Diagnosis of Deep-Seated Lymphoma and Leukemia by Endoscopic Ultrasound–Guided Fine-Needle Aspiration Biopsy

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Abstract

We retrospectively studied the use of endoscopic ultrasound–guided fine-needle aspiration biopsy (EUS-FNAB) as a tool for the diagnosis of deep-seated lymphoma. An on-site assessment at the time of EUS-FNAB was performed by a cytopathologist using Diff-Quik (American Scientific Products, McGraw Park, IL) stain. In addition, Papanicolaou stains were performed on EUS-FNAB smears, immunohistochemical stains were performed on cell blocks, and additional samples were sent for flow cytometric analysis. Final cytologic diagnosis was correlated with surgical pathology and/or clinical follow-up. We evaluated EUS-FNAB specimens of deep-seated lymph nodes, spleen, stomach, and pancreas, and 1 EUS-guided needle core biopsy specimen of a lymph node. Thirteen cases of deep-seated lymphoma were diagnosed, including non-Hodgkin lymphomas and Hodgkin lymphoma. One case of hairy cell leukemia was diagnosed. EUS-FNAB is a minimally invasive, cost-effective, and useful tool for the primary diagnosis or staging of deep-seated lymphomas.

Traditionally, the accepted "gold standard" for the diagnosis of non-Hodgkin lymphoma (NHL) has been based on histomorphologic and immunohistochemical features and flow cytometric immunophenotyping of tissue samples obtained by open surgical biopsy. More recently, fine-needle aspiration biopsy (FNAB) combined with flow cytometry has had an important role in the diagnosis of NHL.[1-6] FNAB has been used extensively to evaluate palpable lymphadenopathy. Although architectural details are lost, routine cytomorphologic studies, coupled with immunohistochemical, flow cytometric, cytogenetic, and molecular studies, permits the diagnosis of lymphoma.[1-8] The evaluation of deep-seated lymphadenopathy can be technically challenging. Computed tomographic–guided fine-needle aspiration has been the tool used to investigate deep-seated lymphadenopathy. Recently, the introduction of endoscopic ultrasound-guided (EUS) FNAB has allowed sampling of deep tissues, including deep-seated lymph nodes.[6,8,9] EUS-FNAB has been demonstrated to be a highly sensitive and specific tool in the diagnosis of metastatic epithelial neoplasms in deep-seated lymph nodes.[8-11] We studied EUS-FNAB as a tool in the diagnosis of deep-seated lymphoma.

Materials and Methods

We retrospectively evaluated 1,261 sequential EUS-FNAB specimens obtained at our institution from August 2000 to September 2004. EUS-FNAB was performed with a curvilinear echoendoscope (UC-30P, Olympus, Mellville, NY). All EUS-FNAB procedures were performed using a 22-gauge needle (Wilson-Cook, Winston Salem, NC). The aspirated sample

was placed on glass slides, and air-dried and alcohol-fixed smears were prepared. Air-dried smears were stained with Diff-Quik (American Scientific Products, McGraw Park, IL). Immediate review of the Diff-Quik–stained smears was performed by a cytopathologist in the endoscopy suite to ensure specimen adequacy and to make a preliminary diagnosis. Alcohol-fixed smears were later stained with Papanicolaou stain. Aspirated material was placed in 10 mL of Cytolyt preservative solution (Cytyc, Boxborough, MA), and from this suspension, ThinPrep (Cytyc) slides were prepared. A cell block was prepared using the fibrin clot method, fixed in formalin, and embedded in paraffin. Next, 5-µm-thick sections were stained with H&E. Immunohistochemical stains were performed on sections from the cell block. Aspirated material also was placed in 10 mL of RPMI medium for 4-color flow cytometry (Becton Dickinson FACSort, San Jose, CA). Final diagnoses were correlated with histologic examination of surgical specimens and/or clinical follow-up.

Table 1
Endoscopic Ultrasound-Guided Fine-Needle Aspiration Biopsies Performed at the University of Alabama at Birmingham, August 2000 to September 2004

Site	No.
Pancreas	564
Deep-seated lymph nodes	385
Thoracic	
Aortopulmonary window	27
Celiac	66
Mediastinal	13
Periesophageal	39
Paratracheal	7
Subcarinal	124
Intra-abdominal	
Gastrohepatic	1
Periduodenal	7
Periductal	6
Perigastric	16
Perihepatic	15
Peripancreatic	64
Other sites	312
Total	1,261

Table 2
Sites of 14 Deep-Seated Lymphoma/Leukemia Cases

Site	No. of Cases
Deep-seated lymph node	9
Celiac	3
Gastrohepatic	1
Mediastinal	1
Paraesophageal	1
Peripancreatic	1
Subcarinal	2
Extranodal	5
Pancreas	2
Spleen	2
Stomach	1

Results

Demographics of Deep-Seated Lymphoma/Leukemia Cases

A total of 1,261 EUS-FNABs were performed at our institution between August 2000 and September 2004. Of these, 564 were from the pancreas, 385 from deep-seated lymph nodes, and 312 from other sites (including gastrointestinal tract, hepatobiliary tree, adrenal gland, kidney, spleen, and lung) **Table 1**. From the 385 lymph nodes, 13 cases of lymphoma (3.4%) and 1 case of leukemia (0.3%) were diagnosed. Of 385 cases, 9 (2.3%) involved lymph nodes; 5 (0.6%) of 876 cases were extranodal. The extranodal cases involved spleen (2 cases), pancreas (2 cases), and stomach (1 case) **Table 2**.

Of the 13 cases of deep-seated lymphoma, 10 were primary diagnoses and 3 cases represented recurrent disease. A case of hairy cell leukemia was a recurrent diagnosis made on an aspirate from a gastrohepatic lymph node. Of the 13 patients with deep-seated lymphoma, 7 were men and 6 were women with an age range of 25 to 81 years (mean age, 59.0 years). The symptoms indicating the need for EUS-FNAB included abdominal pain (9 patients); weight loss (3 patients); mediastinal, splenic, pancreatic, or stomach masses shown by imaging studies (11 patients); dysphagia (1 patient); and fever (1 patient diagnosed with hairy cell leukemia). Recurrent lymphoma or leukemia was suspected in the 3 patients with a history of lymphoma and in the patient with a history of hairy cell leukemia **Table 3**.

EUS-FNAB Diagnosis of Deep-Seated Lymphoma

Of the 13 cases of deep-seated lymphoma, 12 were diagnosed as NHL and 1 as Hodgkin lymphoma **Table 4**. By using cytomorphologic studies in combination with flow cytometry on aspirated samples or immunohistochemical

Table 3
Deep-Seated Lymphoma: Patient Demographics

	No. of Patients*
Sex	
Male	7
Female	6
Age (y)	
Range	25-81
Mean	59.0
Symptoms	
Pain	9
Weight loss	3
Masses shown by imaging studies	11
Dysphagia	1
Suspected lymphoma†	3

* Unless otherwise indicated. The case of hairy cell leukemia is not included in the table.
† Lymphoma suspected in patients with a history of hematopathologic disease.

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analysis performed on cell blocks, 10 of 12 cases of deep-seated lymphoma were diagnosed as NHL. In 2 of the 12 cases, deep-seated lymphoma was not diagnosed by these methods owing to the inability to evaluate the aspirate samples by flow cytometry. In one case, an insufficient number of viable cells precluded the diagnosis, and in the second case, the aspirate sample was unavailable for evaluation. However, evaluation of tissue specimens from these 2 patients later confirmed NHL. Of the 12 cases of NHL, 6 were diagnosed as large B-cell lymphoma **Image 1**, 3 as follicular center cell lymphoma **Image 2**, and 1 as extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALToma) of

Table 4
Diagnoses in 13 Cases of Deep-Seated Lymphoma

Diagnosis	No. of Cases
Non-Hodgkin lymphoma	12
Large B-cell lymphoma	7
Follicular center cell lymphoma	3
Reactive process	1
Malignant lymphoproliferative process	1
Hodgkin lymphoma	1

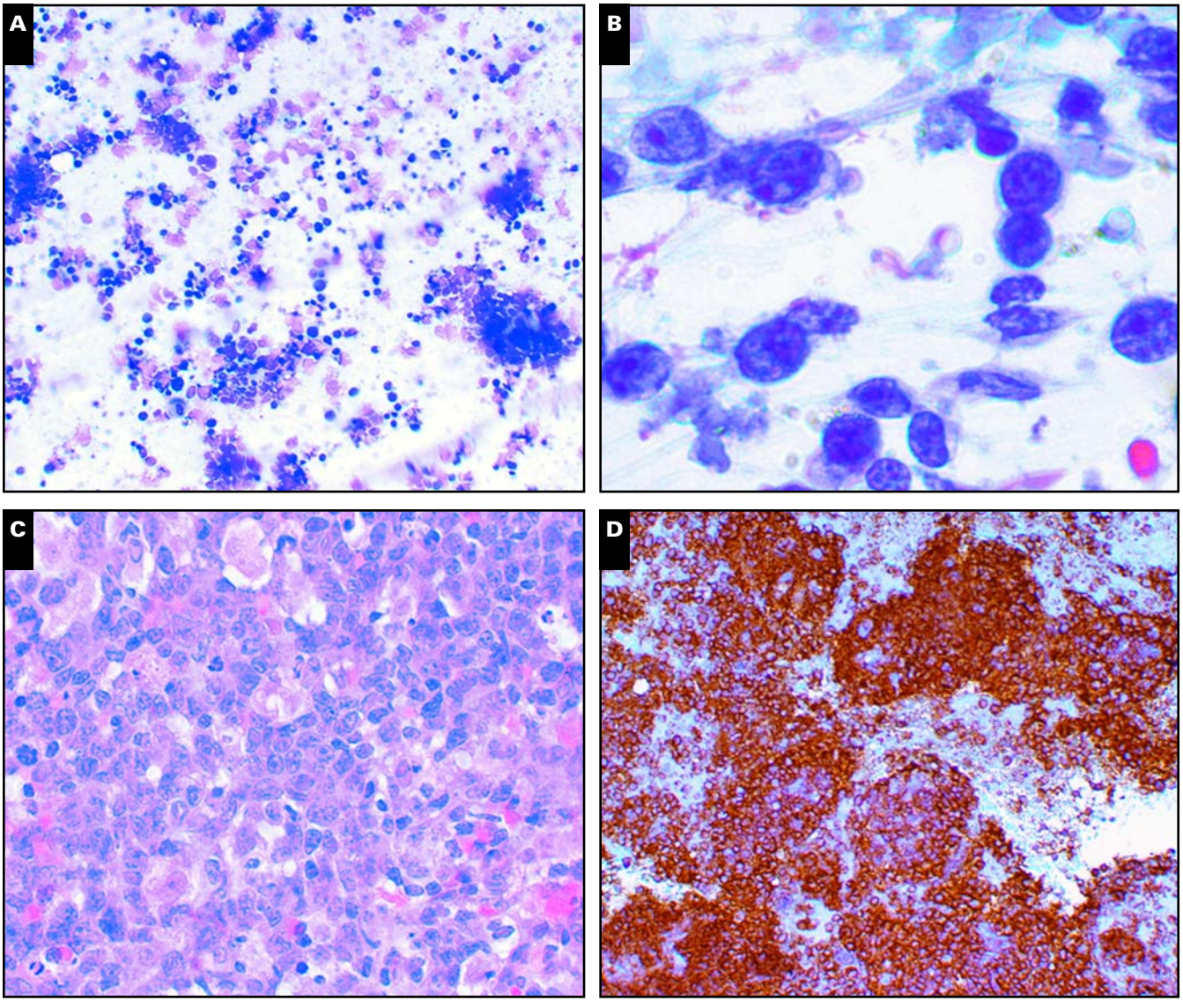

Image 1 Large B-cell lymphoma. **A**, Aspirate smear from enlarged celiac node showing a moderately cellular smear with groups of large cells and many single large cells in the background (Diff-Quik, ×400). **B**, Aspirate smear showing large cells with prominent nucleoli (Papanicolaou, ×1,000). **C**, Cell-block preparation from fine-needle aspirated cells of celiac node (H&E, ×400). **D**, Immunoperoxidase stain for B-cell marker (CD20) shows diffuse positivity in the cell-block preparation of the fine-needle aspirate (×400).

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the stomach. One case of deep-seated lymphoma was diagnosed as Hodgkin lymphoma.

In 8 of 12 cases of NHL, flow cytometry was performed on the EUS-FNAB sample. In 4 cases, immunohistochemical studies were performed on cell blocks. In 10 of the 12 cases, deep-seated lymphoma was diagnosed correctly by EUS-FNAB with ancillary studies (flow cytometry or immunohistochemical analysis). In 2 of 12 cases, deep-seated lymphoma was not diagnosed by these methods.

In 1 of these cases, the patient had a splenic mass noted on imaging studies. EUS-FNAB showed a mixed population of lymphocytes in a bloody background. Flow cytometry was performed on the aspirate, and no clonal population was detected. However, fewer than 100,000 cells were viable, precluding accurate flow cytometric analysis. The EUS-FNAB diagnosis was reactive process, and the surgical pathology diagnosis on the splenectomy specimen was high-grade large B-cell lymphoma. An EUS-FNAB diagnosis of lymphoma

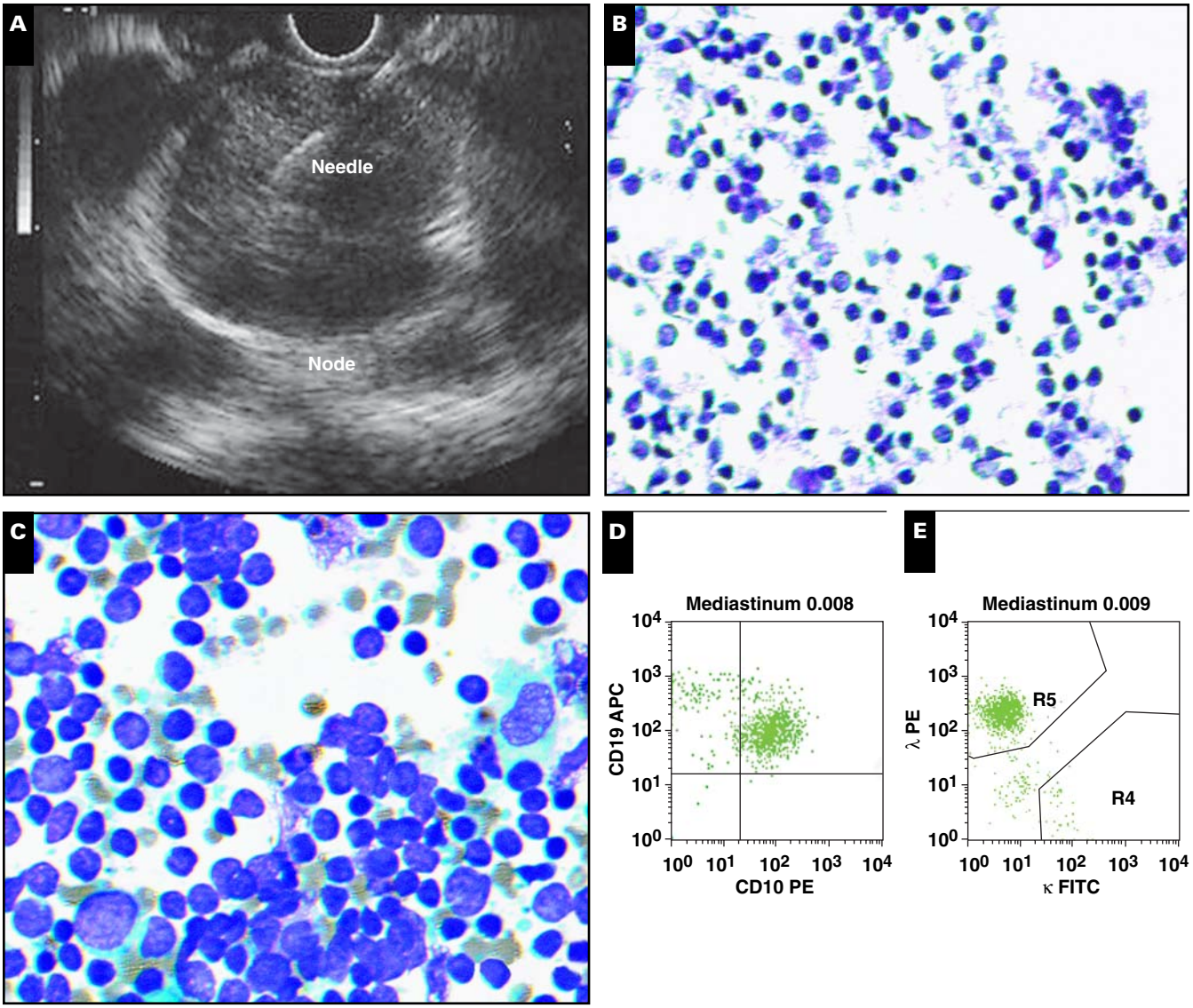

Image 2 Follicular center cell lymphoma. **A**, Real-time, ultrasound-directed fine-needle aspiration biopsy of an enlarged mediastinal lymph node in patient diagnosed with follicular center cell lymphoma. **B**, Aspirate smear from a mediastinal lymph node demonstrating a population of small to intermediate-sized cells (Diff-Quik, ×400). **C**, Aspirate smear showing small to intermediate-sized cells, some of which are cleaved (Papanicolaou, ×400). **D**, Mediastinal lymph node specimen. Scattergram showing distribution of CD10 positivity and CD19 (B-cell marker) positivity in the gated population of cells. **E**, Mediastinal lymph node specimen. Scattergram showing distribution of κ and λ light chain positivity with a predominance of λ light chain in the gated population of cells (κ/λ ratio, 18.0). APC, allophycocyanin; FITC, fluorescein isothiocyanate; PE, phycoerythrin.

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was not made in this case. Possibilities include sampling error and tumor cell destruction owing to the fragile nature of the large cells in large B-cell lymphoma, making flow cytometric diagnosis difficult.

In the second case, the patient had a history of dysphagia and weight loss, and previous EUS revealed a large mass of the gastric cardia. EUS-FNAB was performed on a periesophageal lymph node, and a lymphoproliferative process was noted cytomorphologically. However, only 3 passes could be performed, and the aspirated sample was unavailable for further ancillary studies. The patient underwent surgery, and a high-grade B-cell lymphoma of the stomach was found **Image 3**.

One case was diagnosed as Hodgkin lymphoma. EUS-FNAB was performed on a subcarinal lymph node. An atypical cell population suggestive of Hodgkin lymphoma was noted; however, immunohistochemical stains performed on the cell block were equivocal for a diagnosis of Hodgkin lymphoma. The patient underwent endoscopic ultrasound–guided transesophageal Trucut needle (Wilson Cook, Winston-Salem, NC) biopsy of a mediastinal lymph node, which confirmed the diagnosis of Hodgkin lymphoma.

These data result in a sensitivity of 77% (10 of 13 cases of lymphoma correctly diagnosed) for EUS-FNAB (combined with ancillary studies) in the diagnosis of deep-seated lymphoma **Table 5**. Of the 214 EUS-FNAB specimens diagnosed as benign, 20 (9.3%) were submitted for flow cytometry. Of these, 1 sample was insufficient for analysis, with fewer than 25,000 viable cells. One sample showed an aberrant B-cell population by flow cytometric analysis (expressed CD45, CD19, CD20, CD22, and CD23 but not surface or cytoplasmic immunoglobulin or terminal deoxynucleotidyl transferase), which could represent a reactive process, a lymphoproliferative process, or progressive transformation of a germinal center. The patient then was lost to follow-up. No clonal population was detected by flow cytometry in the remaining 18 cases. These data resulted in a specificity of 100% (no false-positive results), positive predictive value of 100% (18 of 18 cases of benign lymph nodes in samples with sufficient viable cells for analysis correctly diagnosed), and negative predictive value of 86% for EUS-FNAB (combined with ancillary studies) in the diagnosis of deep-seated reactive lymphadenopathy.

EUS-FNAB Diagnosis of Deep-Seated Leukemic Infiltrate

One patient had a history of hairy cell leukemia and was noted to have mediastinal and retroperitoneal adenopathy. EUS-FNAB was performed on a gastrohepatic lymph node. Lymphoid cells with cytoplasmic projections were seen. Flow cytometry confirmed a hairy cell leukemia immunophenotype.

Table 5
Diagnostic Discrimination Values for Endoscopic Ultrasound-Guided Fine-Needle Aspiration Biopsy in Deep-Seated Lymphoma

	Result (%)
Sensitivity	77
Specificity	100
Positive predictive value	100
Negative predictive value	86

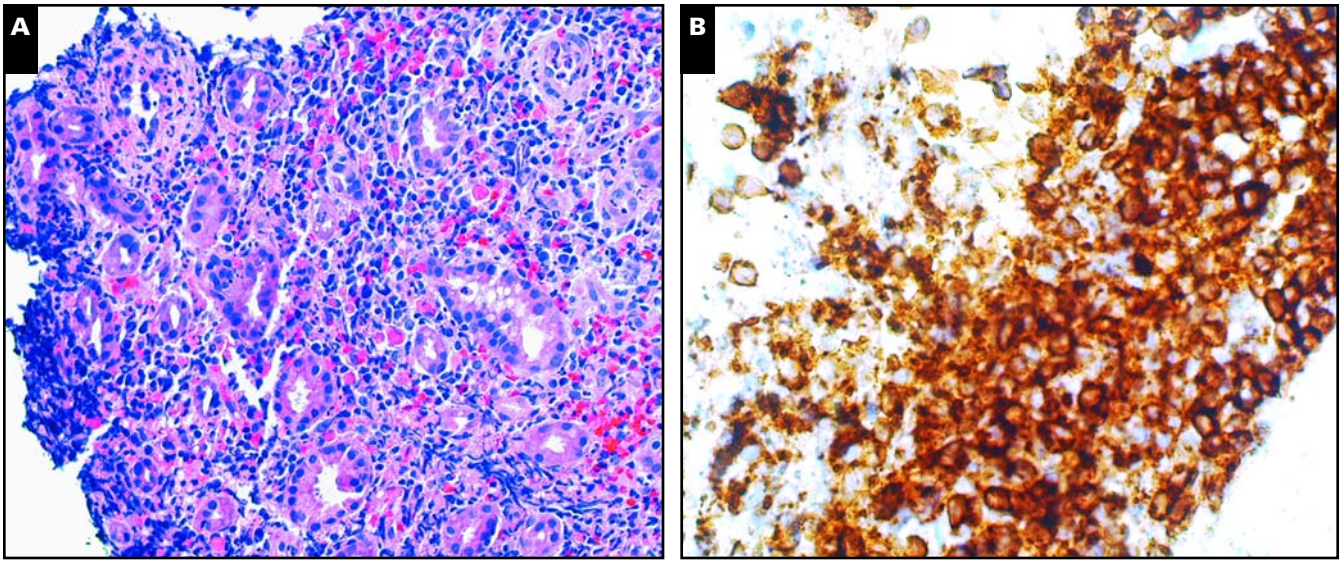

Image 3 Large B-cell lymphoma of stomach. **A**, Gastric biopsy specimen showing infiltration with malignant large lymphoid cells (H&E, ×200). **B**, Immunoperoxidase stain for B-cell marker (CD20) shows diffuse positivity in malignant cells (×400).

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Discussion

Our results indicate that EUS-FNAB, in association with flow cytometry and/or immunohistochemical analysis or Trucut needle core biopsy, is a sensitive (77%) and specific (100%) tool in the diagnosis of primary and recurrent deep-seated NHL and Hodgkin lymphoma. Although the number of cases of benign lymph nodes confirmed by flow cytometry was small, close clinical patient follow-up revealed no clinical evidence of disease, and no further evaluation (surgical) was performed on these patients. The potential definitive diagnosis of lymphoma by aspiration cytology is particularly important for patients whose condition may be too unstable for undergoing general anesthesia and open surgical biopsy, a procedure associated with higher risk. EUS-FNAB has been shown to be a safe procedure without significant complications. It also is less costly than other diagnostic methods, including mediastinoscopic biopsy, thoracotomy with biopsy, and computed tomography–guided biopsy.[12] In addition, EUS-FNAB not only allows access to deep-seated lymph nodes, but also allows sampling of lesions that are small (<25 mm), both of which may be difficult to sample using other techniques.[13]

A diagnosis of deep-seated lymphoma made by EUS-FNAB obviates the need for tissue diagnosis.[1,14] In our study, 77% of the patients with deep-seated lymphoma and 1 patient with a deep-seated hairy cell leukemic infiltrate were given a definitive cytologic diagnosis. Clinical decisions regarding patient triage were based on these cytologic diagnoses. Three patients (23%) required tissue diagnosis. Some investigators do not believe that aspiration cytology can be used to make a diagnosis of lymphoma.[15] Reasons for the preference of surgical lymph node biopsy over FNAB include preservation of tissue architecture and adequate sample for ancillary studies. However, if used with ancillary studies, aspiration cytology has been demonstrated to be a sensitive, specific, and accurate modality for the diagnosis of primary and recurrent lymphoma.[7,14] Demonstration of the immunophenotypic profile can delineate to which subgroup (eg, follicular center cell, mantle cell, small cell) a low-grade lymphoma belongs, which is not possible based on cytomorphologic features alone.

One criticism of FNAB is that observation of the histologic architectural pattern is not possible. Some authors state that adequate grading of follicular center cell lymphoma is not possible on cytologic material.[15] Gong et al[2] proposed criteria for grading follicular center cell lymphomas based on the proportion of immunoblastic cells present in the aspirated sample. We did not attempt to grade our cases of deep-seated follicular center cell lymphoma. When the issue is the possible transformation of a low-grade lymphoma or a recurrent lymphoma, sampling adequacy may be more important than histologic pattern.[16] We were able to distinguish the subgroup (3 cases of follicular center cell lymphoma, no cases of mantle cell or small cell lymphoma) of our cases of low-grade B cell lymphomas based on cytomorphologic and flow cytometric features.

Another criticism of the use of FNAB to diagnose deep-seated lymphoma has been its inability to differentiate large cell transformation of a follicular center cell lymphoma from diffuse large B-cell lymphoma. However, because follicular center cell lymphoma with transformation typically is treated with the same combination chemotherapy used for diffuse large B-cell lymphoma and T cell–rich large B-cell lymphoma, differentiating these 2 entities has little clinical impact for the patients.[16-19] Liu et al[20] successfully classified 9 of 10 cases of NHL by FNAB, which also had histologic confirmation. Their 1 discrepant case originally was diagnosed as follicular center cell lymphoma; the subsequent histologic diagnosis was follicular center cell lymphoma with focal large cell transformation. In this case, the excisional biopsy was performed at a different site 4 months after the original cytologic diagnosis was made. The authors concluded that this case represented transformation or variable histologic features at different locations.

Although grading of follicular NHL is a prognostic factor, it seems that clinical factors might have a more significant role in survival than lymphoma subgroup. In a clinical evaluation of the International Lymphoma Study Group classification of NHL, histologic type and patient characteristics as defined by the International Prognostic Index (eg, older patient age, lactate dehydrogenase level, performance status, and extranodal involvement) predicted patient survival within the NHL subtypes. The study demonstrated significantly different outcomes in terms of overall survival and failure-free survival in an NHL subgroup based on patient clinical characteristics.[16]

EUS-guided Trucut biopsy specimens have rarely been used. Investigators have indicated that the best use of this modality could be to assess lymph nodes.[21] In a case of Hodgkin lymphoma in which a diagnosis by FNAB can be challenging, we demonstrate for the first time that EUS-guided Trucut biopsy of deep-seated lymph nodes is feasible and safe and can provide accurate diagnoses in difficult cases such as Hodgkin lymphoma.[22]

Our experience from the present study indicates that many patients who undergo EUS-FNAB of deep-seated lymph nodes will not undergo subsequent surgical lymph node excision. When cytomorphologic studies were used in combination with ancillary studies, we were able to provide diagnoses on EUS-FNAB samples from deep-seated lymph nodes with good sensitivity and specificity. Our results underscore the importance of this diagnostic modality.

EUS-FNAB used in conjunction with flow cytometry and/or immunohistochemical analysis is a safe, cost-effective, sensitive, and specific method of diagnosis of primary and recurrent deep-seated lymphoma.

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