. The remaining 131 cases (67.8%) revealed a lymphoma that was fully subclassified according to WHO criteria **Table 2**. Most of the cases represented diffuse large B-cell lymphoma, 53 cases (27.5%), and follicular lymphoma, 36 cases (18.7%). Hodgkin lymphoma was diagnosed in 8.3% of cases. Of all lymphoma diagnoses, 75% were fully subclassified **Table 3**. In 26 cases (9.9%), a diagnosis could not be established because the specimens were too small for meaningful analysis or because findings were equivocal for confirming or ruling out lymphoma.

A diagnosis could be obtained in 152 (86.4%) of 176 cases in which the CNB specimen had been obtained from superficial lymph nodes compared with 83 (95%) of 87 of the specimens obtained from deeply seated nodes, which was statistically significant (P = .025). Decisive diagnoses were possible in 127 (83.6%) superficial and 64 (77%) of

Table 3
Diagnoses Made With Corresponding Flow Cytometric, Immunohistochemical, Genetics, and Excisional Biopsy Studies

	Flow Cytometry (n = 137)	Immunohistochemical Analysis (n = 176)	Genetics (n = 20)	Excisional Biopsy (n = 55)
Diagnosis (n = 237)	134	168	20	38
Decisive (n = 193)	111	134	20	23
Lymphoma (n = 131)	76	101	20	14
Benign (n = 62)	35	33	0	9
Incomplete (n = 44)	23	34	0	15
Nondiagnostic (n = 26)	3	8	0	17

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deep nodes (P = .28). The difference was not statistically significant.

Adequate tissue for immunohistochemical and/or flow cytometric studies was obtained in 176 (66.9%) and 137 (52.1%) of cases, respectively (Table 3). The proportion of ancillary studies that had been performed in cases with complete diagnostic subclassification (177/193 [91.7%]) was similar to that of cases with an incomplete diagnosis (41/44 [93.2%]).

Of 131 cases with a decisive lymphoma diagnosis, 76 (58.0%) had adequate material for flow cytometry, compared with 23 (52%) of 44 cases with incomplete lymphoma diagnoses. This difference was not significant (P = .54). Similarly, of 131 cases with a decisive lymphoma diagnosis, 101 (77.1%) underwent immunohistochemical studies, compared with 34 (77%) of 44 cases with incomplete diagnoses.

Genetic testing was performed on 20 CNB samples (7.6%), including 14 FISH studies and 6 polymerase chain reaction studies for *IGH*, *TCR*, or *BCL2* rearrangements. Of the 14 FISH cases, 13 were assessed for a c-*myc* translocation, and 1 case was assessed for t(11;14). With these ancillary studies, a definitive diagnosis could be obtained in all cases.

Decisive and Incomplete Lymphoma Diagnoses

Of the 131 decisive lymphoma diagnoses, 86 represented a new diagnosis and 45 a recurrence (Table 2). Despite a decisive diagnosis, 14 patients underwent a subsequent excisional biopsy. The most common reasons for this management step were a policy by the hematologist-oncologist to only treat patients based on a diagnosis derived from an excisional biopsy or to seek confirmation of the diagnosis, the latter mostly requested by general internists. For the remaining 117 cases, the given diagnosis was sufficient to initiate treatment.

An incomplete lymphoma diagnosis was given in 44 (16.7%) of 263 cases. The majority of these were related to a lymphoma diagnosis, with the majority of these representing a diagnosis of B-cell lymphoma with no further subclassification. In 6 cases, there was a large cell component, and the foremost diagnostic difficulty was related to distinguishing between de novo diffuse large B-cell lymphoma and a grade 3 follicular lymphoma or transformed large B-cell lymphoma from follicular lymphoma. Examples of a decisive diagnosis and an incomplete diagnosis are shown in **Image 1** and **Image 2**, respectively.

Excisional Biopsy

An excisional biopsy was performed in 55 cases after the initial CNB. In 32 cases, the indication was insufficient tissue sampling for diagnosis or equivocal results regarding the malignant or benign nature of the lymph node. In 23 cases, an excisional biopsy was performed despite a decisive diagnosis from the CNB. As mentioned, 14 cases represented fully classified lymphoma cases. The most common reason for performing an excisional biopsy in the 9 benign cases was a discrepancy between the more aggressive clinical manifestations and benign histologic findings. Of the 44 cases with an incomplete diagnosis, 15 had a subsequent excisional biopsy with a decisive diagnosis **Table 4**. The initial CNBs in these 15 cases were all from superficial lymph nodes and were easily accessible for excisional biopsy (Table 4). Of the 29 incomplete cases in which a subsequent excisional biopsy was not done, 21 (72%) had deep-seated pathologic lymph nodes. Follow-up for these cases is provided in **Table 5**. Of the cases in the nondiagnostic group, 17 had a subsequent excisional biopsy for further assessment, and a decisive diagnosis was made for all following this procedure.

In 37 of 38 cases in which excisional biopsy specimens were available as the reference for comparison, the diagnoses were concordant with those of the combination of CNB and FNA and/or flow cytometry. There were no false-positives, and the single discordant case represented a false-negative case in which an initially diagnosed reactive lymphoid infiltrate turned out to be classical Hodgkin lymphoma on excisional biopsy. According to these results, needle biopsy with or without flow cytometry had a sensitivity of 96.5%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 90%.

Clinical and Histologic Follow-up of Cases With Decisive Diagnoses

We were able to retrieve clinical and histologic follow-up in 111 of 117 decisive lymphoma cases and in 41 of 53 decisive benign cases that had not an excisional biopsy for diagnosis confirmation. In the lymphoma group, 45 (40.5%) of 111 cases had a previously documented diagnosis of lymphoma and now had a recurrence or transformation. Of 111 cases, 18 (16.2%) revealed concurrent lymphomatous involvement in other organs, such as peripheral blood or bone marrow. In 14 cases (12.6%), a subsequent recurrence of the same type of lymphoma or transformation was documented. In 17 cases (15.3%), clinical follow-up revealed remission. All of these cases were diffuse large B-cell lymphomas. In 6 cases (5.4%), a complete diagnosis was obtained through decisive ancillary studies, such as cyclin D1 or ALK-1 immunohistochemical analysis or c-*myc* gene rearrangements by in situ hybridization. Of the 111 patients, 7 (6.3%) died of disease, 1 showed radiologically stable lymphadenopathy, and 3 had radiologic progression. In the benign diagnosis cases that had available follow-up, 35 did not receive a subsequent diagnosis of lymphoma; in 5 cases with a distant history of lymphoma, the patients were still lymphoma-free; and 1 patient was diagnosed with subsequent classical Hodgkin lymphoma a year later.

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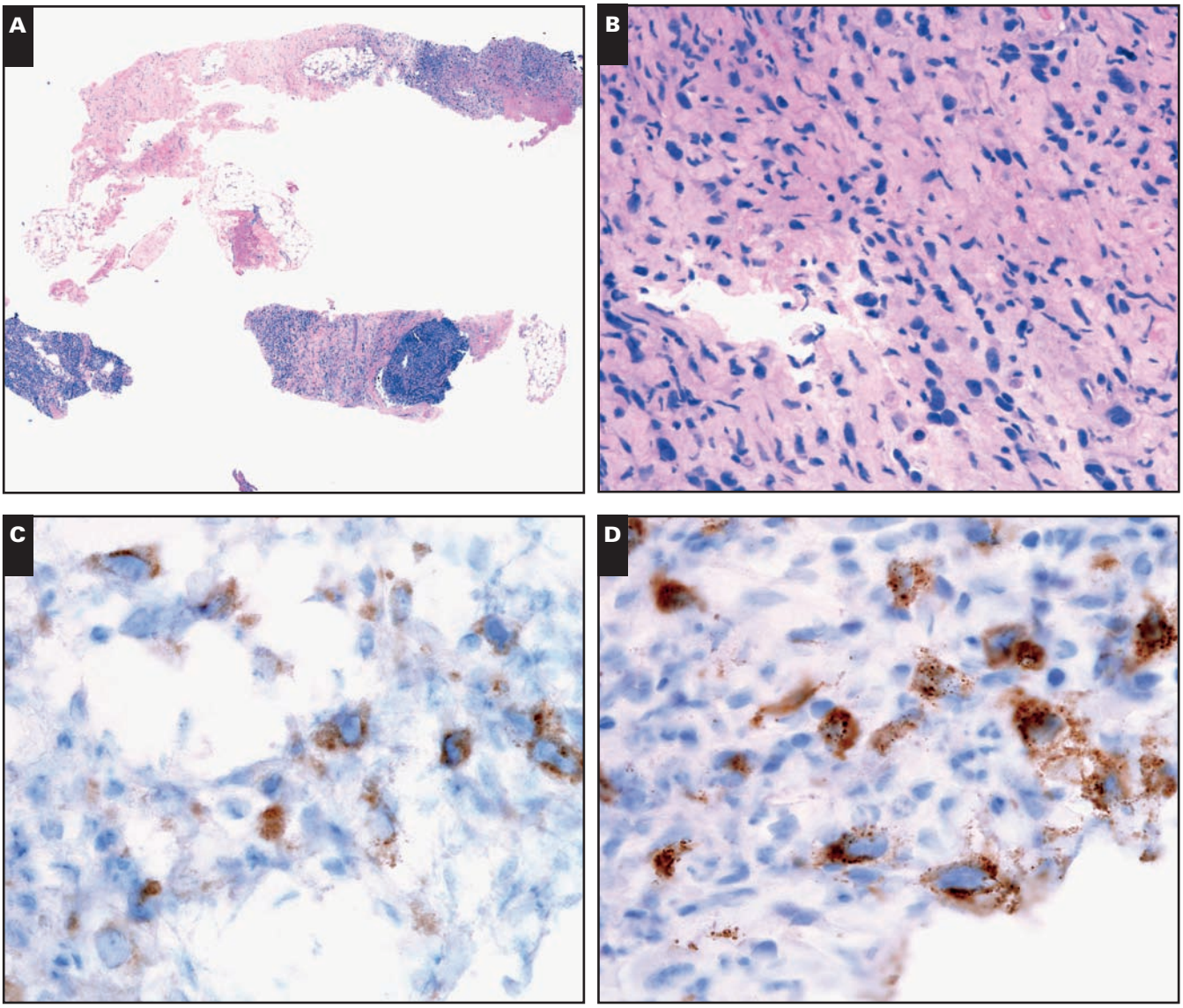

Image 1 **A**, Low-power image of a core needle biopsy specimen obtained from an enlarged left supraclavicular lymph node in a 26-year-old man. The patient also had an anterior mediastinal mass. The tissue is composed of fibroadipose and lymphoid tissue with fibrosis imparting some nodularity (H&E, ×40). **B**, Higher power view of lesional tissue revealing several large, neoplastic, irregular shaped cells, some with a nucleolus, embedded in fibrosis and surrounded by unremarkable small lymphocytes (H&E, ×500). **C**, The immunohistochemical stain for CD30 highlights the large neoplastic cells (×1,000). **D**, The immunohistochemical stain for CD15 also highlights the large cells (×1,000). CD20, anaplastic lymphoma kinase-1 (ALK-1), and placental-like alkaline phosphatase were negative in malignant cells, and a decisive diagnosis of classical Hodgkin lymphoma was made in this case.

Discussion

With 263 cases, our study represents the largest to investigate the diagnostic accuracy of lymph node CNB and/or FNA in association with ancillary studies, namely flow cytometry, immunohistochemical analysis, and genetics, in patients with suspected lymphoma. Of special interest was to find out if tissue retrieval for flow cytometry compromised tissue retrieval for morphologic review.

Our results indicate that CNB and FNA in association with ancillary studies were able to provide a diagnosis in about 90% of cases. However, of all the lymphoma cases diagnosed (n = 175), a specific lymphoma classification was possible in only about 75%, which appears lower than in the majority of studies published reporting an overall diagnostic success rate at about 80%.[4,6,9,10] A few studies report an even higher success rate, between 88% and 98%.[11-13] The reason for the lower diagnostic accuracy in our study may be the smaller

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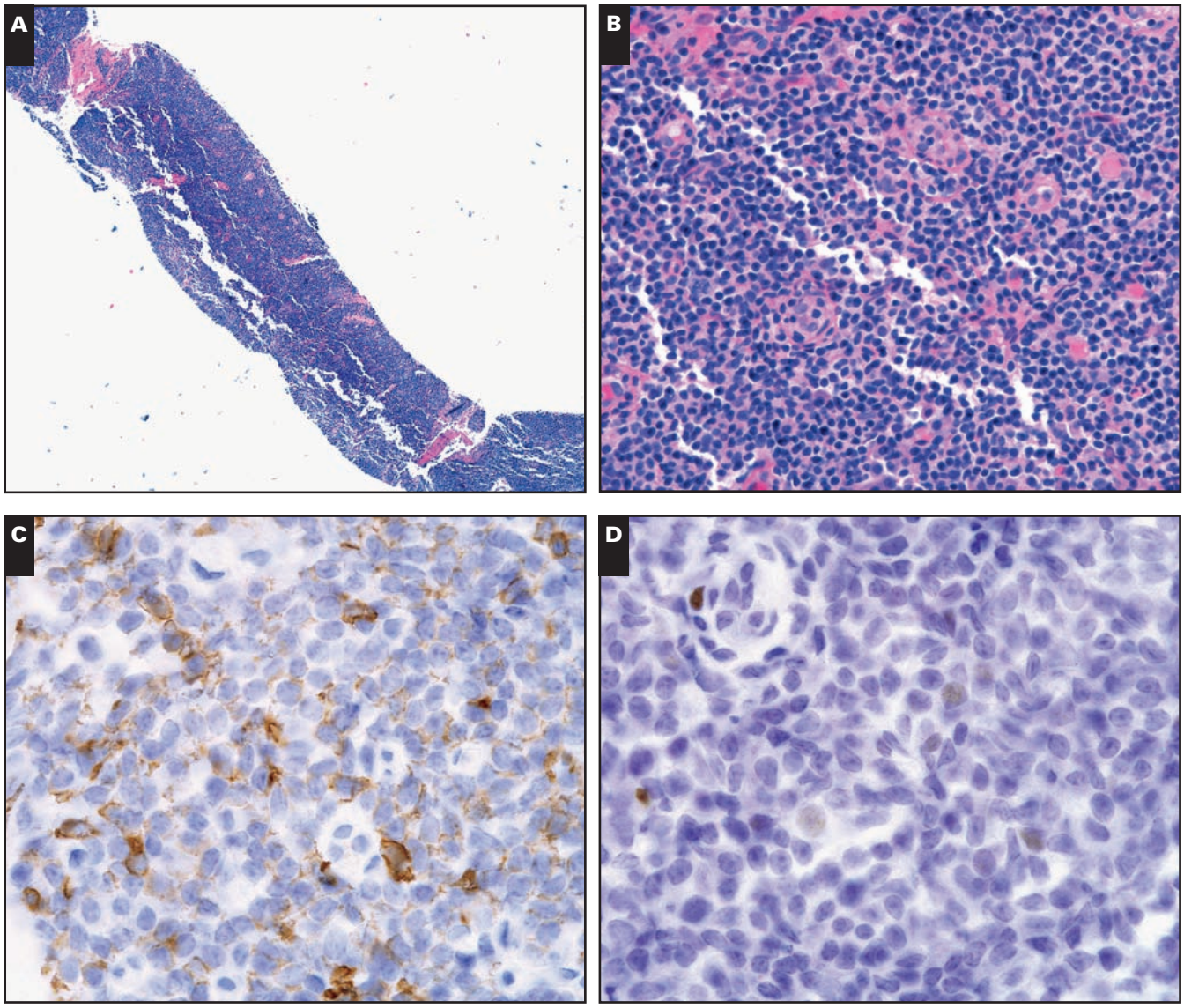

Image 2 **A**, Low-power image of a core needle biopsy specimen obtained from a right inguinal lymph node in a 60-year-old woman. The core reveals dense lymphoid tissue (H&E, ×40). **B**, Higher power view of the lymphoid infiltrate composed of small, round lymphoid cells (H&E, ×500). Corresponding flow cytometry of a concurrent fine-needle aspiration sample revealed a clonal κ immunoglobulin light chain–restricted B-cell population (not shown). Not enough cells were present for full flow cytometric immunophenotyping. **C**, The immunohistochemical stain for CD5 strongly highlights background T lymphocytes and weakly highlights malignant B cells (×1,000). **D**, The immunohistochemical stain for cyclin D1 is negative (×1,000). Based on cytology of malignant lymphoid cells, a diagnosis of small lymphocytic lymphoma was favored. The patient subsequently underwent a bone marrow study that confirmed this diagnosis.

core gauge used and a lesser number of cores submitted to our hematopathology service for morphologic analysis.

While nearly 60% of CNB samples submitted to us consisted of 3 or fewer cylinders and, moreover, only about 65% were obtained with an 18-gauge or larger gauge needle, all of the reported studies reporting higher diagnostic accuracy consistently evaluated 4 to 5 cores, obtained by 18-gauge or larger gauge needles. Demharter et al,[11] for example, were able to subclassify 94% of 64 diagnosed lymphoma cases by evaluating CNBs of 5 cylinders, obtained with a 14-gauge needle. This yielded sufficient tissue for the routine application of 22 immunohistochemical stains, *IGH* and *TCR* polymerase chain reaction studies, and FISH for common lymphoma translocations. Moreover, flow cytometry was not performed to aid in diagnosis. de Larrinoa et al[12] were able to achieve an 88% diagnostic accuracy for their 102 CNB-diagnosed lymphomas. Full subclassification was possible in all of these cases. However, their series included

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Table 4
Characteristics and Other Testing of CNB Specimens With Incomplete Diagnoses in Which Subsequent Excisional Biopsies Were Done

Case No./ Age (y)	LN Location	No. of Cores	CNB Diagnosis	FNA	FC	IHC	Genetics	Excisional Biopsy Diagnosis
1/49	Superficial	2	Suspect Hodgkin	Yes	No	Yes	No	Hodgkin
2/47	Superficial	2	Suspect B-NHL	Yes	Attempted	Yes	No	DLBCL
3/27	Superficial	3	Suspect Hodgkin	Yes	Yes	Attempted	No	Hodgkin
4/53	Superficial	2	B-NHL	Yes	Yes	Yes	No	Follicular
5/19	Superficial	2	Suspect Hodgkin	Yes	No	Yes	No	Hodgkin
6/53	Superficial	2	B-NHL	Yes	Yes	No	No	Marginal
7/24	Superficial	Minute	Suspect Hodgkin	Yes	No	No	No	Hodgkin
8/64	Superficial	Several	Suspect B-NHL	Yes	Attempted	No	No	Follicular
9/29	Superficial	Several	Suspect NHL	Yes	No	Yes	No	Hodgkin
10/69	Superficial	5	Suspect Hodgkin	Yes	Yes	Yes	No	Hodgkin
11/53	Superficial	Minute	Suspect DLBCL	Yes	Yes	Yes	No	DLBCL
12/55	Superficial	2	Suspect Hodgkin	No	No	Yes	No	Hodgkin
13/35	Superficial	3	Lymphoma	Yes	No	Yes	No	Hodgkin
14/58	Superficial	2	B-NHL	Yes	No	Yes	No	Marginal
15/69	Superficial	—	B-NHL	Yes	Yes	No	No	Follicular

CNB, core needle biopsy; DLBCL, diffuse large B-cell lymphoma; FC, flow cytometry; FNA, fine-needle aspiration; IHC, immunohistochemical analysis; LN, lymph node; NHL, non-Hodgkin lymphoma.

Table 5
Follow-up in Cases With an Incomplete CNB Diagnosis in Which Subsequent Excisional Biopsy Was Not Done

Case No./ Age (y)	LN Location	No. of Cores	CNB Diagnosis	FNA Performed	FC Performed	IHC Performed	Follow-up
1/51	Deep	2	PCN vs B-NHL	Yes	No	Yes	Multiple biopsies before diagnosis of DLBCL
2/60	Superficial	1	Suspect SLL	Yes	Yes	Yes	Final diagnosis of CLL on PB
3/79	Superficial	Multiple	Suspect DLBCL	Yes	Yes	Yes	NA
4/63	Superficial	2	CD10+ B-NHL	Yes	Yes	Yes	Final diagnosis of follicular on BM
5/64	Deep	NA	B-NHL	No	No	Yes	Treated based on biopsy; no recurrence
6/88	Deep	2	Suspect B-NHL	Yes	No	Yes	NA
7/63	Superficial	6	Low-grade B-NHL	Yes	Yes	Yes	Refused excisional biopsy; observed
8/74	Deep	2	Low-grade B-NHL	Yes	Yes	No	Unstable condition for excisional biopsy
9/23	Deep	2	Suspect recurrent Hodgkin	Yes	No	Yes	History of Hodgkin, treated
10/83	Deep	1	Suspect Hodgkin	No	No	Yes	NA
11/75	Deep	2	Low-grade B-NHL	Yes	Yes	Yes	Diagnosis of MZL on PB
12/73	Superficial	Minute	B-NHL	Yes	Yes	Yes	NA
13/85	Deep	2	B-NHL	Yes	Yes	Yes	NA
14/50	Deep	Multiple	B-NHL	No	No	No	Diagnosis of low-grade follicular on BM
15/24	Superficial	Multiple	Suspect recurrent Hodgkin	No	No	Yes	Treated owing to clinical progression
16/72	Deep	NA	Suspect Hodgkin	No	Yes	Yes	Observed; concurrent carcinoma diagnosis
17/56	Superficial	Minute	B-NHL	Yes	Yes	No	Unstable condition for excisional biopsy; treated
18/63	Deep	Multiple	Favor LBCL	Yes	Yes	Yes	Unstable condition for excisional biopsy; treated
19/56	Deep	3	B-NHL	Yes	Yes	Yes	Unstable condition for excisional biopsy; treated
20/82	Deep	2	Favor LBCL	Yes	Yes	Yes	Unstable condition for excisional biopsy; treated
21/59	Deep	Multiple	Favor LBCL	Yes	Yes	No	Treated
22/48	Deep	Minute	B-NHL	Yes	Yes	No	Diagnosis of MZL on BM
23/67	Deep	NA	B-NHL	No	Yes	Yes	Observed
24/84	Superficial	Multiple	Suspect Hodgkin	Yes	Yes	Yes	Unstable condition for excisional biopsy; not treated
25/43	Deep	Multiple	Recurrent B-NHL	Yes	Yes	No	Observed
26/63	Deep	3	Worrisome for Hodgkin	No	No	Yes	Observed; unlikely Hodgkin lymphoma
27/74	Deep	NA	Favor LBCL	No	Yes	Yes	Treated
28/36	Deep	2	Suspect recurrent Hodgkin	Yes	No	Yes	Had excisional biopsy with recurrence 1 mo before this CNB
29/79	Deep	Minute	Malignant lymphoma	Yes	Yes	Yes	Recent history of DLBCL

BM, bone marrow; CLL, chronic lymphocytic leukemia; CNB, core needle biopsy; FC, flow cytometry; FNA, fine-needle aspiration; IHC, immunohistochemical analysis; LBCL, large B-cell lymphoma; LN, lymph node; MZL, marginal B-cell lymphoma; NA, not available; NHL, non-Hodgkin lymphoma; PB, peripheral blood; PCN, plasma cell neoplasm; SLL, small lymphocytic lymphoma.

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8 false-negative CNB interpretations that were shown to represent lymphomas on subsequent excisional biopsies. This study used 18-gauge needles for tissue retrieval, and 4 to 5 cores were usually obtained for morphologic review. Again, no flow cytometric immunophenotyping was performed on these cases.

Although flow cytometry from corresponding FNA specimens generally aided in establishing a lymphoma diagnosis in our study, it did not add value to subclassification of a lymphoma because about 52% of cases with an incomplete diagnosis had adequate full immunophenotyping vs about 57% of cases with a decisive diagnosis ($P = .54$). Therefore, it appears that the architectural pattern remains instrumental in complete subclassification. Lack of an architectural pattern owing to small CNB biopsy specimens posed the biggest challenge for our hematopathologists in addressing subclassification of a suspected lymphoma, grading a follicular lymphoma, or differentiating between a grade 3 follicular lymphoma or diffuse large B-cell lymphoma. This finding emphasizes how important it is, despite successful immunophenotyping by flow cytometry, to obtain enough tissue for morphologic confirmation. The importance of not compromising architectural detail by increasingly relying on flow cytometry obtained from FNA is further supported by Hehn et al,[14] who quote the success rate of obtaining a complete histologic diagnosis on only FNA samples using the WHO classification at 29% of their 93 cases investigated. Of the 93 cases, 67 (72%) had a subsequent excisional biopsy, and among the paired comparisons, only 12% were correlated with the excisional biopsy diagnosis. Immunophenotypic analysis was completed in 24 of the 67 paired FNA cases, and only 29% were correlated with subsequent histologic findings on excisional biopsy. We did not assess the diagnostic accuracy of FNA on its own because that was not the scope of our study; however, FNA had a pivotal role in making tissue available for flow cytometry.

The studies by Siebert et al[15] and Lachar et al[8] are similar to our study in that they investigated the usefulness of CNB and/or FNA in combination with the ancillary studies of flow cytometry, immunohistochemical analysis, and genetics in the diagnosis of lymphoma. Of the 60 cases reported by Siebert et al,[15] 38 represented lymphoma diagnoses, of which about 18% were not further subclassified. Flow cytometry was necessary in 20 of the 38 cases, useful in 14 cases, not useful in 2 cases, and misleading in 2 cases. Lachar et al[8] investigated 101 cases, of which a decisive lymphoma diagnosis was established in about 75% of cases. In about 87% of the 101 cases, ancillary studies were performed, including immunohistochemical analysis in 79% and flow cytometry in 32% of cases. Flow cytometry was contributory to the diagnosis in 78% of these cases. Genetic analysis was performed in 9% of all cases and was contributory in 67% of these. Not only did the diagnostic accuracy of 75% in this study correspond to ours but the method of obtaining specimens resembled ours: generally 2 to 4 passes were obtained with mostly 18-gauge needles for morphologic evaluation, after FNA passes were obtained for flow cytometry.

Genetic analysis did not represent a major ancillary tool in the lymphoma workup of a CNB or FNA specimen. Our results indicate that FISH analysis for a c-*myc* gene rearrangement was the most commonly used genetic testing because it can easily assess for the possibility of a Burkitt lymphoma, which requires a treatment regimen different from that for diffuse large B-cell lymphoma. FISH was performed on paraffin-embedded tissue samples and was able to provide reliable results on all requested cases.

Optimization of the diagnosis of a suspected lymphoma by combined CNB and FNA is heavily dependent on a coordinated approach among radiologists, pathologists, and oncologist-hematologists. In addition, optimal results require experienced radiologists and pathologists. Cores should be ample and preferably obtained with a larger gauge needle to allow for architectural interpretation and immunohistochemical studies, if needed. Much of the literature reviewed herein recommends 4 or 5 cores with 14- to 18-gauge needles for morphologic review.[4,6,9,10,16,17] Uniform guidelines for optimal tissue retrieval should be in place among the different clinical departments within the same institution performing the CNB for lymphoma workup. Multiple passes of FNAs may be necessary to yield sufficient material for flow cytometry.

Cytologic evaluation of cell size and shape of lesional cells in the FNA specimen should always be integrated into the overall case interpretation. Immunophenotyping by flow cytometry on FNA samples should not replace morphologic analysis and should be regarded as an adjunct to adequate tissue morphologic analysis. Tissue collection for flow cytometry and FNA evaluation should certainly not come at the expense of adequate core tissue for morphologic analysis because previous studies have demonstrated the superiority of CNB compared with FNA.[14,18] Pathologists should make every effort to adhere to the current WHO classification when making a diagnosis and explain the limitations for not being able to determine a decisive diagnosis. Experienced oncologist-hematologists may still opt to treat patients despite an incomplete diagnosis owing to clinical manifestations. This approach occurred in 60% of cases with an incomplete diagnosis.

We have noticed that CNBs are no longer preferably used for deep-seated lymph nodes in patients who are in poor clinical condition or in any situation that prevents an open surgical biopsy. In fact, our study shows that two thirds of the CNB samples were obtained from superficial sites. It is clear that the trend is moving toward the most cost-effective and safe method of obtaining lymph node tissue for diagnosis, which

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makes close collaboration among radiologists, pathologists, and clinicians even more important.

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