Repeated Endoscopic Ultrasound Guided Fine Needle Aspiration (EUS-FNA) Improved Diagnostic Yield of Inconclusive Initial Cytology for Suspected Pancreatic Cancer and Unknown Intra-Abdominal Lymphadenopathy

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Background*: Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is considered as an effective technique to obtain cytology specimens for definite diagnosis of the patients who were suspected of having pancreatic cancer and unknown intra-abdominal lymphadenopathy. The value of repeated EUS-FNA in these patients who had inconclusive first EUS-FNA cytology is not well established.*

Objective*: To determine the yield of repeated EUS-FNA in obtaining a definite diagnosis in patients with clinically suspect for pancreatic cancer and unknown intra-abdominal lymphadenopathy including the reasons for which initial EUS-FNA failed.*

Material and Method*: The authors retrospectively reviewed the EUS database of Siriraj endoscopy center, from January 2007 to May 2011, to identify all patients who underwent repeated EUS-FNA for high index suspicion of pancreatic cancer and unknown intra-abdominal lymphadenopathy. The inconclusive results of the first EUS-FNA, the factors associated with non-diagnosed versus diagnosed cytology results were compared.*

Results*: A total of 478 EUS- FNA were performed in our institution. Fifteen patients (6M, 9F), mean age of 57 $\pm$ 11.8 years (30-72 years) had repeated EUS-FNA done for the evaluation of possible malignant diseases. Eight of these patients presented with pancreatic masses and the other seven patients had unknown intra-abdominal lymphadenopathy. The second EUS-FNA diagnosed and was truly benign in 4 patients. Repeated EUS-FNA facilitated determination of the true status of the disease in 13 of 15 patients which 9 of whom were malignancy. Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of repeated EUS-FNA for both inconclusive pancreatic masses and unknown intra-abdominal lymphadenopathy were 90%, 100%, 100%, 80% and 92.8% respectively.*

Conclusion*: Repeated EUS-FNA for inconclusive initial cytology of patients with suspected pancreatic cancer and unknown intra-abdominal lymphadenopathy provided a very high yield for definite tissue diagnosis and should be recommended.*

Keywords: *EUS-FNA, Pancreatic mass, Lymphadenopathy, Repeated, Fine needle aspiration, EUS guided, Pancreatic cancer*

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Endoscopic Ultrasound-Guided Fine Needle Aspiration (EUS-FNA) is a safe and accurate technique for tissue diagnosis of pancreatic lesions and intra-abdominal lymphadenopathy[1-4]. EUS-FNA is considered to be preferable than the transcutaneous approach for tissue diagnosis, especially for pancreatic and retroperitoneal lesions, because of its intra-luminal approach, which was closer to the target lesions and thus might lead to lower risk of tumor seeding[5]. The accuracy of EUS-FNA for pancreatic and intra-abdominal lymphadenopathy from previous studies was reported about 83-92%[9,11-13] and 96%[14], respectively. Even with this high accuracy, there still be 5-15 percent of the cases which the first EUS-FNA would be considered as a non diagnosis[3]. Value of repeated EUS-FNA in patients suspected of having a

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malignancy after inconclusive first EUS-FNA is not very well established. Cytological analysis of EUS-FNA specimens may have some variable impact factors including target lesions, endosonographers' skill, needle size, FNA technique and cytological evaluation[6-10]. However, there had been only a few reports regarding the value of repeated EUS FNA for continued suspicious of malignancy, after first inconclusive EUS-FNA, results which showed overall accuracy varied from 61 to 96%[12,13]. This dilemma can also be a problem for Thai gastroenterologists when making a decision whether or not doing repeat EUS-FNA. Therefore, the authors conducted the present study to assess the yield of repeated EUS FNA for tissue diagnosis in the patients suspected of pancreatic cancer, including unknown intra-abdominal lymphadenopathy with initial non diagnosed EUS-FNA cytology. And the authors also would like to determine the reasons for which initial EUS-FNA failed.

Material and Method

The authors maintained contact with Siriraj Institutional Review Board committee (SIRB). The medical records and endoscopic reports of those who underwent EUS FNA from January 2007 to May 2011 were retrospectively reviewed. A Majority of all EUS procedures and a majority of the patients who underwent repeated EUS-FNA, performed by experienced endosonographers were included in the present analysis. The definition of a non-diagnosis included cases in which FNA was attempted in patients with suspected malignancy for one of the following reasons: 1) inadequate sample material was reported, 2) adequate sample material was obtained, but cytology was negative for malignancy (which were reported as 2.1) negative for malignancy, 2.2) inflammation process or reactive process, 2.3) non-diagnosed sampling, 2.4) Atypical cell). All patients gave written informed consents before undergoing the procedures. All of the procedures were performed in the endoscopy suite. All patients got intravenous sedation with propofol administered by anesthesiologists. The EUS was carried out using a curvilinear array echoendoscopes (GF UC-140, Olympus, Tokyo, Japan). Three standard approaches were used for scanning intra-abdominal organs and lymphnodes. The lesions at pancreatic head were punctured through the first part of duodenum. The lesions located at pancreatic body and tail were approached through the gastric wall. The uncinate lesions were punctured through the descending duodenum. The lymphnodes were approached through the gastric or duodenal wall depending on the location of target lymphnode. 22 gauge FNA needles were used (NA 200H-8022, Olympus, Tokyo, Japan). EUS-FNA was carried out under Doppler guidance to avoid intervening vascular structures. The aspirated material was spread onto glass slides with immediate alcohol fixation and also flushed into the formalin containing bottle. An on-site cytologist was not available at Siriraj Hospital, so the aspirated material was sent elsewhere for cytological analysis within 24 hours. Post-procedure, the patient was observed over night and was discharged the day after the procedure. In the presented study, the authors utilized the cytology results which were interpreted by experienced cytopathologists. In case of repeated EUS-FNA, all the procedures carried out were performed by the same endosonographer as the previous procedure. Surgical histopathology (in case of surgery performed), cytology positive for malignancy and/or cytology suspicious for malignancy with clinical follow-up were used as a gold standard for final diagnosis.

Statistical analysis

Descriptive statistics were used to summarize baseline demographic and clinical characteristics. Continuous variables were reported as mean and standard deviation while categorical variables were reported as proportions. Sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated. The factors associated with non-diagnosed versus diagnosed cytology results were compared.

Results

From January 2007 to May 2011, four hundred and seventy eight cases of EUS-FNA were performed in Siriraj Hospital. A total of 15 patients (3.1%) of which 9 were female, mean age of 57 $\pm$ 11.8 (range 30-72 years) had repeated EUS-FNA done due to undiagnosed cytology results of the first approaches. Eight of them underwent EUS-FNA due to being suspected of pancreatic cancer and 7 patients presented with unknown intra-abdominal lymphadenopathy. Two-third of pancreatic masses located at head while the peri-pancreatic and celiac lymphnodes were predominant. The average size of the pancreatic lesions was 3.7 cm in maximal diameter and less than 2 cm for intra-abdominal lympnodes. Two to three passes were made and 9-10 glass slides were obtained per case. There was no statistical significant difference between the first and second needle passes nor number of

slides. The malignant endosonographic characters such as vascular involvement and liver nodules were found in 75% of the patients who had pancreatic mass. No serious complications were encountered in any cases of EUS-FNA. No complications were related with sedation. The patients' demographic data and the details of endosonographic characters were shown in Table 1.

All the patients were followed-up for an average of 21 months (2-48 months). Of the fifteen patients who underwent repeated EUS-FNA, the final diagnosis could be made in 13 cases. One of the second EUS-FNAs was involved inadequate sampling. Another case was false negative cytology for which the final diagnosis was achieved from surgical histopathology that showed adenocarcinoma. Table 2 shows etiological distribution at EUS-FNA and final diagnosis. Regarding the details described in the table below, the sensitivity, specificity, positive predictive value, negative predictive value and accuracy of repeat EUS-FNA for both inconclusive pancreatic masses and unknown intra-abdominal lymphadenopathy were 90%, 100%, 100%, 80% and 92.8% respectively. For subgroup analysis, the sensitivity, specificity, positive predictive value ,negative predictive value and accuracy of repeat EUS-FNA for inconclusive pancreatic masses were 100%, 100%, 100%, 100% and 100% while those of unknown intra-abdominal lymphadenopathy were 75%, 100%, 100%, 75% and 85.7%, respectively. EUS features indicating malignant pancreatic mass was found in all cases of pancreatic cancer although the first EUS-FNA cytology results were inconclusive. However, endosonographic characteristics of malignant lymphnodes were found in both benign and malignant diseases.

Six patients with pancreatic mass of whom EUS-FNA cytology resulted in the final diagnosis of pancreatic cancer (false negative initial study) are shown in Table 3. Regarding the data in Table 3, the authors observed that three of six cases were the cases in which the cytological preparation might be difficult for the cytopathologist to interpret the slides and one of the cases was a small lesion (less than 1 cm in diameter). The number of needle passes might be less for those cases also. Most of the patients underwent repeated EUS-FNA within 8 weeks and in only one case did the tumor increase in size.

Discussion

Regarding the results of the present study, the authors learned that EUS- FNA played an important role in diagnosis of both pancreatic lesions and intra-abdominal lymphadenopathy. This endosonographic technique can be performed safely with minimal complications. This procedure did have some impact on the patient managements and was also beneficial for the attending physician or gastroenterologists for making the decision whether a lesion should be followed-up or the patients should be referred to surgery. Even with the inconclusive cytology results of the initial EUS-FNA, repeating the procedure would still be of benefit for the patients. According to the sensitivity, specificity, negative predictive value and positive predictive value, including the accuracy of the second EUS-FNA mentioned above, the authors should recommend the second attempt for the ones who failed EUS-FNA for the first time. The repeated procedure should be done within 8 weeks after the first procedure to avoid undue progression of the tumor. Anyway, Eloubeidi et al[3] reported that more than three procedures did not increase the yield of EUS-FNA thus the authors should proceed to another options to obtain the final diagnosis, such as CT guided biopsy, laparoscopic tissue samplings or surgical interventions. According to the data in Table 2, if the endosonographic malignant characteristics were seen combined with pancreatic mass in those patients suspected of pancreatic cancer, the likelihood of pancreatic cancer would be very high. Therefore, repeated EUS-FNA might not be necessary if it would not have any impact on the treatment. But this approach could not be applied to the patients with unknown intra-abdominal lymphadenopathy. Because of the small number of patients in the present study, there were some limitations of interpretation especially the reasons for which the first EUS-FNA failed. In general, the yield of EUS-FNA depends on multiple factors such as the location of target lesions which would be related the technical difficulty, the endosonographers's skill, needle size and the number of needle passes. The on-site cytologist for evaluation of the aspirated tissue was also required to increase the adequacy and accuracy of EUS-FNA. In Siriraj Hospital, the authors used the same size of needles and the endosonographer who performed the second EUS-FNA was the same as the one who performed the previous procedure. Therefore, both factors would not be the causes of the initial failure. The authors proposed that the reasons of failed initial EUS-FNA in the presented study might be from inappropriate aspirated tissue preparation/fixation and too small size of the lesion (technical difficulty). To improve the accuracy of

Table 1. The demographic data and endoscopic characteristics of first and second procedures

Data	First EUS-FNA n = 15 (%)		Second EUS-FNA n = 15 (%)	
Sex				
Male	6			
Age (years)	57.2 (30-72)			
Underlying diseases				
None	13			
Diabetes Mellitus	1			
Cirrhosis	1			
Family history of cancer				
No	15			
Provisional diagnosis	Pancreatic Mass n = 8	Lympha-denopathy n = 7	Pancreatic Mass n = 8	Lympha-denopathy n = 7
Pancreatic cancer	8	0		
Metastasis cancer	0	2		
Lymphoma	0	4		
Others (tuberculosis)	0	1		
Endosonographic findings				
Mean Size (cm)	3.7	1.8	3.4	2.2
Location				
Uncinate	0		0	
Head	6		6	
Genu	0		0	
Body	2		2	
Tail	0		0	
Celiac		3		3
Peri-pancreatic		3		3
Aortocaval		1		1
Echogenicity				
Hypoechogenicity	7	5	6	6
Hyperechogenicity	0	0	0	0
Isoechogenicity	1	2	2	1
Heterogenicity				
Homogeneous	7	2	7	2
Heterogeneous	1	5	1	5
Malignant characteristics				
Vascular involvement				
Yes	6	0	6	0
No	2	7	2	7
Liver nodules				
FNA passes	2.1	3.3	2.3	3.3
Amount of glass slides(slide)	10.4	9.3	10.3	10.0

the EUS-FNA in Siriraj Hospital, the authors should reorganize the system of cytological diagnosis, especially as do when an on-site cytopathologist might be required. Finally, the authors would like to propose the managment algorithm for the lesions suspected pancreatic cancer as below (Fig. 1).

Conclusion

Repeated EUS-FNA after initial inconclusive initial cytology of patients with suspected pancreatic

Table 2. The demographic data and cytology results of first and second EUS-FNA, including final diagnosis

No.	Sex	age	Type of lesion	Location	Malignant features from EUS*	Adequacy of sampling	First EUS-FNA cytology	Second EUS-FNA cytology	Final Diagnosis
1	Female	30	LN	peripancreatic	Yes	Yes	Atypical cell	Tuberculosis	Tuberculosis
2	Male	46	LN	Celiac	Yes	Yes	Negative for malignancy	Amyloidosis	Amyloidosis
3	Female	61	Pancreas	HOP	Yes, vascular	Yes	Atypical	Positive for malignancy	Ca pancreas
4	Female	63	Pancreas	Body	Yes, vascular	Yes	Benign	Suspicious for malignancy	Ca pancreas
5	Female	60	LN	Celiac	Yes	Yes	Benign	Suspicious for malignancy	Metastatic adenocarcinoma
6	Male	43	LN	Celiac	Yes	Yes	Non-diagnostic	Benign	Chronic pancreatitis
7	Female	65	LN	Peripancreatic	Yes	Yes	Atypical	Suspicious for lymphoma	Follicular lymphoma
8	Male	72	Pancreas	Body	Yes, liver nodules	Yes	Negative for malignancy	Positive for malignancy	Ca pancreas
9	Male	63	LN	Aortocaval	Yes	Yes	Negative for malignancy	Inflammation	Adenocarcinoma
10	Female	48	Pancreas	HOP	Yes, vascular	No	Inadequate	Suspicious for malignancy	Ca pancreas
11	Male	53	Pancreas	HOP	No	Yes	Negative for malignancy	Benign	Chronic pancreatitis
12	Male	69	Pancreas	HOP	No	Yes	Negative for malignancy	Inadequate	Resolving pancreatitis
13	Female	63	Pancreas	HOP	Yes, vascular	Yes	Negative for malignancy	Positive for malignancy	Ca pancreas
14	Female	60	Pancreas	HOP	Yes, vascular	Yes	Non-diagnostic	Suspicious for malignancy	Ca pancreas
15	Female	62	LN	Peripancreatic	Yes	No	Inadequate	Suspicious for malignancy	Ca pancreas

Abbriviations: LN ; lymphnode , HOP ; Head of Pancreas , Ca; cancer, *EUS features indicating malignant pancreatic mass: vascular involvement, multiple liver nodules, peritoneal nodules with ascites, EUS features indicating malignant lymphadenopathy: round shape, sharp edge, size >1 cm, hypoechoicity

Table 3. The demographic data and cytology results of fault negative initial EUS-FNA

Patients' details (sex, years)	First cytology result	Cytologist 's comment	Location	Number of needle passes	Time to second procedure	Size of masses (cm.)	Tumor status
1 .female, 61	Atypical cells	Excessive thickness, air dry	Head	2	8 weeks	4.0	Stable
2. Female, 63	Benign Cellular change	None	Body	2	2 weeks	2.8	Stable
3. Male, 72	Negative for malignancy	Necrotic tumor tissue	Body	4	3 weeks	4.2	Stable
4. Female, 48	Inadequate	None	Head	2	5 weeks	3.8	Stable
5. Female, 63	Negative for malignancy	None	Head	2	8 weeks	0.8	larger
6. Female, 60	Non-diagnosis	Scant cellularity	Head	2	6 weeks	4.0	Stable

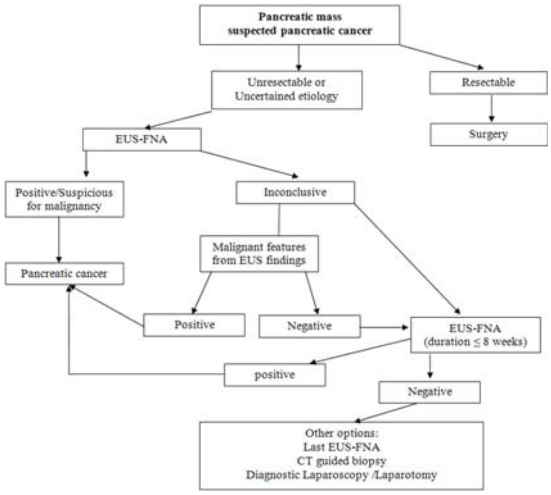

Fig. 1 The proposed algorithm for management of pancreatic mass suspected cancer

cancer and unknown intra-abdominal lymphadenopathy provided a very high yield for definite tissue diagnosis and should be recommended. However, the reasons of initial failure of EUS-FNA in our study were the inappropriate aspirated tissue preparation/fixation and too small size of the lesion (technical difficulties). Thus, an on-site cytopathologist might be required to improve the accuracy of the first EUS-FNA procedure and reduce the total expense for the patients and the hospital.

Potential conflicts of interest

None.

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การตรวจเซลล์วิทยาด้วยวิธีเจาะดูดเนื้อเยื่อตับอ่อนและต่อมน้ำเหลืองผ่านทางกล้องอัลตราซาวด์ซ้ำช่วยเพิ่มโอกาสการวินิจฉัยผู้ป่วยที่สงสัยมะเร็งตับอ่อนหรือต่อมน้ำเหลืองในช่องท้องโตที่ไม่ทราบสาเหตุในกรณีผลการตรวจด้วยวิธีดังกล่าวครั้งแรกไม่ชัดเจน

วรายุ ปรัชญกุล, ทัศนีย์ ศรีประยูร, ปิตุลักษณ์ อัศวกุล, สุพจน์ พงศ์ประสบชัย, นนทลี เผ่าสวัสดิ์, อุดม คชินทร

วัตถุประสงค์: *เพื่อศึกษาการตรวจเซลล์วิทยาด้วยวิธีเจาะดูดเนื้อเยื่อตับอ่อน และต่อมน้ำเหลืองผ่านทางกล้องอัลตราซาวด์ซ้ำในผู้ป่วยที่สงสัยมะเร็งตับอ่อนหรือต่อมน้ำเหลืองในช่องท้องโต ที่ยังไม่ทราบสาเหตุช่วยเพิ่มโอกาสการวินิจฉัยในกรณีที่ผลการตรวจด้วยวิธีดังกล่าวครั้งแรกไม่ชัดเจน รวมทั้งหาปัจจัยที่มีผลทำให้การตรวจครั้งแรกไม่ประสบผลสำเร็จ*

วัสดุและวิธีการ: *ทบทวนเวชระเบียนของผู้ป่วยที่สงสัยว่าเป็นมะเร็งตับอ่อน และผู้ป่วยที่มีต่อมน้ำเหลืองในช่องท้องโตโดยไม่ทราบสาเหตุโดยที่ผู้ป่วยเหล่านั้นต้องเคยได้รับการตรวจส่องกล้องอัลตราซาวด์เพื่อวินิจฉัย โดยวิธีเจาะดูดเนื้อเยื่อตับอ่อน และต่อมน้ำเหลืองผ่านทางกล้องอัลตราซาวด์มาก่อน และได้รับการตรวจซ้ำ เพื่อยืนยันถึงสาเหตุดังกล่าว ระหว่างเดือนมกราคม พ.ศ. 2550 ถึงเดือนพฤษภาคม พ.ศ. 2554 โดยเก็บข้อมูลเพื่อทราบถึงอาการแสดงทางคลินิก ข้อบ่งชี้ในการตรวจซ้ำ ลักษณะรอยโรคที่ตรวจพบทางอัลตราซาวด์ ความเพียงพอของเนื้อเยื่อที่ได้รับจากการใช้เข็มขนาดเล็กเจาะและดูดเซลล์ ผลทางพยาธิวิทยา ภาวะแทรกซ้อนที่เกิดขึ้นหลังการทำ รวมถึงการติดตามผู้ป่วยเพื่อประเมินอาการทางคลินิก จากนั้นนำข้อมูลทั้งหมดมาเปรียบเทียบเพื่อหาปัจจัยที่มีผลให้การตรวจวินิจฉัยครั้งแรกไม่ประสบผลสำเร็จ*

ผลการศึกษา: *จากจำนวนผู้ป่วยที่ได้รับการตรวจเซลล์วิทยาด้วยวิธีเจาะดูดเนื้อเยื่อ ของรอยโรคผ่านทางกล้องอัลตราซาวด์ทั้งสิ้น 478 ราย มีผู้ป่วยที่ได้รับการตรวจทางเซลล์วิทยาด้วยวิธีเจาะดูดเนื้อเยื่อตับอ่อน และต่อมน้ำเหลืองผ่านทางกล้องอัลตราซาวด์ซ้ำทั้งสิ้นจำนวน 15 ราย เป็นชาย 6 ราย และหญิง 9 ราย อายุเฉลี่ย 57 ปี (พิสัยระหว่าง 30-72 ปี) แบ่งตามอาการแสดงทางคลินิกกล่าวคือ ก้อนที่ตับอ่อนจำนวน 8 ราย และต่อมน้ำเหลืองในช่องท้องโตโดยไม่ทราบสาเหตุอีก 7 ราย ผลการตรวจโดยวิธีส่องกล้องอัลตราซาวด์ และตรวจเนื้อเยื่อซ้ำสามารถให้การวินิจฉัยโรคได้ชัดเจนถึง 13 จาก 15 ราย ในจำนวนนี้เป็นโรคที่ไม่ใช่มะเร็งจำนวน 4 ราย คิดเป็นความไว ความจำเพาะและความแม่นยำเท่ากับร้อยละ 90, 100 และ 92.8 ตามลำดับ*

สรุป: *การตรวจเซลล์วิทยาด้วยวิธีเจาะดูดเนื้อเยื่อตับอ่อน และต่อมน้ำเหลืองผ่านทางกล้องอัลตราซาวด์ซ้ำในผู้ป่วยที่สงสัยมะเร็งตับอ่อนหรือต่อมน้ำเหลืองในช่องท้องโตที่ยังไม่ทราบสาเหตุ ช่วยเพิ่มโอกาสการวินิจฉัยในกรณีที่ผลการตรวจด้วยวิธีดังกล่าวครั้งแรกไม่ชัดเจน*