TOPIC HIGHLIGHT

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Endoscopic ultrasonography guided-fine needle aspiration for the diagnosis of solid pancreaticobiliary lesions: Clinical aspects to improve the diagnosis

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Abstract

Endoscopic ultrasonography-guided fine-needle aspiration (EUS-FNA) has been applied to pancreaticobiliary lesions since the 1990s and is in widespread use throughout the world today. We used this method to confirm the pathological evidence of the pancreaticobiliary lesions and to perform suitable therapies. Complications of EUS-FNA are quite rare, but some of them are severe. Operators should master conventional EUS observation and experience a minimum of 20-30 cases of supervised EUS-FNA on non-pancreatic and pancreatic lesions before attempting solo EUS-FNA. Studies conducted on pancreaticobiliary EUS-FNA have focused on selection of suitable instruments (*e.g.*, needle selection) and sampling techniques (*e.g.*, fanning method, suction level, with or without a stylet, optimum number of passes). Today, the diagnostic ability of EUS-FNA is still improving; the detection of pancreatic cancer (PC) currently has a sensitivity of 90%-95% and specificity of 95%-100%. In addition to PC, a variety of rare pancreatic tumors can be discriminated by conducting immunohistochemistry on the FNA materials. A flexible, large caliber needle has been used to obtain a large piece of tissue, which can provide sufficient histological information to be helpful in classifying benign pancreatic lesions. EUS-FNA can supply high diagnostic yields even for biliary lesions or peri-pancreaticobiliary lymph nodes. This review focuses on the clinical aspects of EUS-FNA in the pancreaticobiliary field, with the aim of providing information that can enable more accurate and efficient diagnosis.

Core tip: Since the first attempts in 1990th, the instruments and methodology associated with endoscopic ultrasonography-guided fine needle aspiration have been largely improved for greater safety and efficacy of the procedure and accuracy of diagnosis. Choices of suitable needle and puncture method (fanning, suction, stylet, number of the passes) are critical for the better diagnostic yields of the pancreaticobiliary lesions as well as improving endosonographic skills.

INTRODUCTION

Until the 1990s, the diagnostic accuracy of pancreaticobiliary lesions was limited because of the tissue sampling procedures used, which were mostly endoscopic retrograde cholangiopancreatography (ERCP) or occasionally extra-abdominal approaches [*e.g.*, computed tomography (CT)-guided or ultrasonography (US)-guided procedures]. Forceps biopsy[1] and brush cytology[2] have been applied during ERCP to provide confirmatory histological evidence. However, diagnosis of pancreatic carcinoma (PC), even in recent studies, is limited by a sensitivity of 49%-66% for pancreatic duct brushing cytology, with a complication of 3%-6% of post-ERCP pancreatitis[3,4], and pancreatic duct forceps biopsy has seldom been reported since the 1990s[5]. In contrast, these methods are routinely performed for bile duct carcinoma (BDC) and have achieved excellent diagnostic yields [forceps biopsy (77%-92%)[1,6] and brush cytology (75%-79%)[2,7]].

Endoscopic ultrasonography-guided fine-needle aspiration (EUS-FNA) is a safe and efficient diagnostic tool that provides pathological results for the lesions[8-10]. This method was first reported by Harada *et al*[11] and Caletti *et al*[12] in 1991, who attempted to obtain tissues from para-esophageal lymph nodes of the dog and human gastric submucosal tumors. Today, most of the lesions in or around the gastrointestinal tracts are targets of EUS-FNA[8]. The standard technique simply consists of the following steps: (1) visualization of the target by EUS; (2) selection of the puncture line; (3) needle puncture; (4) removal of the stylet; (5) suction by vacuum syringe; (6) back-and-forth movement of the needle; (7) removal of the needle; and (8) expulsion of the sample from the needle using the stylet[13] (Figure 1). The diagnostic capability of this method for cytopathological diagnosis of PC is highly sensitive and accurate[9,14-17] (sensitivity: 85%; specificity: 98%, by a meta-analysis performed in 2012)[17]; however, it is affected by various factors, such as scope position[13], needle type[18-21], FNA methodology[22-25], characteristics of the lesions, environments surrounding the lesions, on-site pathologist[8,13,15,19,24]. Recently, the instruments associated with EUS-FNA have been improved, including videoscopes[26] and FNA needles[21,27,28], as have the methodologies[19,22,25,29]. Associated studies have confirmed the improvements of EUS-FNA in the pancreaticobiliary field.

DIAGNOSTIC YIELD AND SAFETY OF EUS-FNA FOR SOLID PANCREATIC LESIONS

EUS-FNA is an excellent diagnostic tool for obtaining cytopathological evidence from pancreatic mass lesions. The reported sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy for detecting PCs were 79%-98%, 71%-100%, 96%-100%, 33%-85%, and 82%-98%, respectively[9,15,16,21,22,24,25,29-36]. False negative and false positive rates were 12%-14%[30,37] and 0%-5%[30,31,37,38], respectively.

EUS-FNA can usually be performed safely. When the pancreatic solid mass is targeted, the most common complication is mild pancreatitis and the complication rate ranges from 0%[9,15] to 3.4%[10,28,29,35,39,40]. Targeting of small masses (≤ 20 mm) and endocrine tumors are reported as incurring risks of complications[39]. Rare but serious complications have been reported, such as severe bleeding (0.2%)[41], rupture of pseudoaneurysm[42], pancreatic pseudocyst[43], abscess[35], and cancer seeding[44,45]. Bleeding is a concern with this procedure; however, a prospective study performed on patients taking aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) did not show increased level of bleeding events, suggesting that EUS-FNA is safe in patients taking these anticoagulants[46].

Infectious complication, bacteremia or sepsis is scarcely caused by EUS-FNA for the pancreatobiliary solid lesions. Barawi *et al*[47] and Levy *et al*[48] prospectively examined blood cultures after EUS-FNA near the gastrointestinal tract and reported 5.8% (3/52)[48]-6.0% (6/100)[47] of culture-positive cases. However, all these patients were asymptomatic and the detected bacteria were coagulase negative *Straphylococcus*, *Streptococcus viridans* and so
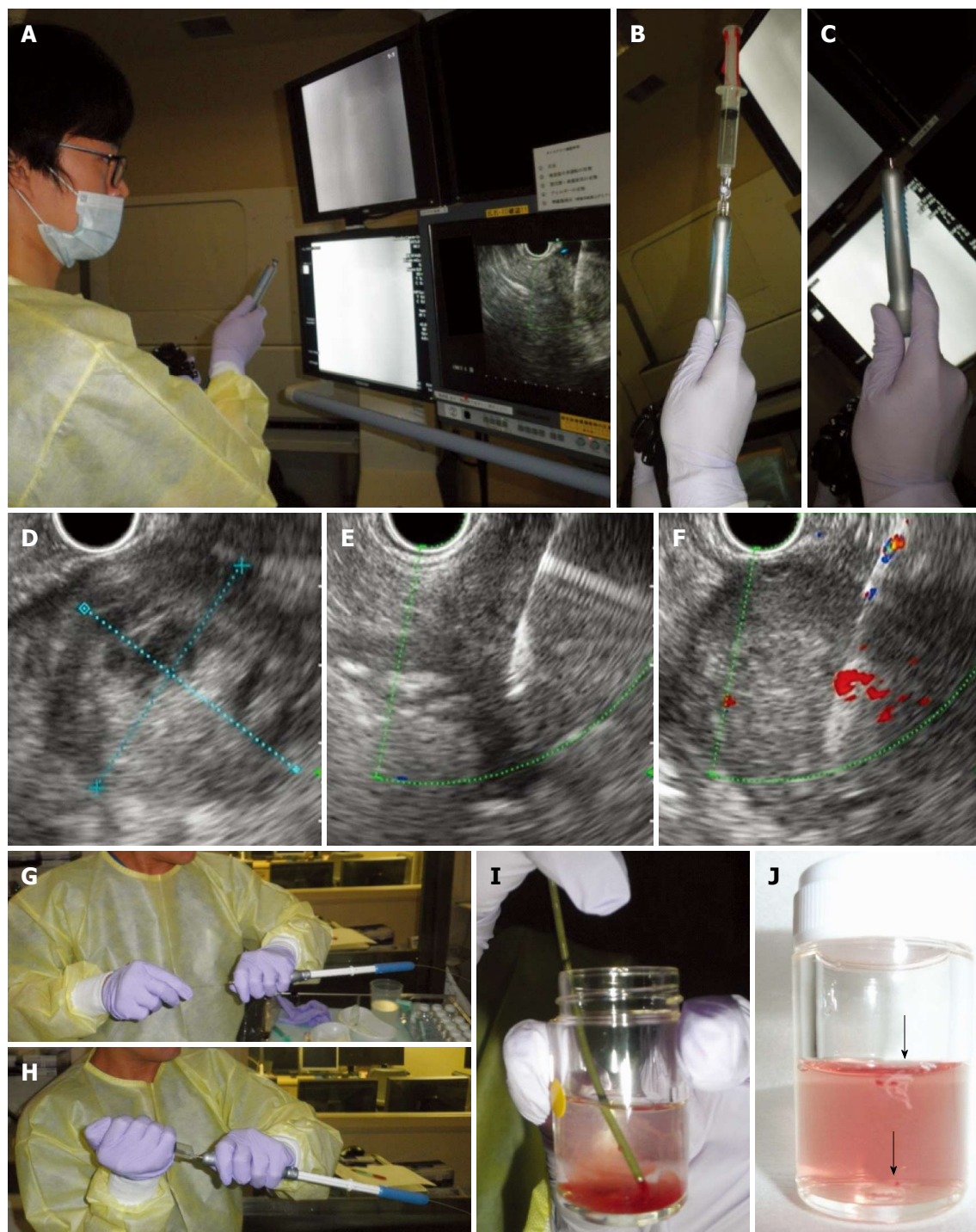
Figure 1 Actual condition of an endosonographer performing endoscopic ultrasonography guided-fine needle aspiration of a solid pancreatic lesion. A: An endosonographer puncturing a pancreatic mass by fine needle aspiration (FNA) needle with a stylet inside the needle; B: An FNA needle aspirated with a 10 mL syringe attached to the top (Suction method); C: An FNA needle without no suction applied by a syringe (Non-suction method); D: A target pancreatic lesion, with a central necrotic area, depicted by ultrasonography and measured for its size; E: A pass at the upper side of the tumor, avoiding the central necrotic area; F: A pass at the lower part of the tumor by the fanning method; G: Expulsion of the aspirated material by insertion of a stylet; H: Flushing out of the residual material with air (This process can often be skipped.); I: A bloody sample extruded into a medium; J: Whitish components separated into another container.

forth, considered to be contaminations. Other studies have noted bacteria-proven sepsis or febrile event only in small proportion of EUS-FNA procedures (0%-1%)[9,10,31,49]. Although the use of antibiotic prophylaxis is still arguable in the cases of cystic lesions, however at least, is not recommended for EUS-FNA of solid pancreatic lesions[48,50].

Tumor seeding is a late complication that is possibly induced by EUS-FNA, and several case reports have demonstrated gastric and/or peritoneal dissemination in cases with cancer at the pancreatic body and tail[44,45,51]. However, to date, no significant effects of EUS-FNA suggestive of increased levels of dissemination or worsening of survival have been found by several retrospective studies. For example, Ngamruengphong *et al*[52] analyzed 2034 patients with surgically resected PC in the Surveillance Epidemiology and End Results (SEER) medical database of the United States during 1998 and 2009; 498 (24%) of these patients underwent EUS-FNA. The study demonstrated a marginally improved prognosis in the EUS-FNA group than in the non-EUS-FNA group, even when the data were adjusted for the tumor site. However, this finding may be simply reflect the current advances in surgery, as cases with PC now tend to undergo more presurgical EUS-FNA. Japanese studies analyzing 82-107 cases of resected PC[53,54] reported no worsening of the incidence of peritoneal dissemination[54] and overall survival[53] in cases where EUS-FNA was used than when it was not (peritoneal dissemination: 17% *vs* 17%[54], overall survival: 1042 d *vs* 557 d; better in the EUS-FNA (+) group, *P* < 0.05[53]). Similar results were obtained in a study that included unresectable cases [217 cytopathologically confirmed PC cases divided into an ERCP group (161 cases) and an EUS-FNA group (56 cases)], and a similar occurrence was noted for peritoneal carcinomatosis in the ERCP group (15% during 545 d) and in the EUS-FNA group (18% during 599 d) (*P* = 0.85)[55].

FACTORS AFFECTING THE DIFFICULTY AND THE LEARNING CURVE OF THE EUS-FNA PROCEDURE

The technical difficulty of EUS-FNA is affected by the location, size[34], hardness, necrosis, and vascularity of the target lesion, by large vessels lining the lesion, by the stability of the scope position[13] and by the needle size[18]. Acute angulation of the scope tip, torsion of the scope shaft, and intensive elevation of the needle sheath hamper smooth needle movement and increase the difficulty of the procedure. Puncture of the pancreas from the stomach is easily performed when the target is large, without disturbing the large

vessels on the puncture line. However, a transduodenal puncture often needs angulation of the scope and needle elevation. In these cases, a 22 or 25 gauge (G) needle[18] is suitable and a flexible 19G needle[20,56] may also work.

Before starting the EUS-FNA procedure, the operator should master the convex-type EUS. In 2001, the American Society of Gastrointestinal Endoscopy recommended that an operator trainee conduct 150 supervised EUS procedures (including 75 pancreaticobiliary indications) and 60 cases of FNA (including 25 pancreatic FNAs) before the determination of competency[57]. The sensitivity of the cytopathological diagnosis of PC increases with the operator's experience and is reported to reach 80% after 20-30 cases of supervised EUS-FNA training[58,59]. Accordingly, a minimum of 20-30 cases of supervised EUS-FNA on non-pancreatic and pancreatic lesions is recommended by the European Society of Gastrointestinal Endoscopy[60].

Selection of FNA needles and puncture methods are important factors associated with the efficacy and accuracy of EUS-FNA diagnosis of solid pancreatic lesions.

SELECTION OF THE FNA NEEDLE

To date, several aspiration biopsy needles and Trucut needles have been used for EUS-FNA or EUS-guided core biopsy (Table 1 and Figure 2). The standard needle for pancreatic EUS-FNA is a 22G, but the needle size is selected by the presumed histological type and location of the targets. In general, a thinner needle (25G) is more flexible and therefore suitable for target lesions that require tight angulation of the scope and/or elevator[18], such as lesions at the pancreatic head. In contrast, a thicker aspiration needle and a Trucut needle (19G) lack flexibility and maneuverability, but can obtain a large piece of tissue, which provides more information for pancreatic pathology. For instance, pancreatic tissues obtained by a 19G FNA needle or core biopsy needle are useful in the diagnosis of pancreatic tumors other than pancreatic adenocarcinoma, tumors surrounded by chronic pancreatitis, lymphoma[61], and autoimmune pancreatitis[28]. However, a flexible 19G needle made of nitinol has recently been used on pancreatic head lesions and enabled satisfactory tissue acquisition from the pancreatic head in 95% of the cases[62]. Another recent advance has been the incorporation of a side port at the needle tip, which promotes efficient tissue acquisition even during the withdrawal manipulation. A comparative study of sampling efficacy from the peripancreatic and gastrointestinal lesions demonstrated that fewer passes were needed for adequate tissue acquisition when using a 22G needle with a side trap than with a 22G standard needle[27].

Table 1 Needles used for endoscopic ultrasonography guided-fine needle aspiration and core biopsy

Manufacturer	Needle		
	Product	Type	Size (gauge)
Boston Scientific	Expect	Aspiration needle	19, 22, 25
	Expect Flex	Aspiration needle	19
Beacon Endoscopic	BNX	Aspiration needle	19, 22, 25
ConMed	Vizeon	Aspiration needle	19, 22
COOK	EchoBrush	Needle with cytology brush	19
	EchoTip ProCore	Aspiration needle with a core trap	19, 22, 25
	EchoTip Ultra	Aspiration needle	19, 22, 25
	QuickCore	Core biopsy needle	19
Hakko	Sonopsy CY	Aspiration needle	21
Medi-globe	Sonotip Pro Control	Aspiration needle	19, 22, 25
	Hancke-Vilmann EUS-FNA System	Aspiration needle	19, 22
Olympus	EZ Shot	Aspiration needle	22
	EZ Shot 2	Aspiration needle	19, 22, 25
	EZ Shot 2 with sideport	Aspiration needle with a sideport	22
	Power Shot	Aspiration needle	22

METHODOLOGY OF EUS-FNA

Fanning method

PC is sometimes accompanied by necrosis, mostly in the central area of the tumor (Figure 1). A previous study using transabdominal ultrasound-guided FNA reported that sampling from the peripheral area of the pancreatic mass improved the diagnostic accuracy[63]. The same is also true with EUS-FNA[60]. In this sense, the fanning method is considered effective, as it collects greater numbers of viable tumor cells. The needle movements within the multiple marginal areas of the mass using the "up-down" dial of the endoscope releases more cells when compared to the standard method that targets one peripheral area of the mass[23] (Figure 1). Bang *et al*[23] demonstrated the efficacy of a fanning method that targeted four marginal sites of the tumor; they needed significantly fewer passes to establish diagnosis than with the standard method [by randomized control trial (RCT) median 1 (interquartile range: 1-1) *vs* 1 (1-3), $P = 0.02$] and found a significantly higher rate of achieving a diagnosis with a single pass (85.7% *vs* 57.7%; $P = 0.02$).

Suction level

The standard EUS-FNA is done with a needle controlled under negative pressure, usually applied with a 10-20 mL syringe[29] (COOK: 10 mL, Boston Scientific and Medi-Globe: 20 mL). However, the suction has been altered to determine its effect on FNA; *i.e.*, by the no suction method[32,36,64,65], slow pull method[22] and high-negative pressure (HNP) method[29]. The no suction method is performed without suction[32,36,64,65] (Figure 1). The slow pull technique applies 10-20 to-and-fro needle movements with simultaneous minimum negative pressure provided by slow and continuous pulling of the stylet from the needle[22]. The HNP method is conducted under a vacuum provided by a 50 mL syringe [in contrast to the normal-negative pressure (NNP) method that uses a 10 mL syringe for vacuum[29].

Recent studies have confirmed that higher amounts of tissue are acquired and that blood contamination increases when the suction level is increased for EUS-FNA of solid pancreatic lesions[22,29,32]. An RCT by Puri *et al*[32] demonstrated a higher sensitivity by adding suction (86% in suction and 67% in non-suction, $P = 0.05$), but subsequent studies[22,65] showed no diagnostic superiority for the suction method. Interestingly, a retrospective study by Nakai *et al*[22] revealed a higher accuracy of the slow-pull method than with the ordinary suction method, but only when using 25G needles (91% *vs* 70%, $P = 0.004$); no difference was noted with a 22G needle. A comparison between HNP and NNP for EUS-FNA of a pancreatic mass using 25G needles confirmed that HNP was superior in terms of adequate tissue acquisition and accurate histological diagnosis compared to NNP (adequate tissue: 90% *vs* 72%, $P = 0.0003$; diagnostic accuracy: 82% *vs* 73%, $P = 0.06$). A high level of blood contamination was recognized in the HNP samples ($P = 0.004$), but the numbers of blood cells did not affect the histological diagnosis. A concern was noted for highly vascular lesions such as pancreatic neuroendocrine tumors (PNETs), as only limited cases have been examined[29]. For the FNA of lymph nodes, the quantity of tissue acquired is usually good and suction is not recommended, in order to reduce blood contamination[66].

With or without a stylet

The stylet is believed to prevent a contamination of the sample with tissue that does not originate from the target lesion; however, procedures for pushing out and withdrawing the stylet are time consuming. Three RCTs[67-69] found no superiority arising from the use of a stylet in terms of tissue contamination and diagnostic yield, and conversely found the adequacy of sample acquisition to be inferior (stylet: 75% *vs* non-stylet: 87%, $P = 0.01$) and saw an increase in blood
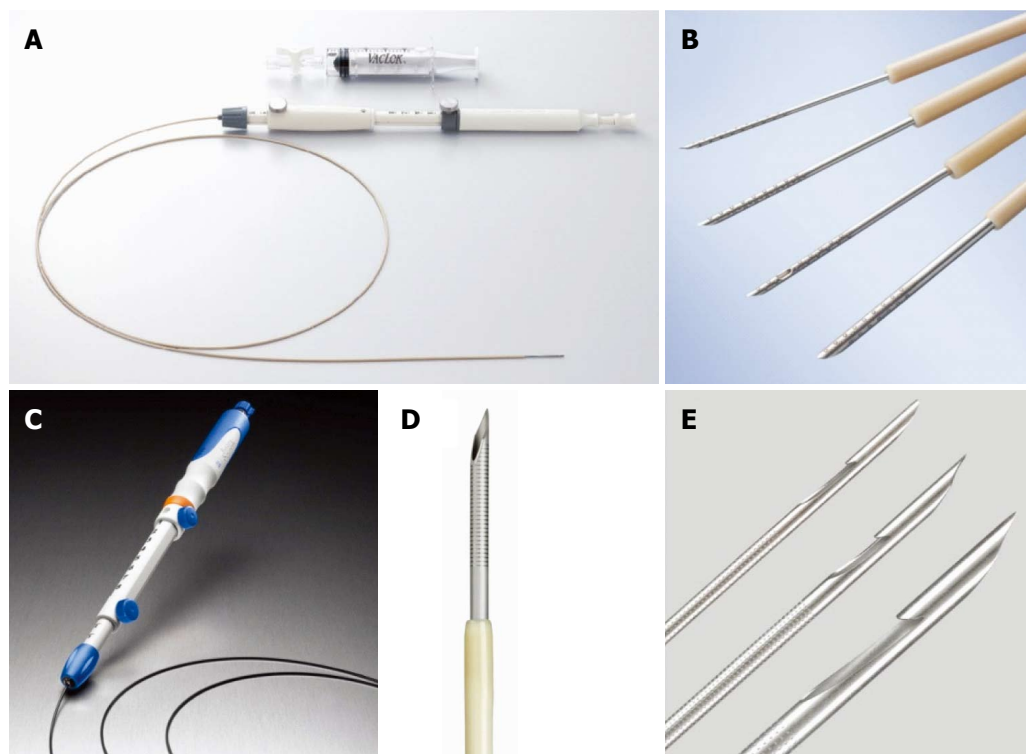
Figure 2 Variations in the fine needle aspiration needles. A: EZ shot 2 (Olympus); B: Different size and shape of needles (EZ shot 2, from top to the bottom: 25 gauge (G), 22G, 22G with a side port); C: Expect™ Slimline (Boston Scientific); D: 19G-flex needle of Expect™; E: Different size of the needles (ProCore, COOK, from top to the bottom: 25G, 22G, 19G).

contamination (75% *vs* 52%, *P* < 0.0001)[68]. However, as mentioned, a slow pull of the stylet during the pass could improve the quality of these FNA samples[22]. A stylet is also useful for pushing the tissues out from the needle onto slides or into a medium (Figure 1).

Number of passes

An on-site pathologist can provide the endo-sonographer with helpful information; for example, if the samples obtained by EUS-FNA contain tissues from the targets or whether additional procedures are needed[15]. However, this system is not feasible at all institutions and not in every tertiary hospital[22,24,70]. In the absence of rapid on-site evaluation (ROSE), knowing the optimum number of passes is critical information[24,25].

Earlier reports during 2000-2004 recommended 5-7 passes[33,71] for cases with a pancreatic mass. Erickson *et al*[71] reported in 2002 that cytological diagnosis of malignancy was obtained in 104 (95%) of 110 cases with PC. The average number of needle passes was 3.4 ± 2.2 (range: 1-10) with ROSE, and the number of passes was affected by the differentiation level of the cancer (well differentiated cancer: 5.5 ± 2.7, moderately differentiated: 2.7 ± 1.2, moderately to poorly differentiated: 3.4 ± 2.1, poorly differentiated: 2.3 ± 1.1) (*P* <0.001)[71]. LeBlanc *et al*[33] reported in 2004 that 7 passes were needed to achieve a sensitivity of 83% and specificity of 100%

from pancreatic and miscellaneous lesions. However, recent studies demonstrated excellent sensitivity (90%-94%)[16,24,25] and specificity (96%-100%)[16,24,25] with fewer numbers of passes. Suzuki *et al*[25] reported an almost equal sensitivity and specificity of 25G needle EUS-FNA for the solid pancreatic lesion between a fixed number of 4 passes (93% and 100%) and a ROSE dependent procedure with a mean of 2.3 passes (94% and 100%). The sensitivity of the first method was increased by adding passes; 53% by 1 pass, 73% by 2 passes, 87% by 3 passes, and 93% by 4 passes. The most recent RCT by Ramesh *et al*[20] reported a similar high sensitivity by cytology obtained until the 2nd pass, with either a 19G flexible needle (92%) or a 25G needle (90%). When on-site cytological information is not available, gross inspection of the whitish component in the obtained materials is simple and useful for judgment of good sampling[24] (Figure 1).

EUS-FNA FOR RARE SOLID PANCREATIC LESIONS

Pancreatic neuroendocrine tumor

The pancreatic neuroendocrine tumor (PNET) accounts for < 3% of all pancreatic neoplasms[72], and is often defined as a well-demarcated, highly vascular tumor based on clinical images. These image characteristics sometimes resemble solid pseudopapillary tumors (SPTs), acinar cell carcinomas (ACCs), and solid-type

serous cystic tumors[73]; the required therapy varies among these tumors. Even within the category of PNET, therapeutic strategy varies by the histological grade (G1, G2 and G3), which is defined by the mitotic index and Ki-67 labeling index[74]. Therefore, EUS-FNA must be able to provide a differential diagnosis as well as accurate grading for treatment of PNET.

PNET has been correctly diagnosed by EUS-FNA at a rate of 77%[75]-90%[76]. Chen *et al*[75] reported that 54 (77%) of 70 histologically confirmed PNETs were preoperatively diagnosed as PNET by EUS-FNA. A cytological diagnosis of the remaining 16 cases of showed suspected PNET (4), no atypical cells (4), atypical cells (3), unsatisfactory material (2), adenocarcinoma (2), and suspected carcinoma (1), suggesting an overall difficulty in the cytological diagnosis of PNET. Chatzipantelis *et al*[77] reported the most helpful cytological findings of PNETs were a richly cellular sample with a monotonous, poorly cohesive population of small or medium-sized cells with granular chromatin (salt and pepper) and plasmacytoid morphology; helpful immunochemical diagnostic markers were neuron-specific enolase, synaptophysin, and chromogranin A.

Figueiredo *et al*[76] reported a large discrepancy between FNA and surgical materials (κ: 0.38, P = 0.003) when using a previous grading system of WHO criteria (2004). However, many recent reports demonstrated high agreement with this grading system. Comparison between EUS-FNA cytology and histology of the resected material showed a concordance of WHO grade and percent agreement of the Ki-67 index of 86% (19/22)[78] and 89% (κ: 0.78)[79], respectively. Histology of EUS-FNA samples and surgical materials also showed high agreement (79%[80]-89%[81]) with the WHO grade based on the Ki-67 index. Hasegawa *et al*[80] analyzed the intratumoral dispersion of the Ki-67 index of PNET and demonstrated a higher level of dispersion in G2 PNET (0.78) than in G1 PNET (0.03) (P < 0.001). In their study, grading concordance increased up to 90% when the FNA sample contained $\geqslant$ 2000 tumor cells, suggesting a necessity for a large amount of sample for accurate grading or prediction of patient's survival[80].

Other pancreatic tumors

ACC[82-84], mixed acinar carcinoma[83-85], SPT[86,87], and intraductal tubullopapillary neoplasm (ITPN)[88,89] can also be diagnosed using EUS-FNA samples. These tumors are histologically similar and often require additional immunostainings for differentiation markers: BCL10, lipase, and trypsin are useful markers specific to ACC[83,84]; ITPN is often positive for cytokeratin 7 and 19, MUC1, and MUC6[88,89], but negative for MUC2 or MUC5AC, which are usually stained in intraductal papillary mucinous neoplasms (IPMNs)[88]. The cytological features of SPT are papillary structures, cercariform cells, large cytoplasmic vacuoles, foam cells, and giant cells. Immunostaining of SPT is usually positive for nuclear β-catenin, but usually negative for chromogranin or lipase[86].

Mass-forming pancreatitis including autoimmune pancreatitis

Mass-forming pancreatitis includes a progressive form of the ordinary chronic pancreatitis or a specific etiology such as focal-type of autoimmune pancreatitis (AIP). The former histologically shows various level of acinar atrophy associated with a progression of the fibrous stroma, sometimes accompanied with ductal dilation, calculi, and squamous metaplasia of the ductal epithelia[72,90]. Autoimmune pancreatitis (AIP) is a unique form of chronic pancreatitis, sometimes resembling pancreatic malignancies on clinical images; it is histologically classified into type 1 (lymphoplasmacytic sclerosing pancreatitis: LPSP) and type 2 (idiopathic duct-centric pancreatitis: IDCP)[91].

Imai *et al*[16] described that EUS-FNA specimens obtained from 21 cases of AIP using 22G needles showed no histology meeting the criteria of LPSP or IDCP, but was beneficial only for eliminating PC. In contrast, Ishikawa *et al*[92] performed EUS-FNA using 22G needles on 47 cases of AIP, and obtained level 1 histological findings of LPSP in 9 cases (19%), level 2 LPSP in 5 cases (11%), and level 2 IDCP in 3 cases (6%). Iwashita *et al*[28] also reported that EUS-FNA using 19G needles supplied adequate tissue for the histological diagnosis of AIP in 43% (19/44) of the cases, suggesting the importance of the amount or size of EUS-FNA samples for proper diagnosis of AIP.

OUTCOME AND MANAGEMENT OF THE PATIENT WITHOUT CONCLUSIVE DIAGNOSIS BY EUS-FNA FOR PANCREATIC LESIONS

EUS-FNA of a pancreatic lesion rarely yields results of "atypical," "indeterminate," or "inconclusive," but this can happen even with enough passes and even with definitive images for PC. Within the previous studies, inconclusive results were recognized in 4.7%-9.2%[93-95] of EUS-FNA for solid or cystic lesions of the pancreas, even in tertiary hospitals. Repeated EUS-FNA[95-97] or careful follow-up[98] is recommended in these cases. Repeated EUS-FNA has been done within 3-4 wk[96,97] after the initial attempt or upon referral to a tertiary center[95]. More than 80% of cases of initially inconclusive results were diagnostic upon repeated EUS-FNA[95-97]. The following reasons are suspected for the failed initial EUS-FNA: mistargeting by the coexisting pancreatitis, technical difficulty in scope positioning, sedation failure, ascites, or collateral vessels, difficulty in cytology (partially cystic, necrotic,

well-differentiated), pathologist's inter-observer variation[96], and inexperienced endosonographers[95]. Reported predictors of malignancy in negative[98] or indeterminate[94] EUS-FNA are high serum levels of CA19-9 ($\geq$ 40 U/mL)[94], associated lymph node swelling, vascular involvement[98], weight loss, and biliary obstruction[93]. FNA does not provide evidence for the absence of cancer; hence, the clinician must follow and/or reexamine, when EUS-FNA results are inconclusive.

EUS-FNA FOR THE BILIARY LESIONS

Biliary stricture

Several studies have been reported the diagnostic ability of EUS-FNA for biliary strictures. Byrne *et al*[99] reported that EUS-FNA performed using 22G needles in 23 resected cases with biliary stricture or masses revealed 11 cases of confirmed malignancy in the surgically materials. The sensitivity and specificity of EUS-FNA were 100%, although a sensitivity of EUS observation alone was 45%. DeWitt *et al*[100], Eloubeidi *et al*[101] and Ohshima *et al*[102] reported on the effects of EUS-FNA in cases with biliary strictures (22-28 cases); most underwent ERCP but gave negative or non-diagnostic results by brush cytology. In their studies, the sensitivity, specificity, PPV, NPV, and accuracy for detecting malignancy in 71%-82% of the cases were 77%-100%, 100%, 100%, 29%-57%, and 79%-88%, respectively[100-102]. No complications were reported in these studies.

Needle tract seeding is also a concern for the biliary cancers. El Chafic *et al*[103] analyzed factors associated with survival in 119 patients with biliary cancer that underwent curative-intent surgery with or without preoperative EUS-FNA (EUS-FNA done in 39 cases), and patient's age, tumor size, and lymph node metastasis were listed as significant prognostic factors but not preoperative EUS-FNA (HR = 1.09, 95%CI: 0.69-1.73, *P* value = 0.7).

However, indications for EUS-FNA in cases with biliary stricture may be limited, as transpapillary biliary sampling (biopsy and brushing cytology) can be done at the same time as biliary drainage, with safety and high sensitivities [forceps biopsy (77%-92%)[1,6] and brush cytology (75%-79%)[2,7]].

Tumorous lesions of the gallbladder

Gallbladder cancer (GBC) is difficult to diagnose with pathological evidence, and sometimes mimics xanthogranulomatous cholecystitis or acute/chronic cholecystitis. Transpapillary approaches such as naso-gallbladder drainage cytology is feasible and highly diagnostic[104,105], but is sometimes technically difficult and is associated with several complications (mild to moderate acute pancreatitis, cholecystitis and cholangitis)[104,106]. EUS-FNA of the gallbladder lesions has been attempted and provided excellent diagnostic rates for detecting GBC (sensitivity: 80%-90% and specificity: 100%)[107-109]. Kim *et al*[108] reported that all 14 cases of lymphadenopathy accompanied with suspected GBC were confirmed as lymph node metastasis by EUS-FNA. Interestingly, Hijioka *et al*[109] reported that 40 (87%) out of 46 cases of GBC was accompanied with lymphadenopathy, of which 36 cases (90%) were confirmed as lymph node metastases by EUS-FNA, in contrast to null lymphadenopathy in 5 cases with xanthogranulomatous cholecystitis. EUS-FNA of the gallbladder wall may have a risk for dissemination in cases with GBC; hence, the first attempt is safer on the regional lymph nodes[109], if swollen.

Tumorous lesions of papilla of Vater

Tumor of the papilla of Vater is usually easy to diagnose if it originates from the duodenal mucosa, as it is exposed to the intestinal lumen and is detectable by the endoscopy. However, some of the ampullary carcinomas originate from ampullary bile duct, ampullary pancreatic duct, or common duct[110] and are not exposed to duodenal lumen at the early stage. In these cases, endoscopic sphincterotomy of the papilla and subsequent forceps biopsy may increase the chance of obtaining cancer tissues[111], but this may not always effective. Ogura *et al*[112] attempted EUS-FNA in 10 patients and diagnosed 3 intra-ampullary carcinomas without complications, after the diagnostic failure of cytology and/or forceps biopsy under ERCP.

CONCLUSION

This review focused on the clinical aspects of EUS-FNA for solid pancreaticobiliary lesions. During the past quarter of a century, the instruments, methodology, and environment concerning EUS-FNA of the pancreaticobiliary field have improved for greater safety and efficacy of the procedure and accuracy of diagnosis. The mastery of endoscopic technique, as well as the correct choice of instrument and method for the target lesion, is essential for better outcomes.

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