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**Methods to increase the** **diagnostic efficiency of endoscopic ultrasound-guided fine-needle aspiration for solid pancreatic lesions: An updated review**

Yang X *et al*. EUS-FNA for pancreatic cancer

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**Abstract**

Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) is a means to procure adequate specimens for histological and cytologic analysis. The ideal EUS-FNA should be safe, accurate, and have a high sample adequacy rate and low adverse events rate. In recent years, many guidelines and trials on EUS-FNA have been published. The purpose of this article is to provide an update on the influence of some of the main factors on the diagnostic efficiency of EUS-FNA as well as a rare but serious complication known as needle tract seeding.

**Key Words:** Endoscopic ultrasound; EUS-FNA; Pancreatic cancer; Diagnostic efficiency

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**Core Tip:** This review evaluates the influencing factors and limitations of endoscopic ultrasound-guided fine-needle aspiration of solid pancreatic lesions. The information presented here highlights multiple factors and the latest results, such as mass size, rapid on-site evaluation, and needle tract seeding for improving diagnostic efficiency. Therefore, this review may be highly beneficial for clinicians focusing on the management of endoscopic ultrasound-guided fine-needle aspiration.

**INTRODUCTION**

Pancreatic cancer is one of the worst solid pancreatic lesions. The incidence of pancreatic cancer is increasing year by year[1], and the 5-year survival rate is no more than 10%[2]. Due to the low early diagnostic rate, approximately 80% of patients are diagnosed when pancreatic cancer has reached an unresectable stage[3]. Therefore, a reliable and widely applicable early assessment of pancreatic cancer is extremely important for personalized therapies[4]. Decades after endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) was designed in the early 1990s by Vilmann *et al*[5], it is considered a recommended method when the diagnosis is unclear in patients with suspected pancreatic cancer following the computed tomography scan pancreatic protocol[6-8]. According to the latest research, genetic testing technology such as whole-exome sequencing and nuclear DNA content assessment can also be used with EUS-FNA[9]. In recent years, many guidelines and trials on EUS-FNA have been published[10,11]. In the past few years, endoscopic ultrasound-guided fine-needle biopsy (EUS-FNB) has become a useful tool. The newer fine-needle biopsy (FNB) needles are equally effective in pancreatic lesions and non-pancreatic lesions, such as subepithelial lesions and abdominal lymph node lesions, which can improve the sample adequacy rate and diagnostic accuracy[12,13]. However, the evidence relating to this is limited and further multiple large sample studies and randomized clinical trials are warranted to improve the diagnostic efficiency of EUS-FNA[14].

**Mass size**

With the development of pancreatic cancer diagnosis technology, early detection of small solid pancreatic lesions is increasingly common. In the past, it was believed that there was no relationship between lesion size and EUS-FNA diagnostic yield[15,16]. However, previous related research was conducted with rapid on-site evaluation (ROSE), in which the procedure was repeated until the representative cells were confirmed from the target lesion. Nevertheless, according to a retrospective cohort study by Crinò *et al*[17], the adequacy, accuracy, and sensitivity of EUS-FNA for solid pancreatic lesions without ROSE are related to the size of the mass. This finding indicates that endoscopists need to be more cautious when diagnosing small solid pancreatic lesions without ROSE, especially in patients with lesions less than 20 mm[6].

**Needle size**

According to the latest guidelines in United Kingdom, Japan, and China, there is still uncertainty regarding the optimal needle size for EUS-FNA in solid pancreatic lesions supported by high-level evidence. Generally, in terms of needle choice, a 19-gauge needle is used for interventional surgery. A 22-gauge needle is usually used for histologic evaluation, while a 25-gauge needle has been widely used in cytologic assessment with ROSE[18,19].

In recent years, due to their manageability and safety, 22-gauge and 25-gauge needles have gained increasing popularity in clinical trials[20]. According to a meta-analysis which included 7 trials with 689 patients and 732 lesions from 2007 to 2014, there was no significant difference between a 22-gauge needle and a 25-gauge needle on cytologic evaluation in terms of diagnostic sensitivity, specificity, sample adequacy, and adverse events[21]. In addition, a retrospective study of 153 patients with pancreatic ductal adenocarcinoma showed that both 22-gauge and 25-gauge needles both provided equal adequate specimens for immunohistochemical analysis[22].

With regard to the 19-gauge needle, it has advantages over the 22-gauge and 25-gauge needle in terms of the size and quality of tissue samples obtained without ROSE[23]. However, as a result of its stiffness and difficulty in use, the 19-gauge needle often fails when performed *via* the transduodenal approach in a bent position, essentially in pancreatic head or uncinate process tumors[23]. To overcome this problem, a flexible 19-gauge needle with a nitinol shaft (19 G Flex) was introduced. However, according to a randomized study by Laquière *et al*[24], the 19 G Flex needle was inferior to a standard 22-gauge needle in diagnosing pancreatic head cancer and was still difficult to use in the transduodenal approach. Intermediate size needles (20-G or 21-G) are on the way[25,26].

**Suction, slow-pull or non-suction**

Suction is commonly used to obtain adequate samples, but it may damage cellular structures and contaminate the sample with blood, clouding cytologic interpretation[27]. Compared with dry suction, wet suction has better sample adequacy and higher diagnostic accuracy without increasing blood contamination[28,29]. In addition, slow-pull and non-suction sampling are techniques that procure samples of good quality with only slight blood contamination[30-32]. According to a prospective randomized trial by Cheng *et al*[30] and a multicenter randomized trial by Saxena *et al*[32], both suction and slow-pull sampling need 2 passes on average and show equivalent sensitivity, specificity, and accuracy. The combination of these two techniques shows better sampling results than each alone. This study also concluded, in contrast to the study by Mohammad Alizadeh *et al*[33], that suction did not increase blood contamination of the sample compared with slow-pull sampling in solid pancreatic lesions.

**With or without stylet**

The use of a stylet during EUS-FNA prolongs the procedure time with an increased risk of unintentional needle stick injury due to repeat passes during reinsertion of the stylet[34]. However, a longer operation time does not mean better diagnostic efficiency. As indicated by prospective studies and meta-analyses, the use of a stylet during EUS-FNA confers no significant difference in terms of technical success, the mean number of needle passes, needle malfunction, complications, adequate sample rate, cellularity, contamination rate, bloodiness, cytological diagnostic accuracy, and histological diagnostic accuracy[35-38].

**Rapid on-site evaluation**

In the past, it was believed that ROSE could help the diagnostic accuracy of pancreatic EUS-FNA and reduce the number of needle passes and inadequate samples[39]. However, recent comprehensive data on the impact of ROSE have been conflicting. In a multicenter randomized controlled trial and a meta-analysis, no statistical difference was demonstrated in diagnostic accuracy, adequacy rate, procedure time, and the average number of needle passes between EUS-FNA with and without ROSE[40,41]. However, a study that considered pancreatic, submucosal upper gastrointestinal tract and adjacent lesions indicated that ROSE does improve the adequacy rate and diagnostic accuracy of EUS-FNA, especially in solid pancreatic lesions[42]. The variety of conclusions among different studies may be related to other factors such as the difficulty in implementing blind methods, additional passes when malignant cells are not detected, and the experience of endoscopists and cytopathologists[43]. Therefore, ROSE alone may not be the predominant factor. It could be considered an essential part of the learning period and in hospitals where the diagnostic accuracy rate is lower than 90%[44].

**Contrast-enhanced harmonic endoscopic ultrasound and elastography**

Contrast-enhanced harmonic endoscopic ultrasound (CEH-EUS) and elastography have been widely used to assist in the diagnosis of pancreatic indeterminate lesions[45]. It can correctly distinguish false negative diagnoses of EUS-FNA, thus improving the diagnostic rate of pancreatic diseases and EUS-FNA[46,47]. CEH-EUS-guided fine-needle aspiration (CEH-EUS-FNA) avoids fibrosis, necrotic areas, and blood vessels in pancreatic lesions, and can locate the sampling site more accurately[48]. Compared with the standard EUS-FNA, it can reduce the number of punctures when obtaining equivalent sufficient samples, thus reducing the incidence of adverse events related to EUS-FNA, such as bleeding, perforation, infection, and pancreatitis *etc*[46,49]. Elastography strain imaging is accessible through EUS, wherein it gauges tissue distortion by the application of a predetermined pressure. The combined utilization of CEH-EUS or elastography appears to enhance the diagnostic capability of EUS[50]. However, a meta-analysis suggested that more studies are needed to assess the combined utilization[51].

**Needle tract seeding**

Apart from common complications such as pancreatitis and bleeding, a rare but serious complication has also received increasing attention since 2003. Cancer recurrence due to needle tract seeding after EUS-FNA was first reported by Hirooka *et al*[52] in a patient with a pancreatic tumor. Since then, relevant studies have been published continuously, discussing the impact of tumor cell seeding *via* the needle tract on short-term prognosis[53]. According to several retrospective studies, although pre-operative EUS-FNA has not been proved to be associated with overall survival or an increased rate of gastric and peritoneal cancer recurrence, its potential long-term prognosis is still non-negligible[54-57]. Furthermore, this phenomenon is unique to tumors in the pancreatic body and tail, considering that the needle tract is not included in the surgical resection of these tumors[58-65]. Therefore, if possible, more attention to the imaging findings of the needle tract in the postoperative follow-up is necessary or including the needle tract during the surgical resection may improve long-term prognosis[66]. In addition, appropriate risk information on needle tract seeding before EUS-FNA is necessary[65].

**EUS-FNB and Macroscopic On-Site Evaluation**

EUS-FNB has become the first choice when multiple immunohistochemical staining is required to assist in the diagnosis of diseases such as autoimmune pancreatitis and pancreatic metastasis[67]. At present, relevant studies have mainly focused on the research and development of puncture needles of different types and shapes. The most common ProCore® biopsy needle improves the adequacy of tissue specimens, and the Acquire® biopsy needle improves the quality of the tissue specimen due to its tip stability and more controllable puncture site[19,67]. However, a study demonstrated that the 22G Acquire® needle achieved better accuracy than the 20G needle due to more pancreatic mass tissue for histologic assay[68].

A trial by Yousri *et al*[69] reported that both FNA and FNB are safe and effective for accurately diagnosing pancreatic and non-pancreatic abnormalities. In comparison to tissue examination alone, FNB demonstrates higher sensitivity and diagnostic accuracy when diagnosing pancreatic lesions. Additionally, FNB can provide a higher quality histological specimen with reduced contamination due to blood. A randomized controlled trial suggested that EUS-FNB without ROSE showed great diagnostic accuracy in solid pancreatic lesions, and ROSE might not be recommended when new FNB needles are used[70]. Although newer FNB needles have the advantage of being self-assisting in diagnosing diseases, standard FNA needles are still very competitive as their high flexibility allows them to puncture difficult target sites and allow for ROSE[25]. A meta-analysis found evidence to suggest that EUS-FNB with ROSE was not significantly better than EUS-FNB with newer end-cutting needles. However, there may still be a potential role for ROSE when reverse bevel needles are utilized[71]. However, ROSE necessitates the presence and expertise of a pathologist, incurs supplementary expenses, and is not accessible in many medical centers. The macroscopic on-site evaluation (MOSE) by an endoscopist was introduced as an alternative to ROSE, and two studies found that MOSE is a complementary technology that reduces the number of needles necessary for sample acquisition and improves diagnostic accuracy in some clinical conditions[71,72] (Table 1).

**Discussion**

EUS-FNA plays a pivotal role in the diagnosis and evaluation of solid pancreatic lesions. Although there are still no globally accepted guidelines for the application of EUS-FNA in solid pancreatic lesions, relevant and clinically meaningful studies on techniques are increasing. The ideal EUS-FNA is safe, accurate, and has a high sample adequacy rate and low adverse events rate. Studies are even exploring its use in cancer diagnosis beyond the digestive system[73-75].

Needle size for EUS-FNA has always been a popular research topic. According to a network meta-analysis involving 27 randomized controlled trials and 2711 patients, there was no significant difference in diagnostic accuracy and sample adequacy among 19-gauge, 22-gauge, and 25-gauge needles[76]. This means that endoscopists can choose the needle size based solely on the purpose of the operation, for instance, interventional surgery, histological evaluation, and cytologic assessment. It is also important to note that although the 19-gauge needle has advantages in terms of the quantity and quality of tissue samples obtained without ROSE, it does not perform well *via* the transduodenal approach in a bent position[23]. Modification of a 19-gauge needle, such as material and shape, to make it flexible and easier to use seems warranted.

Inconsistent findings in studies of ROSE may be due to the difficulty of performing the blind method, additional punctures when no malignant cells are detected, and the difference in the experience of endoscopists and cytopathologists[43]. This prevents ROSE itself from being considered as a major factor affecting the diagnostic accuracy of EUS-FNA, at least without sufficient evidence. However, it is almost certain that ROSE plays a role in the effect of mass size on the accuracy of EUS-FNA. Thus, in hospitals without ROSE, endoscopists should be more cautious in patients with small solid pancreatic lesions[17].

According to a prospective randomized trial by Cheng *et al*[30], there was no statistically significant difference between slow-pull and suction EUS-FNA techniques in terms of safety, accuracy, and blood contamination. Several slow-pull and suction techniques, for instance, wet suction, have also been modified to enhance tissue acquisition or reduce tissue damage[77]. However, sufficient evidence to prove that one technique is superior to another is still required.

As mentioned above, it would be reasonable not to use a stylet during the EUS-FNA process, which may make the operation easier, reduce labor intensity, take less time and be more cost-effective without affecting the quality of the results.

In recent years, although the incidence of needle tract seeding is low, due to its serious consequences, this complication has received more and more attention from endoscopists. This may also be precisely because of its low incidence that the results of its impact on overall survival rate were not obtained in relevant previous studies and meta-analyses[54-57]. In order to fully clarify the clinical characteristics of EUS-FNA posterior needle tract seeding, further prospective studies are warranted. However, in current clinical practice, it is still recommended that attention is paid to needle tract seeding and appropriate risk information is necessary.

Organoids offer a comprehensive depiction of the intricate diversity inherent in tumors, covering their genetic constitution, transcriptional landscape, metabolic dynamics, cytological intricacies, and histological characteristics. Organoids serve as a synthesized representation of multiple tumoral features *in vivo*, thereby serving as a pivotal conduit between fundamental tumor research and clinical applications, such as drug screening[78]. With the exploration and development of new technologies, tissues obtained by EUS can also be used for organoid culture[79].

Tumor organoids are mainly cultured from surgically resected samples, the inherent difficulty in obtaining viable specimens from advanced-stage tumors, such as pancreatic cancer, poses a significant impediment to this approach. In contrast, EUS-FNA is a versatile methodology, applicable across all disease stages, encompassing preoperative, perioperative, post-therapeutic, and recurrent phases. This methodological flexibility means that EUS-FNA is unconstrained by disease staging, thereby facilitating the establishment of a dynamic organoid that faithfully mirrors the temporal progression of the disease[80]. In contrast to traditional methods, these specimens after ROSE can be used immediately in the laboratory to generate organoid cultures, and samples can be taken as the disease progresses, not just after the lesion requires surgical excision.

**CONCLUSION**

In conclusion, short-term outcomes of the factors introduced above are necessary for the improvement of EUS-FNA. Multiple large sample studies and prospective randomized trials are still warranted to discuss cytopathologic support, modification of techniques, materials, and long-term consequences.

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**Footnotes**

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**Table 1 Characteristics of the study**

|  |  |  |  |
| --- | --- | --- | --- |
| **Ref.** | **Number of patients** | **Study design** | **Result (Diagnosis accuracy)** |
| Karsenti *et al*[68] | 60  | Randomized controlled trial | 87% with 22G needle and 67% with 20G needle for FNB, *P* = 0.02 |
| Yousri *et al*[69] | 100  | Prospective study | 98% with FNA and 90% with FNB only depending on tissue |
| Crinò *et al*[70] | 800  | Randomized controlled non-inferiority trial | 96.4% with ROSE and 97.4% without ROSE, *P* = 0.396 |
| Facciorusso *et al*[71] | 2147  | Meta-analysis | FNB with ROSE group better than the FNB only group (OR = 2.49, 1.08-5.73; *P* = 0.03) |

FNA: Fine-needle aspiration; ROSE: Rapid on-site evaluation; FNB: Fine-needle biopsy.