

Role of endoscopic ultrasound-guided fine-needle aspiration in evaluating mediastinal and intra-abdominal lymphadenopathies of unknown origin

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Abstract. The aim of the present study was to evaluate the efficacy of endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) in diagnosing mediastinal and intra-abdominal lymphadenopathies. A total of 154 patients with mediastinal and intra-abdominal lymphadenopathies were included in this retrospective study between February 2010 and March 2015. Malignancy was suspected in the patients as a result of imaging findings and EUS-FNAs were performed to confirm the diagnoses. EUS and EUS-FNA data, as well as hospital medical records, were reviewed. The accuracy of EUS-FNA was 90.8% for diagnosing malignancy and 85.6% for diagnosing benign lymphadenopathy. In combination with flow cytometry (FCM), the accuracy of EUS-FNA to determine lymphoma was 94.2%. Among the malignant lymphadenopathy cases, 80 were caused by metastasis, 19 by lymphoma and 1 by myeloid leukemia. In the 53 benign cases, EUS-FNA revealed a nonspecific inflammatory condition in 27 patients, tuberculosis in 21 patients and Castleman's disease in 5 patients. The factors revealed to be associated with malignant lymphadenopathy included the sex and age of patients, as well as the location and size of the enlarged lymph node. In particular, celiac axis lymphadenopathy was associated with malignancy (23.0% of cases of malignancy, vs. 3.8% of benign lymphadenopathy). EUS-FNA results additionally suggested that the malignant lymph nodes observed in celiac axis were more likely to result from lymphoma (42.1%; 8/19 cases) than metastasis (18.8%; 15/80 cases; P=0.039). By contrast, malignant lymph nodes observed in the mediastinum were more likely to be caused by metastasis (47.5%; 38/80 cases) than

lymphoma (10.5%; 2/19 cases; P=0.004). The results of the present study suggested that EUS-FNA is accurate for differentiating between malignancy and benign lymphadenopathy. Therefore, EUS-FNA in combination with FCM analysis, as a minimally invasive and highly sensitive tool, should be routinely performed for the identification of lymphoma. Additionally, examining the enlarged celiac axis lymph nodes of elderly males, who exhibit an increased risk of malignancy, may be beneficial.

Introduction

Mediastinal and intra-abdominal lymphadenopathies may present with numerous benign or malignant conditions. Benign lymph node enlargement may occur as a response to certain infections, including tuberculosis and fungal infection, as well as several less common diseases, including Castleman's disease and Wegener's granulomatosis. Malignant lymphadenopathy may occur in primary lymphatic diseases such as Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL), as well as in other types of malignancy that have metastasized to regional lymph nodes, including lung, esophageal, breast and pancreatic carcinomas (1,2).

Advanced imaging methods, including computed tomography (CT), magnetic resonance imaging and positron emission tomography-computed tomography (PET-CT), serve important roles in identifying enlarged lymph nodes during the staging workup for lymphoproliferative disorders; however, they may be limited in their ability to differentiate between inflammatory/reactive processes and malignancy (3-5). Thus, tissue obtained percutaneously or surgically is critical to providing a pathological diagnosis, including insight into the possible disease origin. Laparoscopic lymph node biopsy and image-guided needle biopsy (IGNB) have been applied to obtain tissue from enlarged lymph nodes; however, these are invasive and expensive procedures. Endoscopic ultrasound (EUS) has emerged as a novel imaging modality (3,5). A fine-needle aspiration (FNA) biopsy coupled with EUS allows for the sampling of the target lesion with ultrasound guidance in real time, providing minimally invasive access to the lymph nodes within the abdomen and mediastinum (6,7). Compared with IGNB, EUS-FNA exhibits increased

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accuracy for the sampling of anatomically challenging sites, including those close to major blood vessels, which is useful for avoiding injury to the adjacent tissue (7). In addition, EUS-FNA allows access to deep-seated lymph nodes and the sampling of small lesions (<25 mm), which overcomes the limitations of sampling through other techniques (8). Previous studies have demonstrated that, for diagnosing mediastinal and intra-abdominal lymphadenopathies, EUS-FNA exhibits a sensitivity of 79-98.3%, a specificity of 98-100%, a positive predictive value (PPV) of 98-100%, a negative predictive value (NPV) of 62-98.4% and an accuracy of 84-99.4% (9,10).

The aim of the present study was to evaluate the efficacy and safety of EUS-FNA for the diagnosis of mediastinal and intra-abdominal lymphadenopathies of unknown origin.

Patients and methods

Patients. The present retrospective study included 154 patients with mediastinal and intra-abdominal lymphadenopathies that were suspected to be malignant based on imaging data. All patients underwent EUS-FNA at Tongji Hospital (Wuhan, China) between February 2010 and March 2015. The present study was approved by The Ethics Committee of Tongji Hospital and informed consent was obtained from each patient or their legally authorized representative. The following data were collected from the hospital medical record system: Age, sex, EUS findings, EUS-FNA findings, procedure-associated complications and follow-up results.

EUS-FNA procedure. EUS-FNA was performed under deep sedation according to the principles of 'monitored anesthesia care' (11). The patients were anaesthetized with the intravenous administration of Propofol (2 ml/kg). All patients received oxygen during the procedure and their blood pressure and heart rate were monitored.

The EUS-FNA examinations were conducted using an Olympus linear echoendoscope (GF-UCT 240, GF-UCT 260; Olympus Corporation, Tokyo, Japan) platform with the Aloka Processor Alpha-5 (Hitachi, Ltd., Tokyo, Japan). An endosonographer with >5 years of EUS experience, performing >150 EUS-FNAs per year, performed EUS-FNA in the present study. A 22-gauge EchoTip Ultra needle (Cook Endoscopy; Cook Medical Inc., Bloomington, IN, USA) was advanced into lesions with real-time EUS visualization. The endosonographer maneuvered the needle back and forth 20 times within the lesion, applying minimal negative pressure by pulling the needle stylet slowly and continuously. If no specimen was obtained, continuous suction was applied with a 5-10 ml syringe in order to obtain a specimen. Samples were then prepared for histological and cytological examinations. The specimens were expelled from a needle in three steps: i) pushing the stylet into the needle; ii) flushing the needle with 0.1 ml saline; and iii) filling the needle with 2 ml of air. The core tissue was put into the container filled with formaldehyde for histological examination. The fragment of the specimen was put on the glass slide, and another glass slide was used to smear for cytological examinations. In suspected cases of lymphoma, additional samples were obtained for flow cytometry (FCM).

FCM. FCM was performed on a FACScan analyzer (BD Biosciences, Franklin Lakes, NJ, USA) using standard procedures (12,13). The cells were stained with selected monoclonal antibodies as required (30 min at room temperature), from the following: Immunoglobulin κ and λ light chains (cat no. 560950, 1:5; cat no. 562054, 1:5; BD Biosciences, Franklin Lakes, NJ, USA), cluster of differentiation CD2 (cat no. 555327; 1:5; BD Biosciences), CD3 (cat no. 552127; 1:5; BD Biosciences), CD4 (cat no. 561842; 1:20; BD Biosciences), CD5 (cat no. 555353; 1:5; BD Biosciences), CD8 (cat no. 557085; 1:5; BD Biosciences), CD10 (cat no. 561002; 1:5; BD Biosciences), CD11c (cat no. 560999; 1:5; BD Biosciences), CD19 (cat no. 555413; 1:5; BD Biosciences), CD20 (cat no. 560961; 1:5; BD Biosciences), CD23 (cat no. 561146; 1:20; BD Biosciences), CD25 (cat no. 560990; 1:5; BD Biosciences), CD38 (cat no. 555462; 1:5; BD Biosciences), FMC-7 (cat no. 340919; 1:5; BD Biosciences)-a normal B-cell antigen expressed on mature human B cell and it is used in during immunophenotypic analysis and differential diagnosis of lymphomas and leukemias (14), major histocompatibility complex (cat no. ab63567; 1:10; Abcam, Cambridge, MA, USA), class II-DR (HLA-DR) (cat no. 560896; 1:5; BD Biosciences), CD30 (cat no. 555829; 1:5; BD Biosciences), CD45 (cat no. 340953; 1:5; BD Biosciences), CD103 (cat no. 563883; 1:20; BD Biosciences), CD138 (cat no. 564605; 1:20; BD Biosciences), ki-67 (cat no. 612472; 1:5; BD Biosciences), Bcl-2 (cat no. 562679; 1:20; BD Biosciences), CD16 (cat no. 565421; 1:20; BD Biosciences), CD56 (cat no. 565140; 1:20; BD Biosciences), CD57 (cat no. 560845; 1:20; BD Biosciences), CD94 (cat no. 562361; 1:20; BD Biosciences), CD161 (cat no. 551138; 1:5; BD Biosciences), CD158a/h (cat no. 564319; 1:20; BD Biosciences), CD158b (cat no. 566053; 1:20; BD Biosciences), CD158e (cat no. 564103; 1:20; BD Biosciences), T-cell receptor (TCR)ab (cat no. 563826; 1:20; BD Biosciences), and TCRrd (cat no. 564157; 1:5; BD Biosciences).

Follow up. The final diagnosis was typically based on the pathological report obtained from surgery. However, if the patient did not undergo surgery, a 9 month follow-up was performed (15). Final diagnosis was classified as either: i) Malignant lymphadenopathy: EUS-FNA cytology and/or pathology results revealed malignant cells that were confirmed by specific immunohistochemical stains or supported with FCM results; or ii) benign lymphadenopathy: Absence of typical malignant cell morphology with no further signs of malignancy, i.e. the lesions exhibited regression or no further progression at follow-up, as previously described (9,16).

Statistical analysis. EUS-FNA-associated parameters, including the sensitivity, specificity, PPV, NPV and accuracy were evaluated by comparing EUS-FNA results with the final diagnoses. Continuous variables are reported as the mean \pm standard deviation and categorical variables are reported as percentages. Student's t-tests were used to compare continuous variables and categorical variables were compared using χ^2 -tests. A two-tailed P-value of <0.05 was considered to indicate a statistically significant difference. All data were processed and analyzed using SPSS (version 19; IBM Corp., Armonk, NY, USA).

Table I. Patient and lesion characteristics of malignant and benign lymphadenopathy.

Patient characteristics	Malignant lymphadenopathy (n=100)	Benign lymphadenopathy (n=53)	P-value
Sex, n			0.0110 ^a
Male	75	29	
Female	25	24	
Age, years ^b	55±11.9	43±16.8	0.0001 ^a
Lesion location ^c			
Peripancreas	32 (32.0)	21 (39.6)	0.3750
Around the celiac axis	23 (23.0)	2 (3.8)	0.0020 ^a
Hepatic portal	5 (5.0)	8 (15.1)	0.0630
Mediastinum	40 (40.0)	22 (41.5)	0.8640
Endoscopic ultrasound characteristics			
Size, mm ^b	33.9±15.8	24.8±13.8	0.0110 ^a
Sharp margin ^c	85 (85.0)	40 (75.5)	0.1470
Fusion ^c	23 (23.0)	9 (17.0)	0.3840
Echogenic focus ^c	25 (25.0)	15 (28.3)	0.6580
Homogeneous echogenicity ^c	46 (46.0)	22 (41.5)	0.5950
Malignant echo-features ^{c,d}	40 (75.5)	81 (81.0)	0.1530

^aStatistically significant, P<0.05. ^bData are presented as the mean ± standard deviation. ^cData are presented as n (%). ^dMalignant echo-features: Hypochoic, sharp margin, rounded and size >10 mm.

Results

Final diagnoses. A total of 154 patients (105 male, 49 female) were included in the present study. The mean patient age was 49.1 years (22–68 years). The success rate for obtaining pathological material by EUS-FNA was 87.0% (134/154) and adequate cytology samples were obtained in all cases. The mean number of needle passes was 2.9 (range, 1–6). For the 52 patients suspected to be presenting with lymphoma, the EUS-FNA samples were evaluated by cytology, pathology and FCM studies. Overall, a definitive diagnosis was obtained for 153 of the 154 patients (including 31 from surgical pathology and 122 from follow-up). A total of 100 patients were determined to exhibit malignant lymphadenopathy, including 80 cases of metastasis, 19 cases of lymphoma and 1 case of myeloid leukemia. The remaining 53 cases were determined to be benign, including 27 cases of a non-specific inflammatory condition, 21 cases of tuberculosis and 5 cases of Castleman's disease.

Patient and lesion characteristics. As presented in Table I, the incidence of malignant lymphadenopathy in the present study cohort was significantly increased in males compared with females (P=0.011). The observed mean age for patients with malignancy was 55±11.9 years compared with 43±16.8 years for the benign lymphadenopathy (P=0.0001). Other factors associated with malignancy included the location and size of the enlarged lymph node. The mean size of a malignant lymph node was 33.9±15.8 mm, compared with 24.8±13.8 mm for a benign lymph node (P=0.011). In particular, the celiac axis lymphadenopathy was a clear indicator of malignancy (23.0% in malignancy vs. 3.8% in benign lymphadenopathy; P=0.039). However, the echo-features of lymph nodes as assessed during

EUS were not significantly different between malignant and benign cases (P=0.153); therefore, this may not be useful in diagnosing malignancy.

As presented in Table II, it was demonstrated that EUS-FNA may be useful in assessing lymphadenopathy resulting from metastasis and lymphoma in the celiac axis, mediastinum, peripancreatic and hepatic portal areas. Malignant lymph nodes observed in the celiac axis were more likely to have resulted from lymphoma (42.1%; 8/19 cases) than metastasis (18.8%; 15/80 cases; P=0.039). In contrast, malignant lymph nodes observed in the mediastinum were more likely to have been caused by metastasis (47.5%; 38/80 cases) than lymphoma (10.5%; 2/19 cases; P=0.004). EUS-FNA data revealed that the mean size of metastatic lymph nodes was 35.6±16.4 mm, whereas it was 27.2±11.2 mm for lymphoma (P=0.011). Furthermore, lesion fusion was observed in 47.4% of lymphoma cases and 17.5% of metastasis cases (P=0.013). Homogeneous echogenicity was observed in 40.0% of the cases of metastasis and 73.7% of the cases of lymphoma (P=0.011). Thus, these results suggested a potential role for EUS-FNA in distinguishing cases of lymphoma from metastasis in the celiac axis and mediastinum.

Efficacy of EUS-FNA for identifying malignant lymphadenopathy. Among 100 patients diagnosed with malignant lymphadenopathy, the sensitivity, specificity, PPV, NPV and accuracy of EUS-FNA for malignant lymphadenopathy were 86.0, 100.0, 100.0, 79.1 and 90.8%, respectively (Table III).

The predominant cause for lymphadenopathy was metastasis (52.3%; 80/154). The application of EUS-FNA generated a correct diagnosis for 70 patients, including squamous cell carcinoma (n=28), adenocarcinoma (n=32), neuroendocrine

Table II. Patient and lesion characteristics of metastasis and lymphoma.

Patient characteristics	Metastasis (n=80)	Lymphoma (n=19)	P-value
Sex, n			0.2420
Male	62	12	
Female	18	7	
Age, years ^a	54.9±11.9	55.4±12.8	0.8950
Lesion location ^b			
Peripancreas	23 (28.8)	9 (47.4)	0.2800
Around the celiac axis	15 (18.8)	8 (42.1)	0.0390 ^c
Hepatic portal	5 (6.3)	0 (0.0)	0.5800
Mediastinum	38 (47.5)	2 (10.5)	0.0040 ^c
Endoscopic ultrasound characteristics			
Size, mm ^a	35.6±16.4	27.2±11.2	0.0110 ^c
Sharp margin ^b	66 (82.5)	19 (100.0)	0.0650
Fusion ^b	14 (17.5)	9 (47.4)	0.0130 ^c
Echogenic focus ^b	24 (30.0)	1 (5.3)	0.0370 ^c
Homogeneous echogenicity ^b	32 (40.0)	14 (73.7)	0.0110 ^c

^aData are presented as the mean ± standard deviation. ^bData are presented as n (%). ^cStatistically significant, P<0.05.

Table III. Efficacy of endoscopic ultrasound-guided fine-needle aspiration.

Condition	Metric, %				
	Sensitivity	Specificity	PPV	NPV	Accuracy
Malignant lymphadenopathy	86.0	100.0	100.0	79.1	90.8
Metastasis	87.5	100.0	100.0	87.9	93.5
Lymphoma	84.2	100.0	100.0	91.7	94.2
Benign lymphadenopathy	58.5	100.0	100.0	81.9	85.6
Tuberculosis	38.1	100.0	100.0	91.7	92.2
Nonspecific reactive lymphadenopathy	100.0	77.7	54.2	100.0	82.4

PPV, positive predictive value; NPV, negative predictive value.

tumor (n=7) and undifferentiated carcinoma (n=3). Among them, 30 patients had a history of tumors and the recurrence of malignancy was confirmed by EUS-FNA. Notably, the remaining 40 patients had no history of malignancy and no primary lesion had been detected prior to EUS-FNA. Thus, additional examination, including PET-CT, was applied to investigate the tumor origin following EUS-FNA; primary lesions were successfully identified in 35/40 patients. The sensitivity, specificity, PPV, NPV and accuracy of EUS-FNA for metastasis were 87.5, 100.0, 100.0, 87.9 and 93.5%, respectively.

Of the 52 patients suspected to be presenting with lymphoma, 19 patients were confirmed, including 10 patients with diffuse large B-cell lymphomas (DLBCL), 2 patients with HL, 1 patient with T-cell lymphoma and 7 patients with B-cell NHL (B-NHL) without further subclassifications.

The cytological findings included 9 true-positive cases and 3 false-positive cases. Benign lymphadenopathy due to

nonspecific inflammatory reaction was the final diagnosis in the 3 false-positive cases, which was determined by follow-up. The sensitivity, specificity, PPV, NPV and accuracy of EUS-FNA combined with cytology for lymphoma were 47.4, 90.9, 75.0, 75.0 and 75.0%, respectively (Table IV).

Pathology via EUS-FNA provided adequate tissue samples for 84.2% of patients. Notably, the pathology analysis was not sufficient to diagnose 16 cases with malignant lesions, although there was no false-positive result. Only 3 patients were diagnosed correctly (2 cases with DLBCL and 1 case without definite subclassification). The sensitivity, specificity, PPV, NPV and accuracy of EUS-FNA combined with pathology for lymphoma were 15.8, 100.0, 100.0, 67.3 and 69.2%, respectively.

The combined analysis of EUS-FNA and FCM contributed to the correct diagnosis of 16 patients with B-NHL through detecting light-chain restriction expression (Fig. 1). A total of 8 lymphomas were further subtyped as DLBCL due to

Table IV. Efficacy of EUS-FNA in the identification of lymphoma.

Method	n	Final diagnoses, n		Sensitivity, %	Specificity, %	PPV, %	NPV, %	Accuracy, %
		Lymphoma	Non-lymphoma					
EUS-FNA with flow cytometry	52			84.2	100.0	100.0	91.7	94.2
Lymphoma		16	0					
Non-lymphoma		3	33					
EUS-FNA with cytology	52			47.4	90.9	75.0	75.0	75.0
Lymphoma		9	3					
Non-lymphoma		10	30					
EUS-FNA with pathology	52			15.8	100.0	100.0	67.3	69.2
Lymphoma		3	0					
Non-lymphoma		16	33					

EUS-FNA, endoscopic ultrasound-guided fine-needle aspiration; PPV, positive predictive value; NPV, negative predictive value.

the detection of CD3, CD5, CD10, Bcl-2 and CD20 expression by FCM (Fig. 1). There were no false-positive cases. The combined EUS-FNA and FCM method was not able to determine 2 cases of HL or 1 case of T-cell lymphoma, which were ultimately diagnosed with excisional biopsies. The sensitivity, specificity, PPV, NPV and accuracy of EUS-FNA combined with FCM assessing lymphoma were 84.2, 100.0, 100.0, 91.7 and 94.2%, respectively. Thus, the efficacy of the combined analysis is markedly higher than cytology ($P<0.001$) or pathology ($P=0.001$) alone (data not shown).

In one atypical case, a 16-year-old male experienced intermittent abdominal pain for 6 months in the absence of fever. Laboratory investigations were performed on peripheral blood counts, whereas a CT scan revealed retroperitoneal lymphadenopathy. EUS-FNA was therefore recommended to evaluate the enlarged lymph nodes (Fig. 2). Although cytology supported the diagnosis of NHL based on immature lymphocyte proliferation and atypical small, round cells, a definitive conclusion could not be drawn. A subsequent FCM investigation highlighted the presence of abnormal cells with a characteristic phenotype of malignant myeloid origin, which expressed CD117, CD56, CD34, CD13, CD33 and HLA-DR, partially expressed CD19, cMPO, CD11c and CD15, and did not express CD3, CD4, CD8, CD5, CD20, CD23, κ , λ , cCD79a, cCD3 or CD16 (Fig. 2). The patient underwent a bone marrow biopsy and the diagnosis of acute myeloid leukemia was confirmed.

Efficacy of EUS-FNA for benign lymphadenopathy. For the patients with benign lymphadenopathy, the median follow-up period was 10.2 months (range 9–48 months). The sensitivity, specificity, PPV, NPV and accuracy of EUS-FNA for benign lymphadenopathy were 58.5, 100.0, 100.0, 81.9 and 85.6%, respectively. Tuberculosis ($n=21$) was the most common etiology, with the exception of a nonspecific inflammatory condition ($n=27$). The diagnosis of tuberculosis in 8 patients

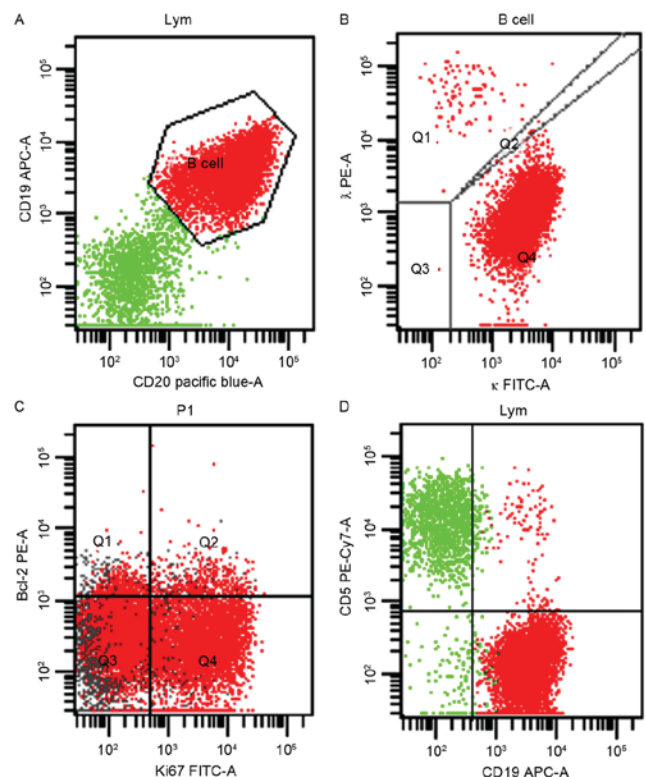


Figure 1. Diffuse large B-cell lymphoma diagnosed using endoscopic ultrasound-guided fine-needle aspiration combined with FCM. (A) FCM scatter plot with CD19/CD20 identified a group of B cells. (B) The group of B cells exhibited restricted κ light-chain expression. (C) FCM identified a population of Bcl-2/Ki-67⁺ cells. (D) FCM scatter plot with CD5/CD19 identified a population of CD5⁺/CD19⁺ cells. FCM, flow cytometry; CD, cluster of differentiation; APC, allophycocyanin; PE, phycoerythrin; FITC, fluorescein isothiocyanate; lym, lymphocyte; P1, target cells.

depended on the histological identification of granulomatous lymphadenitis without acid-fast bacilli (Fig. 3). The

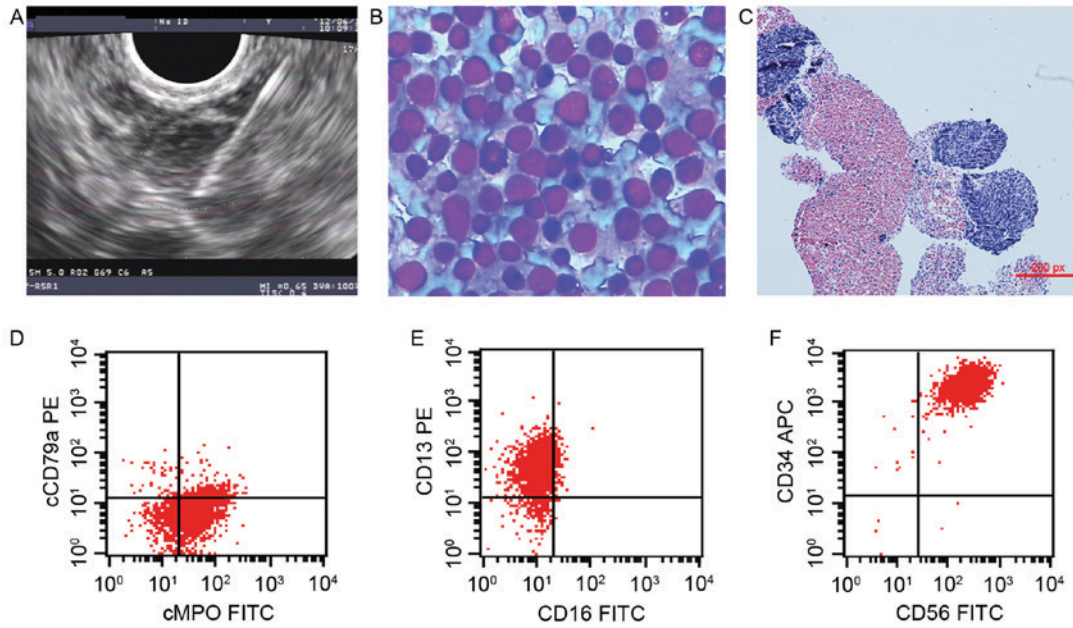


Figure 2. A case of myeloid leukemia accompanied by extramedullary infiltration, diagnosed using EUS-FNA combined with FCM. (A) EUS image of retroperitoneal lymphadenopathy; EUS-FNA was performed using a 22-gauge needle. (B) Cytology identified the active proliferation of immature lymphocytes, suspected to be non-Hodgkin's lymphoma (Liu's stain; magnification, x400). (C) Pathology revealed small, round cells (hematoxylin and eosin stain; magnification, x100). (D) A FCM scatter plot with cCD79a/cMPO revealed cCD79a⁺/cMPO⁺ cells, which suggested that the abnormal cells were derived from the myeloid hematopoietic cells. (E) A FCM scatter plot for CD13/CD16 revealed CD13⁺/CD16⁻ cells, which suggested that the abnormal cells were derived from granulocytes. (F) FCM scatter plot for CD34/CD56 revealed CD34⁺/CD56⁺ cells, which suggested the abnormal cells were precursors. APC, allophycocyanin; PE, phycoerythrin; FITC, fluorescein isothiocyanate; EUS-FNA, endoscopic ultrasound-guided fine-needle aspiration; CD, cluster of differentiation; FCM, flow cytometry; cMPO, myeloperoxidase on the cell membrane.

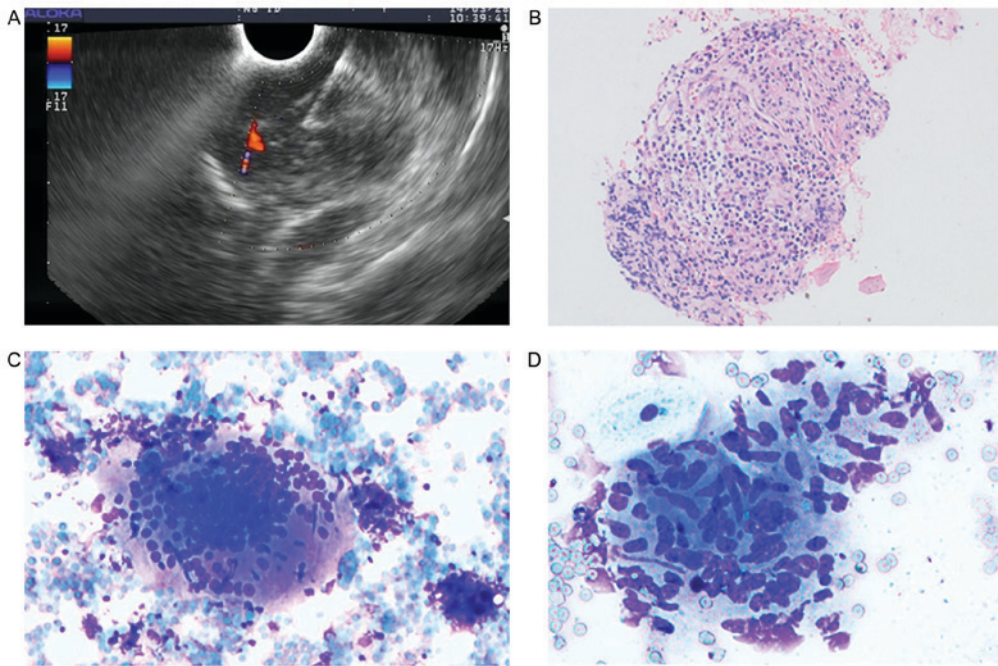


Figure 3. A case of tuberculosis diagnosed by EUS-FNA. (A) EUS images of retroperitoneal lymphadenopathy, presenting as two masses measuring 3.0x1.8 and 1.6x0.8 cm in the mediastinum, with a ill-defined border, heterogeneous echogenicity, echogenic focus and lesion fusion; EUS-FNA was performed with a 22-gauge needle with 5-ml suction pressure. (B) Pathology identified a granuloma (hematoxylin and eosin stain; magnification, x200). Cytology revealed (C) Langerhans giant cells (Liu's stain; magnification, x100) and (D) epithelioid cells (Liu's stain; magnification, x200). EUS-FNA, endoscopic ultrasound-guided fine-needle aspiration.

sensitivity, specificity, PPV, NPV and accuracy of EUS-FNA assessing tuberculosis were 38.1, 100.0, 100.0, 91.7 and 92.2%, respectively.

Complications. None of the patients experienced any severe peri- or post-procedural complications, as previously defined (17), including hemorrhage or infection.

Discussion

EUS-FNA may influence patient management, as a doctor will give patients different advice according to the results of EUS-FNA. For example if the results show benign disease, the doctor may suggest follow-up or conservative treatment. However, if the results indicate malignant disease, the doctor may suggest aggressive treatment. The diagnostic ability of EUS-FNA in regards to lymphadenopathies is well established (3,18). The present retrospective study of 154 patients is one of the largest studies conducted on the topic, to the best of our knowledge. The data collected suggest that EUS-FNA is an efficient and safe method for assessing mediastinal and intra-abdominal lymphadenopathies of unknown origin. In the present study, 65.4% of the enlarged lymph nodes were determined to be malignant. Elderly and male patients were identified to possess an increased risk of malignant disease and, in addition, lymphadenopathy in the region of the celiac axis was more likely to be caused by malignant disease. Echo-features are an important assessment during EUS; however, criteria for their assessment to predict malignant lymph node invasion have yet to be established. Although Catalano *et al* (19) suggested that a number of echo-features, including hypoechoic, sharp borders, rounded contour and size >10 mm, were associated with malignancy, the conclusions were not supported by similar studies (20,21). The results of the present study did not indicate an association between echo-features and malignant lymphadenopathy; however, the size of malignant lymph nodes were significantly increased compared with benign lymph nodes.

Taking into consideration the anatomical and technical challenges associated with accessing the intra-abdomen or mediastinum, the application of EUS-FNA may benefit the patients with lymph node metastasis or lymphoma more than others (15). In the present study, an increased proportion of the enlarged lymph nodes located in the mediastinum were metastatic lymph nodes, whereas enlarged lymph nodes caused by lymphoma were predominantly located around the celiac axis. In addition, metastatic lymph nodes were larger and exhibited echogenic focus, whereas enlarged lymph nodes caused by lymphoma were characterized by lesion fusion and homogeneous echogenicity. These results may provide insight into conducting a differential diagnosis between metastatic lymph nodes and lymphoma in the clinical work-up.

Patients with benign lymphadenopathies do not require intensive treatment; typically, routine patient follow-up is sufficient, depending on the nature of the pathology. By contrast, the patients diagnosed with malignancy require the determination of tumor origin and are subsequently referred for surgical resection when possible, chemo/radiation therapy and/or appropriate palliative care (4,20). PET-CT is considered to be a valuable predictive and prognostic tool for patients with malignancy; however, its sensitivity and NPV are poor (22), and the biopsy result remains the key in guiding the clinician's management choice (17). Consistent with a previous study by Dewitt *et al* (23), the present study demonstrated that EUS-FNA improved the detection of the postoperative recurrence of malignancy, potentially allowing for cancer staging to be performed prior to surgery.

FCM technology allows for the assessment of abnormal cell phenotypes and thus improves the sensitivity, specificity and accuracy of EUS-FNA in diagnosing primary or recurrent lymphoma (1,24,25). In the present study, FCM was performed on samples from 52 patients with suspicions of lymphoma. The sensitivity, specificity, PPV, NPV and accuracy of EUS-FNA combined with FCM for lymphoma were 84.2, 100, 100, 91.7 and 94.2%, respectively. The overall efficacy was markedly increased compared with pathological and cytological analyses.

Another advantage of the combined EUS-FNA and FCM analysis is that it typically provides a sufficient specimen to allow further lymphoma subtyping, so that patients may be given the appropriate therapy (26). Consistent with a previous study on subtyping B-NHL (3), the present study demonstrated that EUS-FNA in combination with FCM markedly improved the diagnosis efficiency in cases of deep-seated lymphoma; 84.2% of the patients with lymphoma were diagnosed with B-NHL, and this method correctly subtyped 50% of B-NHL cases.

There are several limitations for EUS-FNA as an independent tool to provide a definitive diagnosis of lymphoma. Firstly, the diagnosis of HL requires integration of cytomorphology, immunophenotypical and clinical features, and thus may continue to require open biopsies for diagnosis and classification (12). In the present study, 2 cases of HL were ultimately diagnosed using excisional biopsy. Secondly, the diagnosis of T cell lymphomas could not be performed with FCM due to the lack of specific antibodies for detection (12,13). T cell lymphoma rarely occurs in the gastrointestinal tract, mediastinum or retroperitoneum (27); the present study identified only 1 case of T cell lymphoma, which was eventually determined by open biopsy. Third, given the reliance on adequate material for FCM analysis, the quality of sampling requires further improvement. A total of ≥ 3 passes from several angles of the lymph nodes may be necessary to obtain adequate samples (12). In addition, avoiding necrotic tissue and blood contamination during sampling is critical.

In the present study, a rare case of myeloid leukemia with extramedullary leukemic cell infiltration was identified. The patient presented with abdominal pain in the absence of typical myeloid leukemia symptoms, including fever, anemia, bleeding and infection. Although malignant myeloid precursor cells have been demonstrated to infiltrate and proliferate in multiple organ systems (28), limited methods are available to confirm diagnosis. To the best of our knowledge, the present study is the first to demonstrate that EUS-FNA combined with FCM was able to determine the characteristic phenotype of malignant myeloid origin of abnormal cells from the enlarged lymph nodes. This approach may be routinely applied to investigate cases of myeloproliferative neoplasms with unusual clinical manifestations.

For benign lymphadenopathy, extra-pulmonary tuberculosis was the most common etiology besides a nonspecific inflammatory condition in the present study (21 cases). EUS-FNA appeared to be an efficient method for obtaining pathological samples and distinguishing between tuberculosis-induced necrosis and necrosis resulting from malignancy. The diagnostic accuracy in the cohort from the present study was 92.2%; however, the detailed diagnostic criteria for

extra-pulmonary tuberculosis based on EUS-FNA require further study and documentation (2,29,30).

The present study had certain limitations. It was a retrospective study, and surgical pathology was not available for the majority of patients at the time of diagnosis. However, clinical follow-ups were performed for ≥ 9 months.

In conclusion, EUS-FNA is associated with high diagnostic accuracy and low complication rates for evaluating cases of mediastinal and intra-abdominal malignant lymphadenopathy. EUS-FNA in combination with FCM as a minimally invasive and highly sensitive tool should be routinely performed to determine lymphoma, in addition to examining enlarged celiac axis lymph nodes in elderly males, who exhibit an increased risk of malignancy based on the data of the present study.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Author's contributions

JW and BC conceived the study and prepared a draft manuscript. QC and XW assisted with data collection and modification of the manuscript. YW and WH were operational assistants of EUS-FNA and assisted with data collection. All authors contributed to the study design and commented on the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by The Ethics Committee of Tongji Hospital and informed consent was obtained from each patient or their legally authorized representative.

Consent for publication

Informed consent was obtained from each patient or their legally authorized representative.

Competing interests

The authors declare that they have no competing interests.

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