Endoscopic ultrasound guided fine needle aspiration in biliary and pancreatic diseases : pitfalls and performances

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Abstract

Endoscopic ultrasound guided fine needle aspiration (EUS-FNA) has become the most accurate modality for characterization of pancreatic cystic and solid lesions, for differential diagnosis of indeterminate pancreatic masses and for locoregional staging of pancreatic and extrahepatic biliary tumours. EUS-FNA should also be performed in distant lymph nodes, ascites, liver, adrenal and mediastinal metastatic locations. Experienced groups reach a sensitivity over 85% with a 90-100% specificity, a positive predictive value of 98-100%, a negative predictive value of 44-80%, and an accuracy of 75-84% in evaluation of pancreatic masses. Morbidity rate (acute pancreatitis, infection, haemorrhage, perforation) is very low being around 1-2% and risk of peritoneal seeding was shown to be significantly lower than percutaneous CT guided FNA. The performance of this technique is dependent on the endoscopist and cytopathologist experience, the location, size and consistency of the tumour and the number of passes in the lesion. The type of echoendoscope or needle used does not influence the results, whereas it remains debated if presence of the cytopathologist on site might improve FNA performances. These last years, a new liquid-based cytology technique has been developed to process the specimen. Different methods exist to prepare this type of material and all these techniques improve EUS-FNA performance by decreasing the number of inadequate specimens and by increasing the possibility to obtain cell blocks allowing for ancillary techniques such as immunohistochemistry and molecular biology. (Acta gastroenterol. belg., **2004**, 67, **294-300**).

Key words : endosonography, endoscopic ultrasound, EUS, FNA, pancreas, biliary, pathology, cytology, monolayer.

Introduction

Endoscopic ultrasound (EUS) has the unique capability to associate endoscopic viewing and ultrasonographic imaging of the digestive wall and surrounding organs. Since the first independent reports by DiMagno and Strohm in 1980, the evolution seen with EUS over the last 2 decades is impressive (1,2). It is now recognized as the most accurate method for local imaging of mucosal and submucosal lesions and staging of oesophageal, gastric, rectal and pancreaticobiliary cancer.

EUS-FNA was first reported in 1991 (3-5) and is now available in most experienced endoscopy units. In recent years, the technique has even been further developed into injection therapies and guidance of interventional procedures, such as cyst and pancreatobiliary drainage (6,7).

The role of EUS-guided tissue sampling is well established in the characterization of pancreatic cystic and solid lesions, the differential diagnosis of indeterminate pancreatic masses and the locoregional staging of pancreatic and extrahepatic biliary tumours (8). EUS-FNA should also be performed in distant lymph nodes, ascites, liver, adrenal and mediastinal metastatic locations (9-11). New indications include the diagnosis of chronic pancreatitis (12) and combination of diagnosis and treatment, for example celiac block or neurolysis in patients with chronic pancreatitis or non-resectable pancreatic tumours (13,14). The advantages of EUS-FNA over transcutaneous approaches include a better resolution of the pancreatobiliary area which allows targeting very small lesions (3-5 mm), a low morbidity rate and a lower risk of peritoneal seeding during puncture. Peritoneal carcinomatosis may indeed occur more frequently in patients who undergo percutaneous FNA compared with those who have EUS-FNA for the diagnosis of pancreatic cancer (15).

Successful EUS-FNA needs appropriate indications and should only be used to impact the clinical management of the patients (16-18). The main indications of EUS-FNA are detailed in Table 1. In unresectable and resectable pancreatic masses, EUS-FNA is mainly aimed at confirming malignancy and determining the tumour type. The performance of the technique relies mainly on the endoscopist and cytopathologist experience, the location, size and consistency of the tumour and the number of passes in the lesion. The type of echoendoscope or needle used does not influence the results, whereas it remains debated if presence of the pathologist on site improves the performances (19,20).

Performances

Endoscopic ultrasound is the most sensitive method for the detection of pancreatic masses (21, 22), especially in lesions smaller than 2-3 cm. This was confirmed in our institution when compared to magnetic resonance imaging and positron emission tomography in a recent study (Table 2) (23). This high sensitivity allows EUS-FNA to be performed in lesions not even seen with magnetic resonance imaging and computed tomography (24). The sensitivity of EUS-FNA reaches more

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Table 1. — **Indications of EUS-FNA in pancreaticobiliary diseases**

Document malignancy before chemotherapy in unresectable tumours
Confirm malignancy before surgery *(if asked by the surgeon or patient)*
Document absence of malignancy *(if probability is low)*
Differentiate tumour type
– adenocarcinoma
– endocrine tumour
– lymphoma
– metastasis
– rare tumours
Check distant lymph node (M1 disease)
Check ascitis
Check liver
Check mediastinum
Characterize cystic lesions (cytology and biochemical markers)

than 85% with a 90-100% specificity, a positive predictive value of 98-100%, a negative predictive value of 44-80%, and an accuracy of 75-84% in the diagnosis of malignancy in pancreatic masses (Table 3).

Compared with pancreatic and bile duct brushings, EUS-FNA is considered superior in terms of sensitivity. We retrospectively compared the two methods in a subgroup of 26 patients with pancreatic masses of indefinite diagnosis, in whom both methods had been used either successively or during the same endoscopic procedure. Accuracy of bile duct brushing was 65%, pancreatic duct brushing 66.7%, EUS-FNA 73.1%, and of all methods combined 92.3%. There were no false positive results, whereas the false negative rate was 7.7% (2/26). Combination of brush cytology and EUS-FNA should therefore be used in undetermined pancreatic tumours (25). We also showed that ERCP with brush cytology and EUS-FNA could be performed during the same session without any increase in the complication rate (25).

In cystic pancreatic lesions, EUS-FNA has also demonstrated its clinical impact on patient management (26,27). We previously reported the results of EUS-FNA in a cohort of 36 patients evaluated between 1997 and 1999 (28). Patients underwent full assessment including EUS evaluation, cytology and biochemistry of the cystic fluid (amylase, lipase, CEA and CA19-9). Mean lesion size was 37 mm (9-100). EUS was performed under sedation with midazolam and antibiotics were given for 5 days. Final diagnosis was obtained by surgery or endoscopy (33%) or follow-up (67%) during a mean of 13.5 months (range 1-42). Accuracy of diagnosis was 88.9% for EUS, 97.2% for cytology, 77.7% for biochemistry and 97.2% for the combination of all techniques (Table 4). Complications (sepsis) were reported in 2/73 pts (0/36) and treated medically or by endoscopy. At present follow-up, no false negative result has been reported. We concluded that combination of EUS, cytology and biochemical analysis yielded an overall accuracy of 97% in pancreatic cystic lesions, with few side effects, allowing avoidance of further surgical diagnostic procedures in most of the cases (28).

EUS was recently shown to have an interesting impact on cost in the evaluation of pancreatic carcinoma when it is included in the algorithm (29,30). Another study found that the overall costs were reduced by the use of EUS in place of ERCP, also obviating potential complications (31). A recent study similarly found that the use of EUS positively influenced cost savings when a model was made to compare costs associated with EUS fine-needle aspiration (FNA), CT FNA, and

Table 2. — **Detection of pancreatic masses : role of EUS, magnetic resonance imaging and positron emission tomography**

	EUS	FDG-PET	MRI	CT
Sensitivity	48/50 (96%)	44/50 (88%)	34/39 (87%)	7/11 (63%)
Tumors ≤ 2 cm	6/7 (86%)	5/7 (71%)	4/4 (100%)	1/3 (33%)
Tumors 2-3 cm	24/26 (92%)	24/26 (92%)	16/21 (76%)	4/5 (80%)
Tumors ≥ 3 cm	17/17 (100%)	17/17 (100%)	14/14 (100%)	2/3 (66%)
Specificity	10/12 (83%)	8/12 (66%)	9/11 (82%)	–

Table 3. — **Comparison of studies evaluating EUS-FNA of pancreatic masses**

Reference	Year	N	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Chang (65)	1995	20	91	100		94	94
Cahn (16)	1996	50	88				
Bhutani (66)	1997	47	64	100	100		
Williams (67)	1999	144	72	100	100	76	76
Voss (68)	2000	90	75	88	98	74	74
Powis (69)	2000	164	83	90	100	85	85
Erickson (51)	2000	95	88				
Mortensen (70)	2001	22	82				
Raut (71)	2003	233	91	100	100	92	92
Agarwal (58)	2004	86				89	88

PPV : positive predictive value.
NPV : negative predictive value.

Table 4. — **Results of EUS and EUS-FNA in pancreatic cystic lesions**

	EUS	Cytology	Amylase (IU/L)	CEA (ng/ml)	CA199 (U/ml)
Neoplasic (9)	9/9	9/9	79 (5-8014)	1409 (0.2-24247)	999990 (12-2400000)
Mucinous (3)	2/3	2/3	1750 (4-6160)	724 (24-59794)	574219 (250-999999)
Serous (11)	10/11	11/11	71 (22-2000)	3.4 (0.4-180)	264 (5-78574)
Pseudocysts (11)	10/11	11/11	34050 (487-255500)	19 (2.7-900)	23730 (9-2234766)
Endocrine (2)	2/2	2/2	0,27	0,4.2	0,26.5

surgery for staging (32). Interestingly, cost savings appeared to come from the detection of distant malignant adenopathies, and not from direct tumour T-staging. The impact of EUS-guided FNA on clinical management was also studied and showed that FNA precluded surgery, avoided the need for further diagnostic tests, and influenced clinical decisions in more than half of the patients, thus providing substantial cost savings (33). In patients evaluated with CT with or without FNA, EUS-FNA diagnosis was significantly better for identifying and diagnosing pancreatic masses and associated lymph nodes and surgical procedures for diagnosis decreased by 75% after the introduction of EUS-FNA at the authors' institution. Compared with the pre-EUS era, survival was increased significantly by 3 months, which was attributed to the earlier diagnostic capability and greater sensitivity of EUS-FNA (34).

Pitfalls

EUS-FNA is an endoscopic procedure which associates the global morbidity of endoscopy and sedation with the specific morbidity linked to the puncture in the pancreatobiliary area (35). Most complications consist in acute pancreatitis (mainly mild or moderate), infection, haemorrhage and perforation, fortunately with a rate below 1-2% (36,37). The risk of peritoneal seeding was recently shown to be significantly lower than CT guided FNA (38). These complications can somehow be prevented by using the shortest transpancreatic route into the lesion, prophylactic antibiotics in cystic or necrotic lesions and Doppler imaging to avoid vascular vessels or highly vascularized tumoral areas (18).

Factors precluding EUS pancreatic imaging include Zenker diverticulum, upper gastrointestinal strictures, previous surgery such as Billroth II procedures, and intolerance of the patient to endoscopy. FNA should not be performed in lesions located at more than 7 cm from the digestive lumen and should be postponed in patients with coagulation problems. Furthermore some pancreatic lesions are missed by EUS which is not a foolproof method of detecting a pancreatic neoplasm. Possible associated factors that may increase the likelihood of a false-negative EUS examination include chronic pancreatitis, a diffusely infiltrating carcinoma, a prominent ventral/dorsal split and a recent episode (< 4 weeks) of acute pancreatitis. If there is a high clinical suspicion of pancreatic neoplasm, if EUS and other imaging methods are negative, and if the patient does not undergo surgery, a recent study suggests that a repeat EUS after 2-3 months may be useful for detecting an occult pancreatic neoplasm (39).

Performance of EUS-FNA is dependent on the endoscopist and cytopathologist (in)experience, the location, size and consistency of the tumour and the number of passes in the lesion. The type of echoendoscope or needle used does not influence the results, whereas it remains debated if presence of the pathologist on site improves the performances (40). These "pitfalls" will all be detailed below.

Inexperience of the endoscopist

Accurate EUS-FNA requires proper anatomical knowledge of normal and abnormal anatomy in the pancreatobiliary area. A "learning curve" has been demonstrated as with other endoscopic or interventional procedures. EUS-FNA accuracy was significantly improved with more experience from 80 to 92% in a recent multicenter study (36). In another study, the only variable that was found to be a significant predictor of EUS-FNA accuracy in the multivariable model was operator (in)experience (20). A significant increase in sensitivity has been observed after improvement in specific technical skills : shortening of echoendoscope position, scrupulous maintenance of the needle tip US view at all times, swift jabbing punctures, sampling multiple areas of the mass in each pass, and performing more than 10 "jiggles" per needle pass. Sensitivity for the diagnosis of pancreatic cancer was greater than 80% after 25-35 examinations, a level that was maintained afterwards (41).

The current American Society for Gastrointestinal Endoscopy guidelines have proposed that for comprehensive competence in all aspects of EUS at least 150 supervised EUS cases should be performed, with 50 EUS-guided FNA, and at least 75 pancreaticobiliary cases (42). The minimal ASGE recommendations are 25 supervised EUS-FNA pancreatic procedures to become proficient. However it may take more than 100 EUS-FNA to become "comprehensively competent" in all aspects of EUS-FNA and this assumes that basic EUS skills are already in place.

Inexperience of the cytopathologist

The gold standard to evaluate performance of EUS-guided FNA is based on a combination of surgical and/or clinical follow-up. A follow-up tissue confirmation

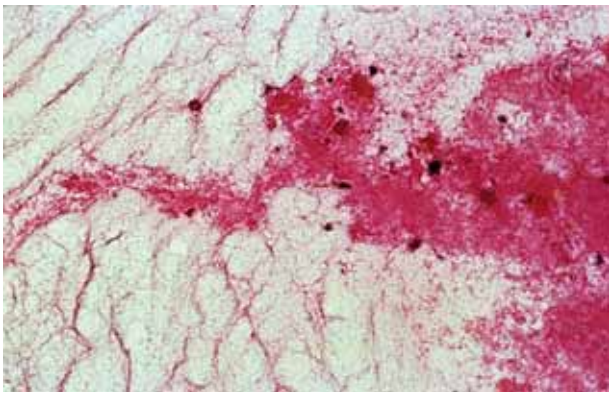

Fig. 1. — Pancreatic FNAB (magnification : ×130) : **mucinous cystic neoplasm** characterized by a mucinous background stained by PAS and a lot of macrophages.

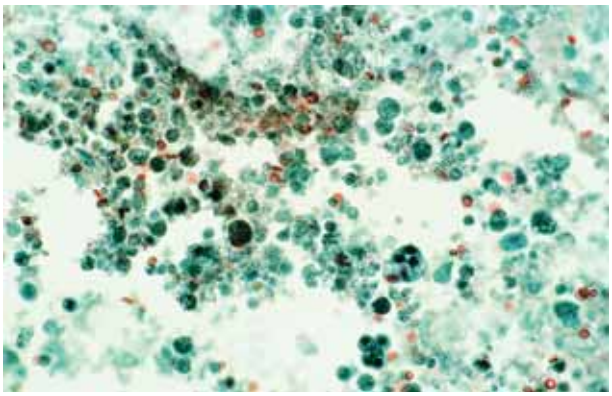

Fig. 2. — Pancreatic FNAB (magnification : ×130) : **cystic adenocarcinoma.** In a dirty background with abundant necrosis, notice macrophages and isolated malignant cells with hyperchromatic nuclei.

for a cytological diagnosis is not always possible and cytology is then the only available tissue confirmation (43). This means that the results of the EUS-FNA is largely dependent on the correct cytologic interpretation of the specimen and therefore on the cytopathologist experience.

It is well known that accuracy of FNA increases when the technique is performed by an experienced clinician and when the slides are reviewed by an experienced cytopathologist (44). The guidelines published in 1997 by the Papanicolaou Society of Cytopathology help to maximise the reliability of FNA procedures. They insist on rapid assessment of aspirates, team approach (cytopathologist, radiologist and clinician), proper training and maintenance of competence, clear and precise communication and rapid turn-around time for reporting (45).

This is particularly important in pancreatic, biliary and hepatic EUS-guided FNA, because these organs are deeply located. Therefore the material obtained may be paucicellular and is frequently contaminated by material from surrounding organs. The experienced cytopathologist is able to recognise these contaminants, such as squamous cells from the oesophagus, digestive glandular cells and mucus from the stomach or duodenum, pancreatic acinar cells or neuroendocrine cells from neuroendocrine hyperplasia observed in chronic pancreatitis and this experience should avoid false-positive diagnoses (43,46).

The role of an experienced cytopathologist is not only to be as performing as possible, but also to identify poor aspirators, who may benefit from targeted training and advice to improve thereby the quality of FNA specimens and patient care (47). On every report the specimen cellularity should therefore be mentioned.

Difficulties for the endoscopist : bad location, small sized or firm lesions

The most difficult punctures occur in a stenotic duodenum or with tumours located in the uncinate process. To access this location, FNA can either be performed in a straight endoscope position from the proximal bulb or the antrum, but with a risk of instability, or in a long position from the distal second part of the duodenum. Angulations of the endoscope may however prevent progression of the needle in the accessory channel or may cause mistargeting of the lesion due to the altered endoscope shape. In such a case, the endoscope has sometimes to be pulled back in the stomach, the needle being advanced in the channel without excessive protrusion to avoid perforation of the duodenal bulb when re-advancing the endoscope in the duodenum. Experience with ERCP techniques will usually facilitate EUS and EUS-FNA of the pancreas head.

Pancreatobiliary EUS-FNA can be challenging for other reasons. Pancreatic tumours may be fibrotic and indurated, resisting penetration by the needle and requiring considerable force to move the needle in the lesion. Risks associated with this problem are perforation induced by these difficult and forceful movements in the duodenum and insufficient cell sampling (17,48). Firm lesions may cause difficulties even in the body and tail of the pancreas. These lesions are indeed accessed with the echoendoscope “floating” in the stomach and losing contact with the tumour through the gastric wall. Placing the patient in a prone position may sometimes improve ultrasonic contact with the lesion during puncture. Difficult penetration in the lesion may be overcome by thinner needles (25-gauge) and automated spring-loaded or trucut needles (48-50). In our experience however, these needles did not provide better specimens, were rather tricky to use in the pancreatic head and could not be easily reused for successive passes.

Several reports showed that the majority of 22-gauge commercial needles were efficient and equivalent in performance and diagnostic yield (51,52). They have to combine excellent acoustic properties to be perfectly seen when targeting the lesion and good mechanical properties such as a good grip, a lock to avoid excessive

penetration in the targeted zone, and a device permitting adaptation to different endoscopes types and lengths.

Size of the lesion may also be challenging. Very small lesions (< 5 mm) should not be punctured. Small lesions of 5-10 mm are accessible but require greater targeting accuracy and may defy FNA because of the tendency of the needle to displace the target during advancement. Sampling of small lymph nodes is especially challenging because they are embedded in loose connective tissue. Similarly, large lesions may be necrotic in their centre. The puncture should target the periphery of the tumour in such cases.

Inadequate FNA technique

The number of passes required to obtain an adequate specimen is highly variable in the literature. Most experts from USA advocate a minimum of 3-5 passes with a range of 1-19 (18,51). A recent paper proposed a minimum of 7 passes in the pancreas and 5 passes in lymph nodes (53). The number of passes is usually determined by the cytologist attending the endoscopy suite. A high number of passes sometimes implicates the use of a second or a third needle. In most European centres these "facilities" are not affordable and the number of passes will depend on the subjective visual assessment of the specimen. In our unit the mean number of passes is 2 (range of 1-4) in a solid pancreatic tumour and 1 (1-2) in cystic lesions, with an accuracy over 88%. We try to limit the number of passes and increase the time spent in the lesion since we observed that precision of targeting and efficacy of puncture decreased during subsequent uses of the same needle.

The technique of EUS-FNA has been well described in the literature (54-57). The lesion has to be placed within the needle tract and Doppler can be used to assess adjacent vascular structures. After the needle has been advanced in the lesion with the stylet slightly retracted, the stylet is pushed back to eject a possible plug and then removed. Suction is applied with a 10-20 mL syringe when the needle is moved back and forth within the lesion. The suction is released when the needle is retracted in the catheter, usually after ten or more back and forth movements in various directions.

Some technical tips may increase the yield of EUS-FNA. In solid tumours the centre may be necrotic and needles passes should therefore target the periphery of the tumour. Movements should be slow to avoid excessive bleeding and lateral movements with the shaft of the echoendoscope should provide varying tracts for the needle. A large accessory channel will facilitate these movements as well as an elevator. In cystic lesions, we prefer to empty the cyst as completely as possible in one single needle pass. The fluid collected is than checked visually and tested for its viscosity. Some of it is sent for biochemical analyses (amylase, lipase, CEA and CA199) and the rest given to the cytopathologist. Thereafter it is crucial to obtain some material from the cystic wall of from any solid component of the cyst, either during the same needle pass or during subsequent passes. Large cysts are sometimes better punctured with a 19-gauge needle which will empty the cyst faster and will provide more material when fluid viscosity is high. These needles are however very stiff and associated with a higher risk of complications for the patient (bleeding) and for the endoscope (accessory channel perforation). They are more difficult to use in the duodenum and should be reserved in pancreaticobiliary disease for specific indications such as cyst puncture and interventional endosonography (58).

Inadequate cytology processing

In the past, the material obtained by FNA in our unit was spread on one or two glass slides and immediately immersed in methanol. The syringe containing the rest of the material was rinsed in 50% alcohol. This material was then centrifuged and cytocentrifuged. All slides were stained with the Papanicolaou method. During this time, the non contributory specimen rate was quite high (about 17%).

These last years a new preparation technique for cytological material has been described : the liquid-based cytology technique. This method has first been applied to Pap-smears permitting an easier lecture of the slides and the possibility of molecular biology studies using the left-over material, such as PCR for HPV-testing. It is now also used for FNA (59). The material obtained by FNA is placed in a conservation fluid, in which the puncture needle can also be rinsed. For cystic lesions, the aspirated fluid can entirely be placed in the vial, whereas for solid lesions, it is better to make one or two smears and then to put the rest of the material in the vial.

Different methods such as Papspin® (Thermo Electron Corporation), Surepath® (Tripath Inc), Thinprep® (Cytyc Inc), Cytoscreen® (Seroa), Turbitec® (Labonord),… can be used to prepare monolayer slides from this material, based on sedimentation, centrifugation or filtration techniques. All these methods permit to obtain a slide on which the cells are evenly dispersed on a rectangular or round surface. If necessary the left-over material can be embedded in paraffin and be used for histochemistry or immunohistochemistry, or even molecular biology (60-62). The advantages of this new method are numerous. There are less non-contributory FNA, because of concentrated material. In our department, the use of a centrifugation-based technique since two years has permitted to lower the rate of non-contributory specimen from 17% to 5%. The obscuring haemorrhagic background can be reduced and the presence of left-over material can be used for complementary studies (59).

Difficulties for the cytologist

Even with these new techniques some lesions remain difficult to diagnose. Neuroendocrine tumours are, by definition, highly vascularized lesions. The FNA

specimen obtained is often very haemorrhagic with only a few diagnostic cells. Tumours with a dense, fibrous stroma give rise to paucicellular smears, because there are only few tumour cells difficult to extract from the fibrosis (63).

As already mentioned, FNA can also be contaminated by material from the different tissues the needle passes through, such as mucus, epithelial cells from the digestive mucosa, mesothelial cells, hepatocytes, ductal cells from the main pancreatic duct or inflammatory cells (46). Most false-positive diagnoses are therefore caused by interpretation errors (64).

Crystalline or darkish material may also be found, directly originating from the biopsy channel of the endoscope or the needle shaft, which may render the lecture of the slides difficult (59). Contamination should be avoided by minimising the number of passes and by performing a proper puncture with the stylet first slightly retracted in the needle during the pass and then pushed forward once the needle is inserted into the lesion to eject the contaminating "plug".

To improve the results, the endoscopist should sample all abnormal appearing tissue around the main tumour such as lymph nodes and hepatic lesions and label them correctly (43).

Conclusions

EUS-FNA is a reliable technique with a high sensitivity, specificity, positive predictive value, and accuracy in the assessment of biliopancreatic tumours. Experience both on the endoscopic and the cytopathologist side is the key factor for improved performance. New liquid-based cytology techniques seem promising to decrease the rate of non contributory specimens but these techniques should not replace a multidisciplinary approach to biliopancreatic disease. Anatomo-clinical correlation remains indeed essential for a correct interpretation of the specimen obtained by FNA.

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