

## Open Access

# Technical Advances in Endoscopic Ultrasound (EUS)-Guided Tissue Acquisition for Pancreatic Cancers: How Can We Get the Best Results with EUS-Guided Fine Needle Aspiration?

Prashant Kedia, Monica Gaidhane and Michel Kahaleh

Division of Gastroenterology and Hepatology, New York Presbyterian Hospital, Weill Cornell Medical College, New York, NY, USA

Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is one of the least invasive and most effective modality in diagnosing pancreatic adenocarcinoma in solid pancreatic lesions, with a higher diagnostic accuracy than cystic tumors. EUS-FNA has been shown to detect tumors less than 3 mm, due to high spatial resolution allowing the detection of very small lesions and vascular invasion, particularly in the pancreatic head and neck, which may not be detected on transverse computed tomography. Furthermore, this minimally invasive procedure is often ideal in the endoscopic procurement of tissue in patients with unresectable tumors. While EUS-FNA has been increasingly used as a diagnostic tool, most studies have collectively looked at all primary pancreatic solid lesions, including lymphomas and pancreatic neuroendocrine neoplasms, whereas very few studies have examined the diagnostic utility of EUS-FNA of pancreatic ductal carcinoma only. As with any novel and advanced endoscopic procedure that may incorporate several practices and approaches, endoscopists have adopted diverse techniques to improve the tissue procurement practice and increase diagnostic accuracy. In this article, we present a review of literature to date and discuss currently practiced EUS-FNA technique, including indications, technical details, equipment, patient selection, and diagnostic accuracy.

**Key Words:** Endosonography; Biopsy, fine-needle; Pancreatic cyst; Lesions; Pancreatic neoplasms

## INTRODUCTION

Despite advancements in medical and surgical therapy in the past few decades, pancreatic cancer continues to be a devastating disease with a dismal prognosis. Unfortunately, the incidence of pancreatic adenocarcinoma is rising and it remains one of the leading causes of cancer-related deaths worldwide.<sup>1,2</sup> Multiple epidemiologic studies have shown the 5-year survival rate to be less than 5%.<sup>1,3</sup> This is in part due to the delayed diagnosis of this cancer in individuals often because of the late onset of symptoms. At initial imaging evaluation, about 80% of patients will already have locally advanced disease or advanced metastasis, thus precluding the possibility

of a curative surgical resection.<sup>4</sup> Of the 20% of patients that appear resectable and index evaluation, less than half end up undergoing curative surgical resection because of regional tumor spreading at the time of surgery.<sup>4</sup> Therefore, early and accurate diagnosis is crucial to provide patients with the necessary time and information to make appropriate treatment decisions and improve their prognosis.

Endoscopic ultrasound (EUS) has been shown to be a valuable imaging tool for the detection of pancreatic lesions. However, it is the capability to perform fine needle aspiration (FNA) and provide a concurrent tissue diagnosis at the time of EUS that has made it an essential tool in the diagnostic algorithm for various visceral malignancies, especially solid pancreatic lesions. Since the first report of EUS-FNA of the pancreas by Vilmann et al.<sup>5</sup> in 1992, EUS-FNA has become the standard of care for tissue diagnosis in the pancreas because it is a safe, effective, and accurate procedure.<sup>6</sup> Various decisions and facets go into performing successful EUS-FNA such as selection of needle size and type, location of the targeted lesion, availability of onsite cytopathology, use of acces-

Received: July 23, 2013 Revised: August 13, 2013

Accepted: August 19, 2013

Correspondence: Michel Kahaleh

Division of Gastroenterology and Hepatology, New York Presbyterian Hospital, Weill Cornell Medical College, 525 E 68th St, New York, NY 10021, USA  
Tel: +1-646-962-4797, Fax: +1-646-962-4000, E-mail: mkahaleh@gmail.com

© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

sories such as stylets and suction, endoscopic technique, and utilization of novel imaging enhancing techniques like elastography and contrast-harmonic EUS. This review will summarize the current literature regarding these various aspects of EUS-FNA and how to optimize tissue acquisition in the pancreas.

## DIAGNOSTIC EUS

The four main objectives of EUS in managing pancreatic lesions include detection, staging, determining surgical resectability, and making a confirmatory tissue diagnosis. The first three goals do not require tissue acquisition. The proximity of the echoendoscope to the pancreas allows for exceptional im-

aging of the head, neck, and uncinate process from the duodenum and the body and tail from the stomach. Multiple studies have shown EUS to be both sensitive and accurate in staging pancreatic lesions. Also the high negative-predictive value of EUS when evaluating pancreatic lesions makes it a reasonable rule out test for malignancy, which can be helpful to clinicians in situations of unclear cross-sectional imaging.<sup>7,8</sup> Although some of the superiority that EUS enjoyed for many years over standard computerized axial tomography (computed tomography, CT) has diminished due to improved cross-sectional imaging technology (i.e., helical CT), many experts still consider it to be the single best test for evaluation of pancreatic lesions.<sup>8,9</sup>

In terms of pancreatic tumor detection, EUS has been shown in prior to studies to be superior to cross-sectional imaging (Table 1).<sup>10-21</sup> Especially for smaller lesions (<3 cm), EUS holds the advantage over CT and transabdominal ultrasound.<sup>22</sup> Although some authors question whether the dominance of EUS over cross-sectional imaging given modern advances in radiographic imaging, a recent systemic review reinforced the conventional data and popular consensus.<sup>23</sup>

Once a lesion has been discovered, staging that lesion and determining whether it is surgically resectable is of utmost importance to establish a prognosis and means of treatment. As with EUS sensitivity, earlier studies of comparing EUS to standard CT showed a clear advantage of EUS in staging tumors and determining resectability (Tables 2, 3).<sup>10-12,16,17,19,21,24-28</sup> Generally EUS consistently performed better than standard CT for staging and determining tumor extension, however the results are less consistent when compared to helical or multidetector CT.<sup>29</sup> In one study, EUS was most accurate at assessing tumor size and lymph node involvement, while helical CT was more precise regarding overall staging, resectability, vascular invasion, distant metastasis, and locoregional exten-

**Table 1.** Sensitivity of Endoscopic Ultrasound Compared to Cross-Sectional Imaging for Detecting Pancreatic Lesions

Author	Number	EUS	CT	Helical/ multidetector CT
Rösch et al., <sup>10</sup> 1992	60	98	85	-
Palazzo et al., <sup>11</sup> 1993	49	91	66	-
Müller et al., <sup>12</sup> 1994	33	94	69	-
Marty et al., <sup>13</sup> 1995	37	92	63	-
Melzer et al., <sup>14</sup> 1996	12	100	83	-
Sugiyama et al., <sup>15</sup> 1997	73	96	86	-
Legmann et al., <sup>16</sup> 1998	30	100	-	92
Gress et al., <sup>17</sup> 1999	81	100	74	-
Mertz et al., <sup>18</sup> 2000	31	93	-	53
Rivadeneira et al., <sup>19</sup> 2003	44	100	-	68
Agarwal et al., <sup>20</sup> 2004	81	100	-	86
Dewitt et al., <sup>21</sup> 2004	80	98	-	86

EUS, endoscopic ultrasound; CT, computed tomography.

**Table 2.** Accuracy of Endoscopic Ultrasound Staging Pancreatic Cancer (T and N) Compared to Cross-Sectional Imaging

Author	Number	EUS (t)	EUS (n)	Standard CT (t)	Standard CT (n)	MD/H CT (t)	MD/H CT (n)
Mukai et al., <sup>24</sup> 1991	26	-	65	-	38	-	-
Rösch et al., <sup>10</sup> 1992	40	-	72	-	38	-	-
Palazzo et al., <sup>11</sup> 1993	64	82	64	45	50	-	-
Müller et al., <sup>12</sup> 1994	16	82	50	56	38	-	-
Legmann et al., <sup>16</sup> 1998	22	90	86	-	-	86	77
Midwinter et al., <sup>25</sup> 1999	23	-	74	-	-	-	65
Gress et al., <sup>17</sup> 1999	81	85	72	30	55	-	-
Rivadeneira et al., <sup>19</sup> 2003	44	-	84	-	-	-	68
Soriano et al., <sup>26</sup> 2004	62	63	67	-	-	73	56
Ramsay et al., <sup>27</sup> 2004	27	63	69	-	-	76	63
Dewitt et al., <sup>21</sup> 2004	53	67	44	-	-	41	47

EUS, endoscopic ultrasound; CT, computed tomography; MD/H, multidetector/helical.

sion.<sup>26</sup> Systemic reviews have also not been able to show a true frontrunner regarding this issue, thus many authors recommend that the most prudent use of these modalities is as complementary rather than competing tools.<sup>8,23</sup>

### EUS-FNA

Although EUS has clearly been established as the most sensitive modality for pancreatic lesions, distinguishing malignant from benign etiologies can be difficult in certain clinical scenarios such as chronic pancreatitis. For this reason, establishing a tissue diagnosis using the cytopathology and histology obtained from EUS-FNA has become both paramount and essential to this technique. EUS-FNA has become widely accepted as a safe, effective, and consistent method of diagnosing pancreatic cancer.<sup>8</sup> A recent meta-analysis evaluated 41 studies with a total of 4,766 patients having undergone EUS-FNA for pancreatic lesions and reported the pooled accuracy to be 86.8% and specificity 95.8% (Table 4).<sup>30-70</sup> This study also found that the accuracy of EUS-FNA is improving over time; in subgroup analysis, EUS-FNA accuracy was higher during 2001 to 2009 than between 1995 to 2000. Another systemic review including studies from the past 10 years confirmed this notion as they reported pooled sensitivity and specificity rates of 92 and 96%.<sup>6</sup> The etiology of this trend is likely multifactorial due to the natural learning curve of this procedure in the endoscopic community, better instruments, and the increased availability of specialized training for EUS-providers.

### EUS-FNA VERSUS OTHER TISSUE SAMPLING MODALITIES

Compared to alternate tissue-acquisition techniques such

**Table 3.** Accuracy of Endoscopic Ultrasound Determination of Surgical Resectability of Pancreatic Lesions Compared to Cross-Sectional Imaging

Author	Number	Modality	Accuracy
Legmann et al., <sup>16</sup> 1998	27	EUS	92
		Helical CT	93
Gress et al., <sup>17</sup> 1999	75	EUS	93
		Standard CT	60
Tierney et al., <sup>28</sup> 2001	24	EUS	83
		Helical CT	75
Soriano et al., <sup>26</sup> 2004	62	EUS	67
		Helical CT	83
Dewitt et al., <sup>21</sup> 2004	53	EUS	77
		Multidetector CT	77
		CT	77

EUS, endoscopic ultrasound; CT, computed tomography.

**Table 4.** Endoscopic Ultrasound-Guided Fine Needle Aspiration Diagnostic Sensitivity and Accuracy for Pancreatic Lesions

Author	Sensitivity	Specificity	Accuracy
Wegener et al., <sup>30</sup> 1995	44	100	-
Giovannini et al., <sup>31</sup> 1995	75	100	-
Cahn et al., <sup>32</sup> 1996	87	100	-
Chang et al., <sup>33</sup> 1997	92	100	95
Bhutani et al., <sup>34</sup> 1997	64	100	-
Baron et al., <sup>35</sup> 1997	95	88	-
Faigel et al., <sup>36</sup> 1997	94	100	-
Wiersema et al., <sup>37</sup> 1997	91	97	-
Binmoeller et al., <sup>38</sup> 1998	68	100	-
Hunerbein et al., <sup>39</sup> 1998	67	100	-
Williams et al., <sup>40</sup> 1999	82	100	85
Suits et al., <sup>41</sup> 1999	96	100	96
Erickson et al., <sup>42</sup> 2000	95	100	-
Voss et al., <sup>43</sup> 2000	68	88	74
Brandwein et al., <sup>44</sup> 2001	79	100	85
Gress et al., <sup>45</sup> 2001	93	100	-
Mallery et al., <sup>46</sup> 2002	74	100	76
Ylagan et al., <sup>47</sup> 2002	78	100	-
Fritscher-Ravens et al., <sup>48</sup> 2002	88	100	-
Harewood et al., <sup>49</sup> 2002	94	71	84
Levy et al., <sup>50</sup> 2003	67	100	60
Afify et al., <sup>51</sup> 2003	80	82	-
Raut et al., <sup>52</sup> 2003	91	100	92
Larghi et al., <sup>53</sup> 2004	86	100	-
Ho et al., <sup>54</sup> 2004	99	100	-
Itoi et al., <sup>55</sup> 2005	100	100	-
DeWitt et al., <sup>56</sup> 2005	100	100	-
Gines et al., <sup>57</sup> 2005	64	100	-
Ryozawa et al., <sup>58</sup> 2005	82	100	84
Varadarajulu et al., <sup>59</sup> 2005	91	94	91
Mitsuhashi et al., <sup>60</sup> 2006	95	100	82
Wittmann et al., <sup>61</sup> 2006	60	80	77
Horwhat et al., <sup>62</sup> 2006	88	35	-
Storch et al., <sup>63</sup> 2006	60	100	76
Aithal et al., <sup>64</sup> 2007	94	100	82
Rocca et al., <sup>65</sup> 2007	90	78	82
Ardengh et al., <sup>66</sup> 2007	78	99	87
Eloubeidi et al., <sup>67</sup> 2007	95	92	94
Agarwal et al., <sup>68</sup> 2008	89	100	-
Holt et al., <sup>69</sup> 2008	69	93	-
Jindal et al., <sup>70</sup> 2008	98	100	-
Pooled values	87	96	87

as percutaneous CT-guided biopsy and endoscopic retrograde cholangiopancreatography (ERCP) brush cytology, EUS-FNA holds an advantage of being more sensitive and less invasive. It is well established that the sensitivity of ERCP brush cytology is quite low and ranges anywhere between 30% to 85%.<sup>71</sup> Therefore, EUS-FNA has supplanted ERCP brush cytology as the primary method of pancreatic tissue acquisition especially in patients without obstructive jaundice. When compared to CT-guided biopsy, EUS-FNA has both the logistical and economic advantage being able to image and obtain tissue during a single-session as opposed to two separate occurrences. Head to head trials have also supported the increased sensitivity of EUS-FNA compared to CT-guided biopsy.<sup>62</sup> Also there is a concern that CT-guided biopsy may confer a higher risk of peritoneal tumor seeding compared to EUS-FNA, which has been shown in retrospective studies to be as high as 16.3% compared to 2.2%.<sup>72</sup> EUS-FNA has also been shown to be more sensitive for detecting and sampling malignant ascites compared to CT, therefore furthering its diagnostic advantage over percutaneous sampling techniques.<sup>73</sup>

EUS-FNA has also proved to be a safe and effective salvage biopsy maneuver in cases of nondiagnostic ERCP brush cytology and CT-guided biopsy. In the study by Harewood and Wiersema<sup>49</sup> 185 patients with pancreatic masses were assessed by CT-guided biopsy or ERCP brush cytology prior to going to EUS-FNA. Of the 58 patients with negative CT-guided biopsies and 36 patients with negative ERCP brush cytology, subsequent EUS-FNA detected malignancy in 90% and 94% of patients.<sup>49</sup> The high sensitivity and accuracy of EUS-FNA after previously negative tissue sampling procedures has been confirmed in other studies as well, which boast sensitivity and accuracy rates of 93% and 88%.<sup>45,74</sup>

## EUS-FNA SAMPLING: TECHNIQUES AND ACCESSORIES

### Positioning and technique

In general, EUS-FNA is performed best when the echoendoscope is in a stable position with a straight tip, thus allowing for easy passage of the FNA needle. This is usually achieved more often in the transesophageal and transgastric position as opposed to the transduodenal. When targeting the uncinate process or pancreatic neck from the duodenal bulb, the tip of the echoendoscope is flexed, thus making needle passage more difficult.<sup>75</sup> Troubleshooting this issue by maneuvering the echoendoscope into the long position may solve the problem, however at the expense of a more precarious scope position. The other option to deal with difficult passage of the needle in torqued or flexed positions is to choose a smaller gauge (G) such as the 25 G needles to allow for easier passage.

It is important during EUS-FNA for the endoscopist to actively attempt to sample multiple sections of a pancreatic lesion rather than mechanically penetrate only one tissue tract. Because neoplastic lesions can be heterogenous in nature, with necrotic, acellular centers, it is important to target multiple areas of the lesion especially the periphery to improve cellular yield.<sup>75</sup> A recently described fanning-technique has become accepted amongst endosonographers as one study has shown it to improve first pass diagnostic rates by almost 30%.<sup>76</sup> The idea is to reposition the needle angle using the dials and elevator intermittently to successively sample from multiple areas of the lesion rather than one singular angle. It is thought that the fanning technique works not only by successively sampling new tracts of tissue, but limiting the amount of blood and artifact from previous tract sites.<sup>75,76</sup>

One recurrent question endosonographers face is the least number of passes that is adequate to sample a pancreatic lesion. In cases where onsite cytopathology is available, this question becomes much easier to answer because there is real-time feedback on FNA yield. However, in cases without that luxury, the risk of causing more cellular injury and possible complications must be weighed against the benefit of improving diagnostic accuracy. As of now, there is no generalized consensus about the optimal number of passes or sampling techniques for EUS-FNA of pancreatic lesions. Various studies have estimated the ideal number of needle passes in the pancreas without onsite cytopathology to be between 3 and 7.<sup>77-79</sup> Wallace et al.<sup>77</sup> recommended 3 needle passes each with back and forth motions of about 30 seconds each to adequately detect pancreatic malignancy.<sup>75</sup> Pellisé Urquiza et al.<sup>78</sup> found that the diagnostic plateau for EUS-FNA is reached after the fourth pass. Leblanc et al.<sup>79</sup> found that the diagnostic sensitivity of 7 and 5 needle passes in the pancreas and lymph nodes to be about 83% and 77%. While there is no consensus, based on the available data, if no onsite cytopathology is available some authors recommend at least 5 to 6 passes in the pancreas and 2 to 3 passes in lymph nodes for adequate EUS-FNA sensitivity.<sup>75</sup>

### Choosing the needle

Unfortunately, there is no single EUS-FNA needle available that is perfect for every pancreatic lesion. Therefore, the endoscopist must use their experience and clinical judgment to decide on the appropriate needle type. Generally when making this decision, they should consider which needle will optimize cellular yield, minimize complications, and specimen contamination, and the need for needle flexibility based on the lesion's location in the pancreas. As stated before, transduodenal puncture to sample pancreatic head, neck, and uncinate lesions may demand more pliable needles due to the

flexed tip of the echoendoscope.

As of now, there are three needle G (19, 22, 25 G) available for EUS-FNA. Multiple prospective studies, including four randomized-controlled trials (RCTs) have been performed to compare the diagnostic yield and accuracy of these needles (Table 5).<sup>80-84</sup> Three of the RCTs compare 22 and 25 G needles without any statistically significant differences in diagnostic accuracy, although there was a trend towards significance when using 25 G needles in pancreatic head/uncinate lesions.<sup>81,82</sup> One prospective study by Sakamoto et al.<sup>84</sup> showed a clear benefit of the 25 G over the 22 G for uncinate processes with reported diagnostic accuracies of 100% versus 33%. These studies also revealed that while the 19 G needle may improve cellular yield compared to the smaller needles, it comes at the expense of decreased efficacy for transduodenal lesions. A recent meta-analysis on the issue found that 25 G needles may have a slight benefit over 22 G in terms of specimen adequacy, however this did not translate into significantly higher diagnostic accuracy or fewer complications.<sup>85</sup> Therefore, the choice between 25 and 22 G needle for pancreatic body and tail masses may be left up to the endoscopist's preference with the caveat that 25 G is probably superior for pancreatic head lesions. On the other hand, 19 G needles may provide higher cellular yields and potentially provide histologic sample, but are not generally effective in pancreatic head lesions.

**Histology: EUS-fine needle biopsy**

Despite the impressive outcomes achieved with EUS-FNA, there are some limitations of obtaining tissue via aspiration for cytology. As mentioned earlier, one limitation is the unclear number of passes required to achieve an adequate sample in the absence of an onsite cytopathologist. This issue is amplified in settings of pancreatic tissue fibrosis and distortion such as chronic pancreatitis when the cellular yield from EUS-FNA is

diminished.<sup>59</sup> Also, while usually 100 cells or less is the minimum number to obtain a pathologic diagnosis, some tests may require additional cells such as RNA extraction.<sup>8</sup> Finally, cytology is devoid of tissue architecture, which may be necessary in certain situations to clarify a diagnosis such as lymphoma, autoimmune pancreatitis, and gastrointestinal stromal tumors.

To address these concerns, various EUS-compatible core biopsy needles have been developed including the 19 G Trucut (Wilson Cook, Winston-Salem, NC, USA) and 19/22 G ProCore (Cook Endoscopy, Winston-Salem, NC, USA). These needles have various design mechanisms that allow for cutting and procurement of a solid core of architecture-preserved tissue. The actual benefit of EUS-guided fine needle biopsy (FNB) for pancreatic lesions is unclear. Various reports have quoted the accuracy rates of EUS-FNB in the pancreas to be between 61% and 89.4% (Table 6).<sup>53,55,61,86-88</sup> The rigidity of 19 G Trucut needle proved to be a limitation for transduodenal sampling as Itoi et al.<sup>55</sup> reported zero percent sensitivity for uncinate lesions. This limitation has been somewhat overcome with the development of more flexible core biopsy needles. One RCT comparing 22 G EUS-FNA and FNB needles for pancreatic lesions found no significant difference in diagnostic yield/adequacy, technical success, and complications.<sup>88</sup> Some authors have shown that the combination of both modalities may improve both sensitivity and accuracy in assessing pancreatic lesions.<sup>61</sup> Thus, as of now, there is no clear indication that EUS-FNB is preferred over EUS-FNA for pancreatic lesions unless histologic analysis is required.

**Using suction**

Conventionally, the use of suction on the FNA needle system had been standard in the endoscopic world because of the intuitive notion that it would increase cellular yield. How-

**Table 5.** Trials Comparing Different Needle Gauges for Endoscopic Ultrasound-Guided Fine Needle Aspiration of Solid Mass Lesions

Author	Study design	Number	Location	Needles, G	Diagnostic accuracy, %	Comments
Siddiqui et al., <sup>80</sup> 2009	RCT	131	All lesions	22 vs. 25	87.5 vs. 9; <i>p</i> =NS	-
Camellini et al., <sup>81</sup> 2011	RCT	127	All lesions	22 vs. 25	77.8 vs. 78.1; <i>p</i> =NS	Targetting lesions of the distal duodenum was better with 25 G
Fabbri et al., <sup>82</sup> 2011	RCT	50	Pancreas	22 vs. 25	86 vs. 94; <i>p</i> =NS	Trend towards significance with 25 G needle
Song et al., <sup>83</sup> 2010	RCT	117	Pancreas/ peripancreas	19 vs. 22	94.5 vs. 78.9; <i>p</i> =0.01 (per-protocol analysis, excluding technical failures)	A 19 G produced significantly higher cellular yield overall, but less successful for pancreatic head masses
Sakamoto et al., <sup>84</sup> 2009	Prospective case series	24	Pancreas	25 vs. 22 vs. 19 (Trucut)	91.7 vs. 79.7 vs. 54.1; <i>p</i> =NS (25 G vs. 22 G) <i>p</i> <0.05 (25 G vs. 19 G)	A 25 G significantly better than others for uncinate masses

G, gauge; RCT, randomized-controlled trial; NS, not significant.



ever, although this has shown to be true in various studies, it comes at a price of decreasing the quality of the specimen due to increased bloodiness. This has been shown in two of the three RCTs performed on this topic (Table 7).<sup>77,89-91</sup> Thus, while there is no consensus on the topic, many authors conclude that suction is of limited value during EUS-FNA pancreas because it reduces the quality of the specimen.<sup>8</sup> However, in cases where cellular yield is low such as fibrotic lesions in chronic pancreatitis, use of suction may be appropriate to improve cellularity and diagnostic yield. In softer lesions, which may contain necrosis and blood, the use of suction is discouraged to minimize distortion of the cellular sample.

### Using the stylet

In every EUS-FNA system, there is a stylet preloaded to the tip of the needle. The stylet is a rigid metal wire that runs through the length of the FNA needle to theoretically prevent any tissue plugs that might prevent adequate sample procurement of the target lesion as the FNA needle traverses visceral organs.<sup>75</sup> Many endoscopists find the use of the stylet tedious and risky as it increases the chance for needle-stick injuries. Three RCTs have been performed looking at the diagnostic yield of performing EUS-FNA in the pancreas with or with-

out and stylet.<sup>89,92-94</sup> All three of these well-designed studies have shown consistent results in that the use of the stylet offers no advantage in terms of diagnostic yield, but does significantly increase the bloodiness of specimens. Therefore, there is no advantage to using the stylet when performing EUS-FNA of the pancreas.

### Onsite cytopathology

Unfortunately, up to 32% of EUS-FNA specimens may be non-diagnostic for a variety of reasons including acellularity and specimen distortion from blood and artifact.<sup>95</sup> Therefore, having a trained onsite cytotechnician or cytopathologist to actively assess the adequacy of samples during the procedure is very valuable, and unfortunately is a luxury that not all endosonographers can enjoy. Multiple studies have revealed that real-time onsite cytopathology increases the diagnostic yield and reduces the number of indeterminate or unsatisfactory samples from EUS-FNA (Table 8).<sup>96-98</sup>

Alsohaibani et al.<sup>97</sup> showed increased diagnostic yield of 22% even if a trained cytotechnician is preparing slides as opposed to an endoscopy nurse or technician. Other studies have also shown that onsite cytopathology can increase diagnostic yield by 15% to 29% along with reducing the number of re-

**Table 6.** Endoscopic Ultrasound-Guided Fine Needle Biopsy Diagnostic Sensitivity and Accuracy for Pancreatic Solid Lesions

Author	Number	Location	Needle, G	Sensitivity	NPV	Accuracy
Larghi et al., <sup>53</sup> 2004	23	Pancreas	19 Trucut	74	-	61
Itoi et al., <sup>55</sup> 2005	16	Pancreas	19 Trucut	69	-	-
Wittmann et al. <sup>61</sup> 2006	83	Pancreas	19 Trucut	81	-	56
Thomas et al., <sup>86</sup> 2009	113	Pancreas	19 Trucut	62.4	25	67.5
Iglesias-Garcia et al., <sup>87</sup> 2011	47	Pancreas	19 Procore	95.7	-	89.4
Bang et al., <sup>88</sup> 2012	28	Pancreat	19 Procore	-	-	89.3

G, gauge; NPV, negative predictive value.

**Table 7.** Trials Evaluating the Role of Suction in Performing Endoscopic Ultrasound-Guided Fine Needle Aspiration

Author	Number	Measure	Suction	No suction	p-value
Mair et al., <sup>89</sup> 1989	100	Diagnostic yield	Increased	Baseline	NS
		Bloodiness	-	-	NS
		Cellularity	-	-	NS
Wallace et al., <sup>77</sup> 2001	43	Diagnostic yield	Unchanged	Unchanged	0.73
		Bloodiness	Uncreased	Baseline	0.0004
		Cellularity	Better	Baseline	0.01
Puri et al., <sup>90</sup> 2009	52	Diagnostic yield	92.3	80	0.1
		Bloodiness	76.9	88.5	0.14
		Cellularity	100	100	1
Kundu et al., <sup>91</sup> 2009	100	Diagnostic yield	78	81	0.61
		Bloodiness	72	50	0.002
		Cellularity	50	59	0.59

NS, not significant.

quired needles passes and possible complications associated with the procedure.<sup>99-101</sup> The combination of increased diagnostic yield and fewer nondiagnostic specimens can translate into fewer needles used, shorter procedure times, fewer repeat procedures, and significant cost reduction. Indeed, the cost-benefit of onsite cytopathology has been evaluated and estimated to be over \$400,000 annually for a single institution in one study.<sup>78,95</sup>

## SLIDE PREPARATION AND FIXATIVE SOLUTIONS

EUS-FNA specimens are prepared in two methods to create smear. For immediate interpretation, the specimens can be air-dried and stained with Diff-Quik (Dade Diagnostics, Miami, FL, USA). Otherwise specimens can be fixed in an alcohol solution, which allows for nuclear preservation and eventually stained with Papanicolaou or H&E stains.<sup>75</sup> If special stains are required, then the sample can be stored in liquid media and prepared for cell block, which involves centrifuge, fixation, sectioning, and staining with H&E. A newer liquid-based cytology (Thin prep; Cytoc Inc., Marlborough, MA, USA and SurePath; TriPath Inc., Burlington, NC, USA) is now available. The benefits of this technique are that it may mitigate human error during preparation, better preserve the cellular integrity, and display cells in a uniform monolayer dispersion.<sup>75</sup> However, the liquid-based technology is costly and its accuracy has not been validated. Multiple studies have shown that traditional smear preparation exhibits a higher diagnostic accuracy (84% to 98% vs. 64% to 67%) when compared directly to liquid-based cytology. More studies are needed to evaluate the efficacy and usefulness of this preparation.

## IMPROVING EUS-FNA: ELASTOGRAPHY, CONTRAST HARMONIC EUS, AND FLUORESCENCE *IN SITU* HYBRIDIZATION

Although EUS-FNA is a good test for evaluating pancreatic lesions, it is not perfect as evidenced by the pooled diagnostic accuracy of about 87% in a recent meta-analysis.<sup>29</sup> Because making the diagnosis of pancreatic malignancy is so crucial

to overall prognosis, various complementary imaging and analytic technologies have been developed to try and improve this limitation.

Two novel imaging technologies now available for EUS include elastography and contrast harmonic echo. Elastography capitalizes on the fact that diseased tissue such as malignancy can lead to altered mechanical properties of the tissue through remodeling, inflammation, and fibrosis.<sup>102</sup> Thus, elastography can measure tissue stiffness and help differentiate benign versus malignant tissue without having to actually physically sample the lesion. Using the hue-histogram produced by the technology, which correlates with tissue elasticity, some studies have been able to show accuracy rates up to 89% to distinguish benign versus malignant pancreatic lesions and lymph nodes.<sup>103,104</sup> A variable known as the strain ratio, which is a calculated quotient of the lesion's relative stiffness, has been shown to have a sensitivity and specificity of 100% and 92.9% in diagnosing pancreatic malignancies.<sup>105</sup> Contrast-harmonic echo (CHE) is an imaging modality that enhances vascular imaging during EUS. Intravenous contrast agents containing gas-filled microbubbles are injected into peripheral veins thus allowing improved EUS visualization of the microvasculature, which may help diagnose malignant lesions. By evaluating the echogenicity and enhancement of pancreatic lesions during CHE, Fusaroli et al.<sup>106</sup> were able to show improved detection (96%) and accuracy (82%) for pancreatic adenocarcinomas. While elastography and CHE are promising technologies, they are still relatively new and require further research to establish their role in evaluating pancreatic masses. As of now, they should be used as complementary tools to EUS-FNA in specialized centers that have experts trained in their usage.

Another enhancement on the interpretation side of EUS-FNA has been the development of fluorescence *in situ* hybridization (FISH). FISH is a technique that can detect various chromosomal abnormalities by using specific, fluorescently-labeled DNA probes. Polysomy and trisomy FISH (aside from trisomy 7) have been shown to be independent predictors of malignancy with high specificities.<sup>107</sup> Multiple studies have shown that in cases of indeterminate cytology, FISH can provide a diagnosis up to 79% of cases without compromising specificity.<sup>108,109</sup> Further cost-effectiveness and efficacy studies are required to determine which in application of

**Table 8.** Studies Evaluating the Role of Onsite Cytopathology at the Time of Endoscopic Ultrasound-Guided Fine Needle Aspiration

Author	Number	Diagnostic yield with vs. without OCP, %	Unsatisfactory, %	Indeterminate, %
Klapman et al., <sup>96</sup> 2003	198	78 vs. 32, $p=0.001$	10 vs. 12, $p=0.9$	9 vs. 20, $p=0.003$
Alsohaibani et al., <sup>97</sup> 2009	104	77 vs. 53, $p=0.01$	23 vs. 47, $p=0.001$	0 vs. 17, $p=NS$
Iglesias-Garcia et al., <sup>98</sup> 2011	182	97 vs. 86, $p=0.01$	2.1 vs. 10.3, $p=0.02$	1 vs. 13, $p=0.002$

OCP, on-site cytopathologist; NS, not significant.

FISH is reasonable.

## PITTFALLS OF EUS-FNA: NONDIAGNOSTIC SAMPLING

Unfortunately, even the most experienced endoscopists cannot perform EUS-FNA with 100% sensitivity due to the inherent limitations of this technology. Lower diagnostic yields are inevitable in certain clinical diagnoses like chronic pancreatitis, which complicates sampling due to increased parenchymal lobularity and calcification.<sup>59</sup> Also transduodenal EUS-FNA can be challenging due to the position of the endoscope, passage of the needle, and visualization of the target lesion. Cytology samples, although adequate, may not be definitive in many cases. And finally, the lack of an onsite cytopathologist, often in the community setting, puts endosonographers at an immediate disadvantage in terms of procuring a diagnostic sample.

There is no universally accepted guideline on how to manage patients with suspected pancreatic cancer, but negative FNA sampling. The management options include repeating the EUS-FNA, attempting CT-guided biopsy, or proceeding to surgical exploration. In healthy, surgically-resectable patients, the decision to go to surgery is relatively straightforward. However, in less healthy/older patients with an unclear clinical picture or margin of tumor, that may be poor surgical candidates, the situation becomes more challenging. Generally, performing CT-guided biopsy is avoided because of the risk of peritoneal tumor seeding.<sup>72</sup> Therefore, in these cases, repeating the EUS-FNA may be the most prudent approach, with reported diagnostic rates of 61% to 84%.<sup>110-112</sup>

For newly trained endosonographers practicing in settings without onsite cytopathology, and thus are susceptible to lower EUS-FNA diagnostic yields, consideration should be given to learning how to self-interpret cytopathology specimens. In these cases, EUS-FNA method should be optimized including performing the appropriate number of passes (at least six to seven in the pancreas and three in lymph nodes) along with the fanning technique to minimize insufficient specimens.<sup>75</sup> Use of the 19 G core biopsy needle may also improve cellular yield. It is important for proceduralists performing EUS-FNA to understand the indications and relevant literature behind the tools they are using to ensure the best outcomes of the procedure and minimize nondiagnostic sampling.

## CONCLUSIONS

EUS-FNA has become a necessary tool for the complete and accurate evaluation of pancreatic lesions. Although it may be the single best test, starting with cross-sectional imaging to

initially evaluate a pancreatic lesion will complement further EUS-FNA assessment. EUS-FNA has established itself as the least invasive, safest, and most effective tool for tissue acquisition in the pancreas. It should be performed by trained endosonographers using the proper tools and techniques. Generally, the use of suction, stylets, and core biopsy needles has not proven to improve diagnostic accuracy. However, utilization of onsite cytopathology, the fanning technique, and appropriate needle gauge depending on the lesion's location improves outcomes. Research is ongoing on how to improve EUS-FNA in terms of novel imaging (elastography, CHE) and sample interpretation (FISH). EUS-FNA has established itself as the first-line procedure for tissue acquisition in the pancreas and will continue to improve as further research is performed to improve its outcomes.

## Conflicts of Interest

Dr. Michel Kahaleh has received grant support from Boston Scientific, Fujinon, EMcison, Xlumena Inc., MaunaKea, W.L. Gore, Cook Endoscopy, Aspire Bariatrics, GIDynamics, and MI Tech. He is a consultant for Xlumena Inc. and Boston Scientific.

## REFERENCES

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin* 2009;59:225-249.
2. Helmstaedter L, Riemann JF. Pancreatic cancer: EUS and early diagnosis. *Langenbecks Arch Surg* 2008;393:923-927.
3. Gudjonsson B. Cancer of the pancreas. 50 years of surgery. *Cancer* 1987;60:2284-2303.
4. National Cancer Institute. SEER Cancer Statistics Review 1975-2010 [Internet]. Bethesda: National Cancer Institute; 2013 [updated 2013 Jun 14]. Available from: [http://seer.cancer.gov/csr/1975\\_2010/](http://seer.cancer.gov/csr/1975_2010/).
5. Vilmann P, Jacobsen GK, Henriksen FW, Hancke S. Endoscopic ultrasonography with guided fine needle aspiration biopsy in pancreatic disease. *Gastrointest Endosc* 1992;38:172-173.
6. Chen J, Yang R, Lu Y, Xia Y, Zhou H. Diagnostic accuracy of endoscopic ultrasound-guided fine-needle aspiration for solid pancreatic lesion: a systematic review. *J Cancer Res Clin Oncol* 2012;138:1433-1441.
7. Klapman JB, Chang KJ, Lee JG, Nguyen P. Negative predictive value of endoscopic ultrasound in a large series of patients with a clinical suspicion of pancreatic cancer. *Am J Gastroenterol* 2005;100:2658-2661.
8. Hasan MK, Hawes RH. EUS-guided FNA of solid pancreas tumors. *Gastrointest Endosc Clin N Am* 2012;22:155-167.
9. Hunt GC, Faigel DO. Assessment of EUS for diagnosing, staging, and determining resectability of pancreatic cancer: a review. *Gastrointest Endosc* 2002;55:232-237.
10. Rösch T, Braig C, Gain T, et al. Staging of pancreatic and ampullary carcinoma by endoscopic ultrasonography: comparison with conventional sonography, computed tomography, and angiography. *Gastroenterology* 1992;102:188-199.
11. Palazzo L, Roseau G, Gayet B, et al. Endoscopic ultrasonography in the diagnosis and staging of pancreatic adenocarcinoma: results of a prospective study with comparison to ultrasonography and CT scan. *Endoscopy* 1993;25:143-150.
12. Müller MF, Meyenberger C, Bertschinger P, Schaer R, Marincek B. Pancreatic tumors: evaluation with endoscopic US, CT, and MR imaging. *Radiology* 1994;190:745-751.
13. Marty O, Aubertin JM, Bouillot JL, Hernigou A, Bloch F, Petite JP. Pro-



- spective comparison of ultrasound endoscopy and computed tomography in the assessment of locoregional invasiveness of malignant ampullar and pancreatic tumors verified surgically. *Gastroenterol Clin Biol* 1995;19:197-203.
14. Melzer E, Avidan B, Heyman Z, Coret A, Bar-Meir S. Preoperative assessment of blood vessel involvement in patients with pancreatic cancer. *Isr J Med Sci* 1996;32:1086-1088.
  15. Sugiyama M, Hagi H, Atomi Y, Saito M. Diagnosis of portal venous invasion by pancreatobiliary carcinoma: value of endoscopic ultrasonography. *Abdom Imaging* 1997;22:434-438.
  16. Legmann P, Vignaux O, Doussset B, et al. Pancreatic tumors: comparison of dual-phase helical CT and endoscopic sonography. *AJR Am J Roentgenol* 1998;170:1315-1322.
  17. Gress FG, Hawes RH, Savides TJ, et al. Role of EUS in the preoperative staging of pancreatic cancer: a large single-center experience. *Gastrointest Endosc* 1999;50:786-791.
  18. Mertz HR, Sechopoulos P, Delbeke D, Leach SD. EUS, PET, and CT scanning for evaluation of pancreatic adenocarcinoma. *Gastrointest Endosc* 2000;52:367-371.
  19. Rivadeneira DE, Pochapin M, Grobmyer SR, et al. Comparison of linear array endoscopic ultrasound and helical computed tomography for the staging of periampullary malignancies. *Ann Surg Oncol* 2003;10:890-897.
  20. Agarwal B, Abu-Hamda E, Molke KL, Correa AM, Ho L. Endoscopic ultrasound-guided fine needle aspiration and multidetector spiral CT in the diagnosis of pancreatic cancer. *Am J Gastroenterol* 2004;99:844-850.
  21. DeWitt J, Devereaux B, Chriswell M, et al. Comparison of endoscopic ultrasonography and multidetector computed tomography for detecting and staging pancreatic cancer. *Ann Intern Med* 2004;141:753-763.
  22. Volmar KE, Vollmer RT, Jowell PS, Nelson RC, Xie HB. Pancreatic FNA in 1000 cases: a comparison of imaging modalities. *Gastrointest Endosc* 2005;61:854-861.
  23. Dewitt J, Devereaux BM, Lehman GA, Sherman S, Imperiale TF. Comparison of endoscopic ultrasound and computed tomography for the preoperative evaluation of pancreatic cancer: a systematic review. *Clin Gastroenterol Hepatol* 2006;4:717-725.
  24. Mukai H, Nakajima M, Yasuda K, et al. Preoperative diagnosis and staging of pancreatic cancer by endoscopic ultrasonography (EUS): a comparative study with other diagnostic tools. *Nihon Shokakibyō Gakkai Zasshi* 1991;88:2132-2142.
  25. Midwinter MJ, Beveridge CJ, Wilsdon JB, Bennett MK, Baudouin CJ, Charnley RM. Correlation between spiral computed tomography, endoscopic ultrasonography and findings at operation in pancreatic and ampullary tumours. *Br J Surg* 1999;86:189-193.
  26. Soriano A, Castells A, Ayuso C, et al. Preoperative staging and tumor resectability assessment of pancreatic cancer: prospective study comparing endoscopic ultrasonography, helical computed tomography, magnetic resonance imaging, and angiography. *Am J Gastroenterol* 2004;99:492-501.
  27. Ramsay D, Marshall M, Song S, et al. Identification and staging of pancreatic tumours using computed tomography, endoscopic ultrasound and mangafodipir trisodium-enhanced magnetic resonance imaging. *Australas Radiol* 2004;48:154-161.
  28. Tierney WM, Francis IR, Eckhauser F, Elta G, Nostrant TT, Scheiman JM. The accuracy of EUS and helical CT in the assessment of vascular invasion by peripapillary malignancy. *Gastrointest Endosc* 2001;53:182-188.
  29. Puli SR, Bechtold ML, Buxbaum JL, Eloubeidi MA. How good is endoscopic ultrasound-guided fine-needle aspiration in diagnosing the correct etiology for a solid pancreatic mass?: a meta-analysis and systematic review. *Pancreas* 2013;42:20-26.
  30. Wegener M, Pfaffenbach B, Adamek RJ. Endosonographically guided transduodenal and transgastral fine-needle aspiration puncture of focal pancreatic lesions. *Bildgebung* 1995;62:110-115.
  31. Giovannini M, Seitz JF, Monges G, Perrier H, Rabbia I. Fine-needle aspiration cytology guided by endoscopic ultrasonography: results in 141 patients. *Endoscopy* 1995;27:171-177.
  32. Cahn M, Chang K, Nguyen P, Butler J. Impact of endoscopic ultrasound with fine-needle aspiration on the surgical management of pancreatic cancer. *Am J Surg* 1996;172:470-472.
  33. Chang KJ, Nguyen P, Erickson RA, Durbin TE, Katz KD. The clinical utility of endoscopic ultrasound-guided fine-needle aspiration in the diagnosis and staging of pancreatic carcinoma. *Gastrointest Endosc* 1997;45:387-393.
  34. Bhutani MS, Hawes RH, Baron PL, et al. Endoscopic ultrasound guided fine needle aspiration of malignant pancreatic lesions. *Endoscopy* 1997;29:854-858.
  35. Baron PL, Aabakken LE, Cole DJ, et al. Differentiation of benign from malignant pancreatic masses by endoscopic ultrasound. *Ann Surg Oncol* 1997;4:639-643.
  36. Faigel DO, Ginsberg GG, Bentz JS, Gupta PK, Smith DB, Kochman ML. Endoscopic ultrasound-guided real-time fine-needle aspiration biopsy of the pancreas in cancer patients with pancreatic lesions. *J Clin Oncol* 1997;15:1439-1443.
  37. Wiersema MJ, Vilman P, Giovannini M, Chang KJ, Wiersema LM. Endosonography-guided fine-needle aspiration biopsy: diagnostic accuracy and complication assessment. *Gastroenterology* 1997;112:1087-1095.
  38. Binmoeller KF, Thul R, Rathod V, et al. Endoscopic ultrasound-guided, 18-gauge, fine needle aspiration biopsy of the pancreas using a 2.8 mm channel convex array echoendoscope. *Gastrointest Endosc* 1998;47:121-127.
  39. Hünerbein M, Dohmoto M, Haensch W, Schlag PM. Endosonography-guided biopsy of mediastinal and pancreatic tumors. *Endoscopy* 1998;30:32-36.
  40. Williams DB, Sahai AV, Aabakken L, et al. Endoscopic ultrasound guided fine needle aspiration biopsy: a large single centre experience. *Gut* 1999;44:720-726.
  41. Suits J, Frazee R, Erickson RA. Endoscopic ultrasound and fine needle aspiration for the evaluation of pancreatic masses. *Arch Surg* 1999;134:639-642.
  42. Erickson RA, Sayage-Rabie L, Beissner RS. Factors predicting the number of EUS-guided fine-needle passes for diagnosis of pancreatic malignancies. *Gastrointest Endosc* 2000;51:184-190.
  43. Voss M, Hammel P, Molas G, et al. Value of endoscopic ultrasound guided fine needle aspiration biopsy in the diagnosis of solid pancreatic masses. *Gut* 2000;46:244-249.
  44. Brandwein SL, Farrell JJ, Centeno BA, Brugge WR. Detection and tumor staging of malignancy in cystic, intraductal, and solid tumors of the pancreas by EUS. *Gastrointest Endosc* 2001;53:722-727.
  45. Gress F, Gottlieb K, Sherman S, Lehman G. Endoscopic ultrasonography-guided fine-needle aspiration biopsy of suspected pancreatic cancer. *Ann Intern Med* 2001;134:459-464.
  46. Mallery JS, Centeno BA, Hahn PF, Chang Y, Warsaw AL, Brugge WR. Pancreatic tissue sampling guided by EUS, CT/US, and surgery: a comparison of sensitivity and specificity. *Gastrointest Endosc* 2002;56:218-224.
  47. Ylagan LR, Edmundowicz S, Kasal K, Walsh D, Lu DW. Endoscopic ultrasound guided fine-needle aspiration cytology of pancreatic carcinoma: a 3-year experience and review of the literature. *Cancer* 2002;96:362-369.
  48. Fritscher-Ravens A, Brand L, Knöfel WT, et al. Comparison of endoscopic ultrasound-guided fine needle aspiration for focal pancreatic lesions in patients with normal parenchyma and chronic pancreatitis. *Am J Gastroenterol* 2002;97:2768-2775.
  49. Harewood GC, Wiersema MJ. Endosonography-guided fine needle aspiration biopsy in the evaluation of pancreatic masses. *Am J Gastroenterol* 2002;97:1386-1391.
  50. Levy MJ, Jondal ML, Clain J, Wiersema MJ. Preliminary experience

- with an EUS-guided trucut biopsy needle compared with EUS-guided FNA. *Gastrointest Endosc* 2003;57:101-106.
51. Afify AM, al-Khafaji BM, Kim B, Scheiman JM. Endoscopic ultrasound-guided fine needle aspiration of the pancreas: diagnostic utility and accuracy. *Acta Cytol* 2003;47:341-348.
  52. Raut CP, Grau AM, Staerckel GA, et al. Diagnostic accuracy of endoscopic ultrasound-guided fine-needle aspiration in patients with presumed pancreatic cancer. *J Gastrointest Surg* 2003;7:118-126.
  53. Larghi A, Verna EC, Stavropoulos SN, Rotterdam H, Lightdale CJ, Stevens PD. EUS-guided trucut needle biopsies in patients with solid pancreatic masses: a prospective study. *Gastrointest Endosc* 2004;59:185-190.
  54. Ho S, Bonasera R, Michael H, et al. The accuracy of endoscopic ultrasound (EUS)-guided fine needle aspiration (FNA) for diagnosing solid pancreatic lesions using a new 25-gauge needle system. *Gastrointest Endosc* 2004;59:P222.
  55. Itoi T, Itokawa F, Sofuni A, et al. Puncture of solid pancreatic tumors guided by endoscopic ultrasonography: a pilot study series comparing Trucut and 19-gauge and 22-gauge aspiration needles. *Endoscopy* 2005;37:362-366.
  56. DeWitt J, Jowell P, Leblanc J, et al. EUS-guided FNA of pancreatic metastases: a multicenter experience. *Gastrointest Endosc* 2005;61:689-696.
  57. Ginès A, Wiersema MJ, Clain JE, Pochron NL, Rajan E, Levy MJ. Prospective study of a Trucut needle for performing EUS-guided biopsy with EUS-guided FNA rescue. *Gastrointest Endosc* 2005;62:597-601.
  58. Ryozaawa S, Kitoh H, Gondo T, et al. Usefulness of endoscopic ultrasound-guided fine-needle aspiration biopsy for the diagnosis of pancreatic cancer. *J Gastroenterol* 2005;40:907-911.
  59. Varadarajulu S, Tamhane A, Eloubeidi MA. Yield of EUS-guided FNA of pancreatic masses in the presence or the absence of chronic pancreatitis. *Gastrointest Endosc* 2005;62:728-736.
  60. Mitsuhashi T, Ghafari S, Chang CY, Gu M. Endoscopic ultrasound-guided fine needle aspiration of the pancreas: cytomorphological evaluation with emphasis on adequacy assessment, diagnostic criteria and contamination from the gastrointestinal tract. *Cytopathology* 2006;17:34-41.
  61. Wittmann J, Kocjan G, Sgouros SN, Deheragoda M, Pereira SP. Endoscopic ultrasound-guided tissue sampling by combined fine needle aspiration and trucut needle biopsy: a prospective study. *Cytopathology* 2006;17:27-33.
  62. Horwhat JD, Paulson EK, McGrath K, et al. A randomized comparison of EUS-guided FNA versus CT or US-guided FNA for the evaluation of pancreatic mass lesions. *Gastrointest Endosc* 2006;63:966-975.
  63. Storch I, Jorda M, Thurer R, et al. Advantage of EUS Trucut biopsy combined with fine-needle aspiration without immediate on-site cytopathologic examination. *Gastrointest Endosc* 2006;64:505-511.
  64. Aithal GP, Anagnostopoulos GK, Tam W, et al. EUS-guided tissue sampling: comparison of "dual sampling" (Trucut biopsy plus FNA) with "sequential sampling" (Trucut biopsy and then FNA as required). *Endoscopy* 2007;39:725-730.
  65. Rocca R, De Angelis C, Daperno M, et al. Endoscopic ultrasound-fine needle aspiration (EUS-FNA) for pancreatic lesions: effectiveness in clinical practice. *Dig Liver Dis* 2007;39:768-774.
  66. Ardengh JC, Lopes CV, de Lima LF, et al. Diagnosis of pancreatic tumors by endoscopic ultrasound-guided fine-needle aspiration. *World J Gastroenterol* 2007;13:3112-3116.
  67. Eloubeidi MA, Varadarajulu S, Desai S, et al. A prospective evaluation of an algorithm incorporating routine preoperative endoscopic ultrasound-guided fine needle aspiration in suspected pancreatic cancer. *J Gastrointest Surg* 2007;11:813-819.
  68. Agarwal B, Krishna NB, Labundy JL, Safdar R, Akduman EI. EUS and/or EUS-guided FNA in patients with CT and/or magnetic resonance imaging findings of enlarged pancreatic head or dilated pancreatic duct with or without a dilated common bile duct. *Gastrointest Endosc* 2008;68:237-242.
  69. Holt EW, Macklin EA, Brugge WR. Variables affecting the accuracy of EUS-guided FNA in the diagnosis of focal pancreatic masses. *Gastrointest Endosc* 2008;67:AB218-AB219.
  70. Jindal R, Goodman AJ, Yusuf TE, Pavey DA, Michael H, Gress FG. Clinical utility of endoscopic ultrasound-guided fine needle aspiration in pancreatic masses using a 25-gauge needle system: a multicenter experience. *Gastrointest Endosc* 2008;67:AB218.
  71. Athanassiadou P, Grapsa D. Value of endoscopic retrograde cholangiopancreatography-guided brushings in preoperative assessment of pancreaticobiliary strictures: what's new? *Acta Cytol* 2008;52:24-34.
  72. Micames C, Jowell PS, White R, et al. Lower frequency of peritoneal carcinomatosis in patients with pancreatic cancer diagnosed by EUS-guided FNA vs. percutaneous FNA. *Gastrointest Endosc* 2003;58:690-695.
  73. DeWitt J, LeBlanc J, McHenry L, McGreevy K, Sherman S. Endoscopic ultrasound-guided fine-needle aspiration of ascites. *Clin Gastroenterol Hepatol* 2007;5:609-615.
  74. Meijer OL, Weersma RK, van der Jagt EJ, van Dullemen HM. Endoscopic ultrasonography in suspected pancreatic malignancy and indeterminate CT. *Neth J Med* 2010;68:360-364.
  75. Varadarajulu S, Fockens P, Hawes RH. Best practices in endoscopic ultrasound-guided fine-needle aspiration. *Clin Gastroenterol Hepatol* 2012;10:697-703.
  76. Bang JY, Magee SH, Ramesh J, Trevino JM, Varadarajulu S. Randomized trial comparing fanning with standard technique for endoscopic ultrasound-guided fine-needle aspiration of solid pancreatic mass lesions. *Endoscopy* 2013;45:445-450.
  77. Wallace MB, Kennedy T, Durkalski V, et al. Randomized controlled trial of EUS-guided fine needle aspiration techniques for the detection of malignant lymphadenopathy. *Gastrointest Endosc* 2001;54:441-447.
  78. Pellisé Urquiza M, Fernández-Esparrach G, Solé M, et al. Endoscopic ultrasound-guided fine needle aspiration: predictive factors of accurate diagnosis and cost-minimization analysis of on-site pathologist. *Gastroenterol Hepatol* 2007;30:319-324.
  79. LeBlanc JK, Ciaccia D, Al-Assi MT, et al. Optimal number of EUS-guided fine needle passes needed to obtain a correct diagnosis. *Gastrointest Endosc* 2004;59:475-481.
  80. Siddiqui UD, Rossi F, Rosenthal LS, Padda MS, Murali-Dharan V, Aslanian HR. EUS-guided FNA of solid pancreatic masses: a prospective, randomized trial comparing 22-gauge and 25-gauge needles. *Gastrointest Endosc* 2009;70:1093-1097.
  81. Camellini L, Carlinfante G, Azzolini F, et al. A randomized clinical trial comparing 22G and 25G needles in endoscopic ultrasound-guided fine-needle aspiration of solid lesions. *Endoscopy* 2011;43:709-715.
  82. Fabbri C, Polifemo AM, Luigiano C, et al. Endoscopic ultrasound-guided fine needle aspiration with 22- and 25-gauge needles in solid pancreatic masses: a prospective comparative study with randomisation of needle sequence. *Dig Liver Dis* 2011;43:647-652.
  83. Song TJ, Kim JH, Lee SS, et al. The prospective randomized, controlled trial of endoscopic ultrasound-guided fine-needle aspiration using 22G and 19G aspiration needles for solid pancreatic or peripancreatic masses. *Am J Gastroenterol* 2010;105:1739-1745.
  84. Sakamoto H, Kitano M, Komaki T, et al. Prospective comparative study of the EUS guided 25-gauge FNA needle with the 19-gauge Trucut needle and 22-gauge FNA needle in patients with solid pancreatic masses. *J Gastroenterol Hepatol* 2009;24:384-390.
  85. Afolter KE, Schmidt RL, Matynia AP, Adler DG, Factor RE. Needle size has only a limited effect on outcomes in EUS-guided fine needle aspiration: a systematic review and meta-analysis. *Dig Dis Sci* 2013;58:1026-1034.
  86. Thomas T, Kaye PV, Raganath K, Aithal G. Efficacy, safety, and predictive factors for a positive yield of EUS-guided Trucut biopsy: a large tertiary referral center experience. *Am J Gastroenterol* 2009;104:584-591.

87. Iglesias-Garcia J, Poley JW, Larghi A, et al. Feasibility and yield of a new EUS histology needle: results from a multicenter, pooled, cohort study. *Gastrointest Endosc* 2011;73:1189-1196.
88. Bang JY, Hebert-Magee S, Trevino J, Ramesh J, Varadarajulu S. Randomized trial comparing the 22-gauge aspiration and 22-gauge biopsy needles for EUS-guided sampling of solid pancreatic mass lesions. *Gastrointest Endosc* 2012;76:321-327.
89. Mair S, Dunbar F, Becker PJ, Du Plessis W. Fine needle cytology: is aspiration suction necessary? a study of 100 masses in various sites. *Acta Cytol* 1989;33:809-813.
90. Puri R, Vilmann P, Săftoiu A, et al. Randomized controlled trial of endoscopic ultrasound-guided fine-needle sampling with or without suction for better cytological diagnosis. *Scand J Gastroenterol* 2009;44:499-504.
91. Kundu S, Conway J, Evans JA, Perkins LA, Geisinger K, Mishra G. A prospective, blinded, randomized trial assessing the yield of endoscopic ultrasound guided fine needle sampling (EUS-FNS) of solid lesions with suction versus no suction. *Gastrointest Endosc* 2009;69:AB323-AB324.
92. Sahai AV, Paquin SC, Garipey G. A prospective comparison of endoscopic ultrasound-guided fine needle aspiration results obtained in the same lesion, with and without the needle stylet. *Endoscopy* 2010;42:900-903.
93. Rastogi A, Wani S, Gupta N, et al. A prospective, single-blind, randomized, controlled trial of EUS-guided FNA with and without a stylet. *Gastrointest Endosc* 2011;74:58-64.
94. Wani S, Gupta N, Gaddam S, et al. A comparative study of endoscopic ultrasound guided fine needle aspiration with and without a stylet. *Dig Dis Sci* 2011;56:2409-2414.
95. Nasuti JF, Gupta PK, Baloch ZW. Diagnostic value and cost-effectiveness of on-site evaluation of fine-needle aspiration specimens: review of 5,688 cases. *Diagn Cytopathol* 2002;27:1-4.
96. Klapman JB, Logrono R, Dye CE, Waxman I. Clinical impact of on-site cytopathology interpretation on endoscopic ultrasound-guided fine needle aspiration. *Am J Gastroenterol* 2003;98:1289-1294.
97. Alsohaibani F, Girgis S, Sandha GS. Does onsite cytotechnology evaluation improve the accuracy of endoscopic ultrasound-guided fine-needle aspiration biopsy? *Can J Gastroenterol* 2009;23:26-30.
98. Iglesias-Garcia J, Dominguez-Munoz JE, Abdulkader I, et al. Influence of on-site cytopathology evaluation on the diagnostic accuracy of endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) of solid pancreatic masses. *Am J Gastroenterol* 2011;106:1705-1710.
99. Chang KJ, Katz KD, Durbin TE, et al. Endoscopic ultrasound-guided fine-needle aspiration. *Gastrointest Endosc* 1994;40:694-699.
100. Erickson RA, Sayage-Rabie L, Beissner RS. Factors predicting the number of EUS-guided fine-needle passes for diagnosis of pancreatic malignancies. *Gastrointest Endosc* 2000;51:184-190.
101. LeBlanc JK, Emerson RE, Dewitt J, et al. A prospective study comparing rapid assessment of smears and ThinPrep for endoscopic ultrasound-guided fine-needle aspirates. *Endoscopy* 2010;42:389-394.
102. Iglesias-Garcia J, Domínguez-Muñoz JE. Endoscopic ultrasound image enhancement elastography. *Gastrointest Endosc Clin N Am* 2012;22:333-348.
103. Giovannini M, Thomas B, Erwan B, et al. Endoscopic ultrasound elastography for evaluation of lymph nodes and pancreatic masses: a multicenter study. *World J Gastroenterol* 2009;15:1587-1593.
104. Săftoiu A, Vilmann P, Ciurea T, et al. Dynamic analysis of EUS used for the differentiation of benign and malignant lymph nodes. *Gastrointest Endosc* 2007;66:291-300.
105. Iglesias-Garcia J, Larino-Noia J, Abdulkader I, Forteza J, Dominguez-Munoz JE. Quantitative endoscopic ultrasound elastography: an accurate method for the differentiation of solid pancreatic masses. *Gastroenterology* 2010;139:1172-1180.
106. Fusaroli P, Spada A, Mancino MG, Caletti G. Contrast harmonic echo-endoscopic ultrasound improves accuracy in diagnosis of solid pancreatic masses. *Clin Gastroenterol Hepatol* 2010;8:629-634.
107. Fritcher EG, Kipp BR, Halling KC, et al. A multivariable model using advanced cytologic methods for the evaluation of indeterminate pancreaticobiliary strictures. *Gastroenterology* 2009;136:2180-2186.
108. Reicher S, Boyar FZ, Albitar M, et al. Fluorescence in situ hybridization and K-ras analyses improve diagnostic yield of endoscopic ultrasound-guided fine-needle aspiration of solid pancreatic masses. *Pancreas* 2011;40:1057-1062.
109. Kubiliun N, Ribeiro A, Fan YS, et al. EUS-FNA with rescue fluorescence in situ hybridization for the diagnosis of pancreatic carcinoma in patients with inconclusive on-site cytopathology results. *Gastrointest Endosc* 2011;74:541-547.
110. Eloubeidi MA, Varadarajulu S, Desai S, Wilcox CM. Value of repeat endoscopic ultrasound-guided fine needle aspiration for suspected pancreatic cancer. *J Gastroenterol Hepatol* 2008;23:567-570.
111. DeWitt J, McGreevy K, Sherman S, LeBlanc J. Utility of a repeated EUS at a tertiary-referral center. *Gastrointest Endosc* 2008;67:610-619.
112. Nicaud M, Hou W, Collins D, Wagh MS, Chauhan S, Draganov PV. The utility of repeat endoscopic ultrasound-guided fine needle aspiration for suspected pancreatic cancer. *Gastroenterol Res Pract* 2010;2010:268290.