The Contrast-Enhanced Harmonic Endoscopic Ultrasonography Fine-Needle Aspiration Technique

Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) is useful for the diagnosis of pancreatic masses. According to three meta-analyses, the sensitivity, specificity, and accuracy of EUS-FNA are 84–92%, 96–98%, and 86–91%, respectively. However, the occurrence of false-negative and false-positive results indicates that the diagnostic performance of EUS-FNA needs to be improved. Contrast-enhanced harmonic endoscopic ultrasonography (CH-EUS) is used for the characterization of pancreatic masses and can be applied to improve the performance of EUS-FNA.

Keywords: avascular ; contrast ; endoscopic ultrasonography ; endoscopic ultrasound-guided fine-needle aspiration

1. Introduction

Endoscopic ultrasonography (EUS) allows detailed visualization of the pancreas and the localization of pancreatic solid masses. Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) was first applied clinically by Vilmann et al. in 1992 [1], and is currently widely used for the pathological diagnosis of pancreatic solid masses. According to three meta-analyses evaluating the diagnostic performance of EUS-FNA for pancreatic masses, its sensitivity, specificity, and accuracy range between 84–92%, 96–98%, and 86–91%, respectively [2]. Thus, EUS-FNA is associated with a few false-negative and false-positive results. Contrast-enhanced harmonic endoscopic ultrasonography (CH-EUS) allows the visualization of intratumor blood flow using an ultrasound contrast agent, such as Perflubutane microspheres, and is applied for the identification and characterization of pancreatobiliary masses [3][4]. Although EUS-FNA is usually performed under EUS guidance, CH-EUS can be used to guide the needle to a specific site in the tumor to improve specimen collection.

2. The CH-EUS-FNA Technique

In selecting the literature, the following search terms were used in PubMed: contrast (title or abstract) OR contrast-enhanced (title or abstract) OR contrast-enhanced harmonic (title or abstract) OR CE-EUS (title or abstract) OR CH-EUS (title or abstract) OR CEH-EUS (title or abstract) AND endoscopic ultrasound (title or abstract) OR EUS (title or abstract) OR endosonography (title or abstract or MeSH terms) OR endoscopic ultrasonography (title or abstract) AND FNA (title or abstract) OR FNB (title or abstract) OR fine needle aspiration (title or abstract) OR fine needle biopsy (title or abstract) OR sampling (title or abstract). Then, after sequential screening of abstracts and texts, eight studies were determined as shown in **Table 1** [5][6][7][8][9][10][11][12]. In most studies, CH-EUS-FNA was performed in the late phase of CH-EUS (**Table 1**), suggesting that CH-EUS-FNA was performed after evaluation of blood flow in the pancreatic mass in late-phase CH-EUS. However, in one study, CH-EUS-FNA was performed in the early arterial phase [12].

Table 1. Studies on CH-EUS-FNA for pancreatic masses.

Reference	Study Design	Number of Patients, n		Number of Cases with Avascular Area, n (%)	Experience of Endosonographer	Needle-Gauge	Contrast Agent	Timing of CH-EUS-FNA	Target of CH-EUS-FNA	Outcome Measu
		CH-EUS-FNA	EUS-FNA							**CH-EUS-FNA**
										Sensitivity Sp
Napoleon et al., 2010 [5]	Prospective	35	0	No data	No data	22	Sulphur hexafluoride microbubbles	Late phase	No data	79.0%
Gincul et al., 2014 [6]	Prospective	100	0	No data	No data	22	Sulphur hexafluoride microbubbles	Late phase	Hypo-enhanced area	96.0%
Hou et al., 2015 [7]	Retrospective	58	105	No data	No data	22	Sulphur hexafluoride microbubbles	No data	Hypo-enhanced area	81.6%

Reference	Study Design	Number of Patients, n		Number of Cases with Avascular Area, n (%)	Experience of Endosonographer	Needle-Gauge	Contrast Agent	Timing of CH-EUS-FNA	Target of CH-EUS-FNA	Outcome Measu... CH-EUS-FNA	
		CH-EUS-FNA	EUS-FNA							Sensitivity	Sp...
Sugimoto et al., 2015 [8]	Prospective	20	20	20/20 (100%)	Less than 100 EUS-FNA	22	Perflubutane microspheres	Late phase	Avoiding avascular area	90.0%	N...
Seicean et al., 2015 [9]	Prospective	51 (both were performed on the same patients)		No data	No data	22	Sulphur hexafluoride microbubbles	Late phase	Avoiding avascular area	82.9%	
Facciorusso et al., 2020 [10]	Retrospective	103	103	No data	20 years of experience	22	Sulphur hexafluoride microbubbles	No data	Hypo-enhanced area	87.6%	
Seicean et al., 2020 [11]	Prospective	75	75	No data	Over 7000 EUS-FNA and 500 CH-EUS	22	Sulphur hexafluoride microbubbles	Late phase	Avoiding avascular area	87.6%	
Itonaga et al., 2020 [12]	Prospective	93 (both were performed on the same patients)		34/93 (41.5%)	Over 300 EUS-FNA	22	Perflubutane microspheres	Early phase	Avoiding avascular area	84.9%	

CH-EUS-FNA, contrast-enhanced harmonic endoscopic ultrasound-guided fine-needle aspiration; EUS-FNA, endoscopic ultrasound-guided fine-needle aspiration; NS, not significant.

A prolonged contrast period is important for EUS-FNA, which normally requires more than two passes. Second-generation ultrasound contrast agents such as Sulphur hexafluoride microbubbles, Perflutren lipid microspheres, and Perflubutane microspheres resonate under low acoustic power and generate a second harmonic component, which provides at least several minutes of contrast effect [13][14]. Unlike other contrast medias, perflubutane microspheres have the advantage of obtaining a Kupffer image. Perflubutane microspheres allow contrast-enhanced ultrasound evaluations at early phase, late phase, and Kupffer phase. The early, late, and Kupffer phases are defined as 10–30 s, 30–120 s, and 10 min after injection of the contrast agent, respectively [15]. There are no Kupffer cells in the pancreas; therefore, early and late phases are used for CH-EUS evaluations for pancreatic lesions and the significance of this advantage in the diagnosis of pancreatic tumors is not presently known. Thus, any second-generation ultrasound contrast agents can be used for the diagnosis of pancreatic tumors. In EUS-FNA, the fanning technique (sampling multiple areas with each needle pass) is recommended to obtain tumor tissue from a hot spot [16]. However, CH-EUS-FNA has the advantage that any avascular area can be avoided and the fanning technique is not always applicable. It remains unclear whether the early or late phase of CH-EUS is more appropriate for identifying the avascular area, with only one study showing that the diagnostic sensitivity of CH-EUS-FNA performed in the early phase was better than that of conventional EUS-FNA (**Table 1**). Nevertheless, the endosonographers are required to observe both the early and late phases for comprehensive assessment in actual clinical practice: the contrast effect of both phased should be taken into consideration when determining the portion of pancreatic masses to undergo EUS-FNA.

3. Diagnostic Capability of CH-EUS-FNA

Three meta-analyses that included a large number of studies reported that CH-EUS shows superior performance for the diagnosis of solid masses [14][17][18]. Eight reports evaluated the pathological diagnostic performance of CH-EUS-FNA for pancreatic masses (**Table 1**) [5][6][7][8][9][10][11][12], with these including six prospective studies and two retrospective ones, although two of the prospective studies were single-arm designs. The number of patients in these studies ranged from 35 to 225. Most studies did not describe the number of cases with avascular areas, but Sugimoto et al. reported that 20 consecutive cases evaluated with CH-EUS-FNA had avascular areas, and Itonaga et al. reported that 41.5% (34/93) of cases had an avascular area. However, the definition of avascular area was ambiguous in these two studies. Previously, Kamata et al. defined tumors with an avascular area as those with a non-enhancing area ≥5 mm on CH-EUS, and reported that 16.4% (48/292) of pancreatic masses had an avascular area [19]. The variation in the proportion of cases with an avascular area could be due to differences in the definition. In the eight studies listed in **Table 1**, CH-EUS-FNA was performed by expert endosonographers using a 22-gauge EUS-FNA needle, whereas data obtained using an EUS-fine needle biopsy (FNB) needle are lacking. Regarding the puncture site, most studies reported avoiding avascular areas, and three studies reported detecting a hypo-enhanced area. Puncturing the hypo-enhanced area, which indicates pancreatic cancer, is reasonable, especially in pancreatic masses without an avascular area. Two prospective studies (Napoleon et al., 2010 and Gincul et al., 2014) demonstrated the feasibility of CH-EUS-FNA in a single-arm study [5][6],

whereas six studies compared the diagnostic accuracy of CH-EUS-FNA and EUS-FNA. Among these studies, two performed both CH-EUS-FNA and EUS-FNA in the same patients (Seicean et al., 2015 and Itonaga et al., 2020) [9][12]. The sensitivity of CH-EUS-FNA ranged from 79% to 96%, and its specificity from 90% to 100%. Six studies showed that the sensitivity of CH-EUS-FNA was higher than that of EUS-FNA, but only one study showed that the difference was significant (p = 0.003; **Table 1**). However, in this study showing a significant difference, the sensitivity of normal EUS-FNA was particularly low at 68.8%, which could be attributed to the fact that a single pass was used to compare the diagnostic performance of the two methods, rather than the multiple passes used in other studies. Moreover, the first pass was performed using EUS-FNA and the second pass using early-phase CH-EUS with the avascular area confirmed. The specimen obtained by single pass was used for evaluation, and EUS-FNA was performed prior to CH-EUS-FNA. In summary, the added value of CH-EUS-FNA in comparison with EUS-FNA remains unclear, and further studies are needed.

The precision of EUS-FNA is considered to be contingent upon the proficiency of the endosonographers. Additionally, the assessment of pancreatic lesions through CH-EUS and the detection of the avascular area are also subject to their examination skills. Thus, standardization of the procedures and diagnostic proficiency is imperative to gauge the impact of ultrasound contrast agents in CH-EUS. In addition, improvements in examination equipment such as endoscopes and EUS-FNA needles may also have an impact on CH-EUS-FNA in the future.

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