ORIGINAL ARTICLE—ALIMENTARY TRACT

Usefulness of endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) for undiagnosed intra-abdominal lymphadenopathy

Osamu Nakahara · Kenji Yamao · Vikram Bhatia · Akira Sawaki · Nobumasa Mizuno · Tadayuki Takagi · Yasuhiro Shimizu · Takashi Koshikawa · Yasushi Yatabe · Hideo Baba

Abstract

Background The differentiation between benign and malignant abdominal lymph nodes is difficult, especially if no primary site is evident or if cancer resection was remote in time. The aim of this study was to evaluate the yield of endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) in patients with undiagnosed intra-abdominal lymphadenopathy.

Methods Fifty-seven consecutive patients with undiagnosed abdominal lymphadenopathy who were registered in our EUS-FNA database from January 1997 to December 2007 were reviewed. EUS-FNA was carried out using a 22-G needle. The final pathological diagnosis was based on the cytopathological, histological, and immunohistochemical (IHC) findings.

Results Adequate specimens were obtained in 93% cases. The final diagnoses included local recurrence of malignancy after resection ($n = 16$), lymphoma ($n = 12$), and benign/reactive changes ($n = 17$). The sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy of EUS-FNA were 94, 100, 100, 90 and 96%, respectively. In addition, it was also possible to classify lymphoma subtypes in 83% of cases. No complications occurred during the procedures.

Conclusions EUS-FNA is clinically very useful for establishing the diagnosis of abdominal lymphadenopathy of unknown cause and can provide sufficient tissue for IHC and subtyping of lymphomas.

Keywords EUS-FNA · Lymphadenopathy · Cytology · Histology

O. Nakahara · K. Yamao (✉) · A. Sawaki ·
N. Mizuno · T. Takagi
Department of Gastroenterology,
Aichi Cancer Center Hospital, 1-1 Kanokoden,
Chikusa-ku, Nagoya 464-8681, Japan
e-mail: kyamao@aichi-cc.jp

V. Bhatia
Institute of Liver and Biliary Sciences, New Delhi, India

Y. Shimizu
Department of Gastrointestinal Surgery,
Aichi Cancer Center Hospital, Nagoya, Japan

T. Koshikawa
Aichi Prefectural College of Nursing and Health,
Nagoya, Japan

Y. Yatabe
Department of Pathology and Genetics,
Aichi Cancer Center Hospital, Nagoya, Japan

O. Nakahara · H. Baba
Department of Gastroenterological Surgery
Graduate School of Medical Sciences,
Kumamoto University, Kumamoto, Japan

Introduction

Because of rapid developments in diagnostic imaging modalities such as ultrasound (US), computed tomographic (CT) scan, and positron emission tomography (PET), increasing number of cases of enlarged abdominal lymph nodes of uncertain origin are being detected. When no primary malignant lesion is evident, the differential diagnosis of abdominal lymphadenopathy can be difficult. Until now, in order to make a definite diagnosis, open laparotomy or laparoscopic tissue sampling had to be performed, but these methods are very invasive and are not cost-effective.

Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) was developed in the 1990s for the diagnosis

of peri-luminal lymphadenopathy adjacent to the gastro-intestinal tract [1, 2]. Although the specimens obtained by EUS-FNA can usually differentiate between benign and malignant lymphadenopathy, some cases such as malignant lymphoma can be difficult to diagnose and subtype. EUS-FNA material was originally used for cytology alone, but in the last few years EUS-FNA material has also been used for histological review. In the present study, we evaluated performance characteristics of EUS-FNA for the diagnosis of undiagnosed abdominal lymphadenopathy which could not be diagnosed by other modalities.

Patients and methods

EUS-FNA was performed in 1257 patients from January 1997 to December 2007 at the Aichi Cancer Center Hospital, Nagoya. Patients who underwent EUS-FNA for undiagnosed abdominal lymphadenopathy origin were identified from our prospectively maintained database. Table 1 shows the inclusion and exclusion criteria for undiagnosed lymphadenopathy used in the present study. All patients had given written informed consent to undergo EUS-FNA, and repeat consent for analysis in the present study was not taken. The study was approved by the institutional review board of our hospital.

EUS protocol

EUS-FNA was performed as previously described, using a 7.5 MHz convex linear array echoendoscope (GF-UCT240, Olympus Optical Co. Ltd., Tokyo, Japan) and a 22-G needle (NA-200H-8022, Olympus) [3–5]. Three standard scanning positions were used for evaluating intra-abdominal lymphadenopathy: (1) Posterior gastric wall to detect celiac axis and upper peri-aortic nodes, (2) Gastric antrum and duodenal bulb to detect enlarged lymph nodes around the pancreatic head and portal vein, and (3) Descending duodenum to detect lymph nodes around the inferior vena cava, superior mesenteric artery, and the uncinate process. Lymph nodes were sampled by the trans-gastric route for position 1, and by the trans-duodenal route for positions 2

Table 1 Inclusion and exclusion criteria of EUS-FNA for undiagnosed lymphadenopathy

Inclusion criteria
Lymph node swelling detected by EUS before EUS-FNA
A primary lesion not obviously identified on chest/abdominal CT and upper/lower gastrointestinal endoscopy
Exclusion criteria
Active primary cancer identified before EUS-FNA
Superficial lymphadenopathy present simultaneously

and 3. The aspirated material was divided into 2 parts for cytopathologic and histopathologic assessment, respectively. For all 57 patients, the aspirated material was immediately evaluated by a cytopathologist (TK, YY) for rapid on site diagnosis [4, 5]. The aspirated material was later stained using Papanicolaou's method. For histopathologic evaluation, the material aspirated with a 22-G needle was directly fixed in 10% formalin in a specimen bottle and later embedded in paraffin. The sections were then stained with hematoxylin-eosin (H&E), as for standard tissue sections. If the cause of lymphadenopathy was considered to be hematopoietic disease, additional samples for flow cytometry were also collected. Immunohistochemical (IHC) staining was selected based on the results of the cytology and the H&E stain. When insufficient material was obtained for a definitive pathological diagnosis, other methods such as CT-guided or US-guided biopsy were attempted.

In each case the final diagnosis of benign or malignant disease was based either on pathology or on a follow up of 12 or more months. Malignancy was diagnosed based on the results of EUS-FNA or percutaneous sampling. Cases with non-malignant aspirates were followed up clinically and with serial imaging. If the lymphadenopathy either regressed or disappeared, and the patient remained clinically well for 12 or more months, a benign etiology was confirmed.

Comparing the results of EUS-FNA with the final diagnosis, the sensitivity, specificity and accuracy of EUS-FNA were calculated.

Patient characteristics

There were 57 patients (31 men, 26 women) with mean age of 62 years (range 32–87 years). The average long axis size of the lymph nodes was 25 mm (range 8–81 mm). We aspirated lymph nodes in 37 patients from position 1 (transgastric), in 12 patients from position 2 (duodenal bulb), and in 8 patients from position 3 (descending duodenum).

The final diagnosis was malignant lymphadenopathy in 37 patients (65%) and benign lymphadenopathy in 20 patients (35%). The mean ($\pm$SD) long axis diameters of the lymph nodes in the benign and malignant groups were 20 ± 10 mm and 27 ± 15 mm, respectively ($p = 0.06$) (Table 2).

The etiologic sub-groups among patients with malignant lymphadenopathy included post-operative abdominal nodal recurrence in 16 patients, lymphoma in 12 patients, metastatic cancer with unknown primary in 4 patients, and malignant nodes due to concomitant cancer in 5 patients (gastric cancer, breast cancer, duodenal cancer, testicular cancer, and ovarian cancer). The etiology of benign abdominal lymphadenopathy included reactive nodes in 17 patients, tuberculosis in 2 patients, and sarcoidosis in 1 patient (Table 3).

Table 2 Patients' characteristics

Male: Female = 31:26		
Mean age: 62 years (range 32–87 years)		
Average lymph node diameter: 25 mm (range 8–81 mm)		
Malignant:Benign = 37:20		
Average lymph node diameter according to malignant or benign status		
Malignant	$n = 37$	27 ± 15 mm
Benign	$n = 20$	20 ± 10 mm

Table 3 Final diagnosis of undiagnosed lymphadenopathy ($n = 57$)

Malignant	$n = 37$	Benign	$n = 20$
Local recurrence	16	Reactive change	17
Lymphoma	12	Tuberculosis	2
Concomitant cancer	5	Sarcoidosis	1
Gastric Ca.	1		
Breast Ca.	1		
Duodenal Ca.	1		
Testicular Ca.	1		
Ovarian Ca.	1		
Ca. of primary unknown	4		

Results

Diagnostic accuracy

The average number of needle passes was 2.3 (range 1–6), and adequate specimens were obtained using EUS-FNA in 93% (53 of 57) of the patients. For the 53 patients with adequate samples, the sensitivity, specificity and accuracy of EUS-FNA were 94% (32 of 34 patients), 100% (19 of 19 patients) and 96% (51 of 53 patients), respectively (Table 4). Among the 4 patients with inadequate samples, diffuse large B-cell lymphoma (DLBCL) was diagnosed using US-guided and CT-guided percutaneous aspiration in 2 patients, sarcoidosis was diagnosed using CT-guided percutaneous aspiration in 1 patient, and recurrence of cholangiocarcinoma in 1 patient based on his clinical course.

Post-operative abdominal lymph node recurrence

Fourteen of 16 patients (88%) with metastatic lymphadenopathy were diagnosed by EUS-FNA. The primary lesions included 4 cholangiocarcinomas, 3 pancreatic cancers, 2 gastric cancers, 2 cancers of the papilla of Vater, 2 colon cancers, 1 lung cancer, 1 esophageal cancer, and 1 ovarian cancer (Table 5). One cholangiocarcinoma and one gastric cancer could not be diagnosed due to insufficient material.

Table 4 Diagnosis accuracy of EUS-FNA for lymphadenopathy

Adequate specimen	53/57 = 93%
Sensitivity	32/34 = 94%
Specificity	19/19 = 100%
Accuracy	51/53 = 96%
Accuracy of the cytology	47/53 = 89%
Accuracy of the histology	45/53 = 85%

Table 5 Final diagnosis of lymphadenopathy suspicious for cancer recurrence

Primary lesion	n	Mean Interval of recurrence (range)
Cholangio Ca.	4	1 y 5 m (1–2 y)
Pancreatic Ca.	3	1 y 6 m (6 m–2 y 3 m)
Gastric Ca.	2	7 y 3 m (2 y 7 m–12 y)
Vater Ca.	2	1 y 10 m (1 y8 m–2 y)
Colon Ca.	2	2 y 3 m (2 y–2 y6 m)
Lung Ca.	1	2 y 1 m
Esophageal Ca.	1	2 y
Ovarian Ca.	1	16 y
Totals	16	3 y 3 m (6 m–16 y)

y year, *m* month

The mean interval of recurrence after surgical resection of the primary tumor was 3.25 years (range 6 months–16 years).

The pathological diagnosis was adenocarcinoma in 12 patients, and one each of squamous cell carcinoma and acinar cell carcinoma in patients with resected esophageal cancer and pancreatic cancer, respectively.

Malignant lymphoma

Ten of 12 patients (83%) with lymphoma were diagnosed by EUS-FNA. On cytology, 4 patients were Class V (malignant lymphoma), 1 patient was Class IV (suspicious of malignancy except ductal carcinoma), 4 patients were Class IIIb (suspicious of malignant lymphoma), 1 patient was Class III (atypia, cannot exclude malignancy), and 1 patient was Class II (reactive change) (Table 6).

On IHC studies using CD3, CD5, CD10, CD20 and BCL2 monoclonal antibodies, 5 lymphomas were subtyped as follicular lymphomas and 3 lymphomas were subtyped as diffuse large B-cell lymphomas. The overall diagnostic accuracy of EUS-FNA for diagnosing lymphoma was 83%. Two cases of diffuse large B-cell lymphoma (DLBCL) could not be diagnosed by EUS-FNA due to insufficient material. These were eventually diagnosed using another modality (US or CT).

Table 6 Malignant lymphoma ($n = 12$)

Pt	Sex	Age (y)	FNA			Final diagnosis	Immunostain	Able to make final diagnosis
			Cytology	Comment	Histology			
1	M	36	Class V	Malignant lymphoma	FL	FL	CD3(–), CD5(–), CD10(+), CD20(+), BCL2(+)	Yes
2	F	45	Class V	Malignant lymphoma	DLBCL	DLBCL	CD3(–), CD5(–), CD10(+), CD20(+), BCL2(+)	Yes
3	M	56	Class V	Malignant lymphoma	Suspicious of malignant lymphoma	DLBCL	CD3(–), CD5(–), CD10(+), CD20(+), BCL2(–)	Yes
4	M	76	Class V	Malignant lymphoma	DLBCL	DLBCL	CD3(–), CD5(–), CD10(–), CD20(+), BCL2(+)	Yes
5	M	70	Class IV	Suspicious of malignancy except ductal carcinoma	Inflammatory change	DLBCL[a]	CD3(–), CD5(–), CD10(–), CD20(+), BCL2(±)	No
6	F	60	Class IIIb	S/O Malignant lymphoma	FL	FL	CD3(–), CD5(–), CD10(+), CD20(+), BCL2(+)	Yes
7	F	33	Class IIIb	S/O Malignant Lymphoma Hodgkin's type	No apparent evidence of malignancy	Hodgkin's lymphoma	Not stained	Yes
8	M	66	Class IIIb	S/O Malignant Lymphoma	FL	FL	CD3(–), CD5(–), CD10(+), CD20(+), BCL2(+)	Yes
9	M	43	Class IIIb	S/O Malignant Lymphoma	DLBCL	DLBCL	Not stained	Yes
10	M	56	Class III	Atypia, cannot exclude malignancy	FL	FL	CD3(–), CD5(–), CD10(+), CD20(+), BCL2(+)	Yes
11	F	57	Class II	–	FL	FL	CD3(–), CD5(–), CD10(+), CD20(+), BCL2(+)	Yes
12	M	58	Class II	Reactive change	Insufficient material	DLBCL[b]	CD3(–), CD5(–), CD10(–), CD20(–), BCL2(+)	No

FL follicular lymphoma, *DLBCL* diffuse large B-cell lymphoma

[a] Diagnosed by Echo-guided

[b] Diagnosed by CT-guided

Benign lymphadenopathy

A total of 20 patients had benign lymphadenopathy. Seventeen patients (85%) had reactive lymphadenopathy, 2 had tuberculosis, and 1 had sarcoidosis. The median follow-up period was 6.9 months (range 6.1–71 months). Tuberculosis was diagnosed by polymerase chain reaction, and sarcoidosis was diagnosed on histopathology.

Complications

Procedure-related complication occurred in only one patient (2%). This patient required a total of 10 days hospitalization due to submucosal hemorrhage, but no blood transfusion or surgical intervention was needed.

Discussion

When lymphadenopathy is detected on imaging modalities such as US, CT or PET, a pathological diagnosis is very important for the patient's treatment. Catalano et al. reported that lymph node size ≥ 10 mm, well defined margins, round or oval shape, and low echogenicity were morphologic predictors of malignancy [6]. When an enlarged lymph node fulfils all of these criteria, malignancy can be diagnosed with high accuracy. However, a malignant lymph node does not always fulfill all of these criteria, while benign lymph nodes sometimes fulfill all of them. Thus, in an individual patient, morphology alone is seldom helpful.

Since the first description of EUS-FNA in 1992, the list of lesions that can be targeted by EUS-FNA has grown to include pancreatic mass lesions, submucosal tumors, mediastinal mass lesions, intra-abdominal or intra-thoracic lymph nodes and ascites [7]. The diagnosis of postoperative recurrence of carcinoma is often difficult even with CT, MRI or fluorine-18 fluoro-deoxyglucose positron emission tomography (FDG-PET) follow-up studies [8, 9]. Even though PET is considered to be the better predictive method for local recurrence after operation, its sensitivity and negative prediction value for malignant

lymphadenopathy are very low [10]. Dewitt et al. reported that EUS-FNA could diagnose recurrence of carcinoma with intra-abdominal or mediastinal lymphadenopathy [11]. In the present study, EUS-FNA was performed in patients with undiagnosed lymphadenopathy, and the overall accuracy rate was 96%. Moreover, a diagnosis of postoperative recurrence could be made from 6 months to 16 years after the surgery. The effectiveness of this method is based on the fact that EUS can be used to identify and sample lymph nodes a few millimeters in size. EUS-FNA is also a safer modality for tissue sampling compared to CT or US guided aspirations because of its higher spatial resolution and shorter needle tract to the target. Also, interposed vessels can be easily avoided by real time imaging with EUS. Hence, if the target lesions are peri-luminal we choose EUS-FNA as the first modality for obtaining a tissue diagnosis.

Malignant lymphoma includes a variety of subtypes, many of which can be cured by chemotherapy and/or radiation therapy. An accurate identification of the subtype of lymphoma should be made because treatment is subtype specific [12]. Immunohistochemistry is necessary for identifying the specific histologic type of lymphoma. Ribeiro et al. [13] reported that malignant lymphoma could be diagnosed by cytology and flow cytometry with an accuracy rate of 81%. In the present study, the diagnostic accuracy rate of cytology for malignant lymphoma was 83%. Usually it is believed that histology is a more sensitive technique than cytology for obtaining a final diagnosis of several diseases. However, cytology has been reported to be an equal or more sensitive technique than histology for the diagnosis of breast or thyroid cancer [14]. We think that the results of cytology are equal to or better than histology for the following reasons: Firstly, we are able to make a smear and evaluate the sample adequacy for diagnosis on-site. If the EUS-FNA specimen is inadequate, the target can be punctured again. Secondly, FNA can sample a wide area of the target by moving the needle. Thirdly, the FNA cytology material can also be processed as a cell block and evaluated with conventional histology.

The optimal needle size (19 G, 22 G, 25 G) that should be used for FNA cytology is controversial. Yasuda et al. reported that a 19-G needle is enough to obtain an adequate tissue sample for both H&E and IHC staining [15]. They could diagnose 44 of 48 malignant lymphomas sampled. Up to now, a 22-G needle has usually been used for EUS-FNA because the specimens obtained by EUS-FNA with this size of needle are sufficient to make a differential diagnosis between malignant and benign lesions. In the present study, sufficient material was obtained and immune stained with a

22 G needle, so that 10 of 12 malignant lymphoma patients could be diagnosed.

Conclusion

EUS-FNA is very useful for making a final diagnosis of undiagnosed abdominal lymphadenopathy. It can also obtain adequate material for IHC staining using a 22-G needle. The material obtained from EUS-FNA is sufficient for classifying the subtype of malignant lymphoma in a large percentage of patients.

References

1. Tio TL, Sie LH, Tytgat GN. Endosonography and cytology in diagnosing and staging pancreatic body and tail carcinoma. Preliminary results of endosonographic guided puncture. Dig Dis Sci. 1993;38:59–64.
2. Caletti G, Odegaard S, Rösch T, Sivak MV, Tio TL, Yasuda K. Endoscopic ultrasonography (EUS): a summary of the conclusions of the working party for the tenth world congress of gastroenterology Los Angeles, California October, 1994. The working group on endoscopic ultrasonography. Am J Gastroenterol. 1994;89(8 Suppl):S138–43.
3. Harewood GC, Wiersema MJ. Endosonography-guided fine needle aspiration biopsy in the evaluation of pancreatic mass. Am J Gastroenterol. 2002;97:1386–91.
4. Tada M, Komatsu Y, Kawabe T, Sasahira N, Isayama H, Toda N. Quantitative analysis of K-ras gene mutation in pancreatic tissue obtained by endoscopic ultrasonography-guided fine needle aspiration: clinical utility diagnosis of pancreatic tumor. Am J Gastroenterol. 2002;97:1386–90.
5. Chang KJ, Nguyen P, Erickson RA, Durbin TE, Katz KD. The clinical utility of endoscopic ultrasound-guided fine-needle aspiration in the diagnosis and staging of pancreatic carcinoma. Gastrointest Endosc. 1997;45:387–93.
6. Catalano MF, Sivak MV Jr, Rice T, Gragg LA, Van Dam J. Endosonographic features predictive of lymph node metastasis. Gastrointest Endosc. 1994;40:442–6.
7. Vilmann P, Hancke S, Henriksen FW, Jacobsen GK. Endoscopic ultrasonography-guided fine-needle aspiration biopsy of lesions in the upper gastrointestinal tract. Gastrointest Endosc. 1995;41:230–5.
8. Bluemke DA, Abrams RA, Yeo CJ, Cameron JL, Fishman EK. Recurrent pancreatic adenocarcinoma: spiral CT evaluation following the Whipple procedure. Radiographics. 1997;17:303–13.
9. Belli P, Costantini M, Romani M, Marano P, Pastore G. Magnetic resonance imaging in breast cancer recurrence. Breast Cancer Res Treat. 2002;73:223–35.
10. De Potter T, Flamen P, Van Cutsem E, Penninckx F, Filez L, Bormans G, et al. Whole-body PET with FDG for the diagnosis of recurrent gastric cancer. Eur J Nucl Med Mol Imaging. 2002;29:525–9.
11. Dewitt J, Ghorai S, Kahi C, Leblanc J, McHenry L, Chappo J, et al. EUS-FNA of recurrent postoperative extraluminal and metastatic malignancy. Gastrointest Endosc. 2003;58:542–8.

12. Kwong YL. Predicting the outcome in non-Hodgkin lymphoma with molecular markers. Br J Haematol. 2007;137:273–87.

13. Ribeiro A, Vazquez-Sequeiros E, Wiersema LM, Wang KK, Clain JE, Wiersema MJ. EUS-guided fine-needle aspiration combined with flow cytometry and immunocytochemistry in the diagnosis of lymphoma. Gastrointest Endosc. 2001;53:485–91.

14. Richard DM. The art and science of cytopathology. Aspiration cytology. Chicago: ASCP; 1996.

15. Yasuda I, Tsurumi H, Omar S, Iwashita T, Kojima Y, Yamada T, et al. Endoscopic ultrasound-guided fine-needle aspiration biopsy for lymphadenopathy of unknown origin. Endoscopy. 2006;38:919–24.