Optimizing EUS-guided Tissue Sampling

novel devices and techniques

PRISCILLA A. VAN RIET

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Colophon

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nieuwe instrumenten en technieken

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Chapter 1

**General introduction,
aims and outline of the thesis**

GENERAL INTRODUCTION, AIMS AND OUTLINE OF THE THESIS

Endoscopic ultrasound (EUS)-guided tissue sampling was introduced in the nineties and offers a minimal invasive and accurate modality for real-time tissue acquisition [1, 2]. Since Vilmann first described its performance in solid pancreatic lesions, the technique has considerably evolved [3]. Today, its use is continuously growing, with an expanding role of tissue analysis in the era of patient tailored medicine [4]. Although EUS-guided tissue sampling can indeed provide a tissue diagnosis with a high level of diagnostic accuracy, its outcome strongly depends on the skills and experience of the performer, the sampling tools and techniques, and the way the tissue is handled and processed [5]. Consequently, EUS-guided tissue sampling has been subject to numerous innovations.

Adjusting and improving the design of EUS-needles has been and still is a major focus of innovation. Traditionally, tissue sampling was performed using fine needle aspiration (FNA) devices, which mainly harvest loose target cells for cytologic evaluation. Unfortunately, its yield depends on rapid on-site tissue evaluation (ROSE) by a dedicated pathologist, which is not generally available in most EUS-centers [6-9]. Furthermore, cytology is suboptimal for the identification of tumor invasion or the diagnosing and staging of specific diseases that require additional (immunohistochemical and molecular) testing, such as auto-immune pancreatitis, submucosal or stromal lesions, and neuro-endocrine tumors [10-12].

Fine needle biopsy (FNB) devices were introduced to overcome these limitations by offering the possibility to harvest histologically intact tissue fragments rather than loose target cells. Although the first devices, the TruCut^{tm} (Travenol Laboratories, 1980) and Quick-Core$^{®}$ (Cook Medical, 2003) needles achieved acceptable diagnostic accuracy rates, their use was hampered by a rigid design, and somewhat difficult deployment of the cutting and firing system [13-15]. Consequently, the ProCore reversed bevel needle (Cook Medical, Ireland) was introduced in 2012. This needle has a reverse bevel located at the lateral side near the tip, which collects tissue when the needle is moved in a retrograde motion. However, the diagnostic performance of the ProCore needle was not convincingly better than the conventional FNA needles [16-20].

As a response to this, several novel FNB needles were designed and introduced. The first was an adjusted ProCore needle, only available as 20-gauge (diameter), which has a forward facing rather than a reversed bevel, and a more flexible design (Cook Medical, 2015). Secondly, the Fork-tip or SharkCore needle (Medtronic, 2016) was introduced, which has a characteristic prominent long tip-edge and an opposing beveled tip-edge with a total six distal cutting-edge surfaces. Last, the Franseen or Acquire needle (Boston Scientific, 2017) was launched, which has a large crown-tip with three cutting edges and a long insertion length. Due to the relatively recent introduction of the newest ProCore, Acquire and SharkCore needle, evidence on their performance is limited.

Parallel to these needle design innovations, EUS-sampling techniques evolved. One adaptation is the application of negative pressure. With the ‘slow pull technique, the stylet is slowly

removed during sampling to create negative pressure at the tip of the needle, which should promote the harvest of tissue. Another way to increase negative pressure is through suction applied by using a vacuum syringe at the proximal end of the sampling device. So far, there is no convincing evidence for the benefit of either technique, or superiority of one over the other [21]. In addition, the 'fanning technique' was introduced, which is named after the fan-like-movement that is made with the needle within the lesion, allowing the lesion to be targeted from different angles, and collecting tissue from different areas of the target lesion. This technique has been proven to increase the diagnostic accuracy of EUS-guided tissue sampling, and is recommended by the European Society for Gastroenterology (ESGE) [22].

Another field of interest is EUS-tissue preservation and processing. Traditionally, specimens were collected with FNA-needles, and handled using the so-called 'smear technique'. Here, the collected material is smeared onto a glass slide and stained for pathological analysis. Unfortunately, smears are sensitive to preparation and contamination artifacts, causing suboptimal diagnostic accuracy rates [23, 24]. Obviously, it would be ideal to assign a dedicated pathologist to handle this on-site, but, as previously mentioned, many centers lack this service due to reimbursement and cost issues [6-9].

A way to bypass the vulnerable smear-preparation is to collect the sample in a liquid-based medium, the so-called liquid-based cytology (LBC) technique, i.e. ThinPrep, SurePath, Cellprep plus, and cell block. LBC makes samples less vulnerable to contamination or artifacts, since debris, blood and exudates can easily be removed from the collected tissue sample [25]. Furthermore, it allows for ancillary tissue tests, such as immunohistochemistry or molecular testing, that could previously only be performed on histological samples. Although these LBC tissue preparation techniques have proven their value in other specialties, such as gynaecology, its diagnostic benefit in gastroenterology remains to be established [24, 26-35].

Although innovations have evolved rapidly, the number of well-conducted studies to assess their value are running behind. Some adaptations may impact others. For example, if the new generation FNB needles turn out to outperform FNA, LBC preparations may become redundant.

AIMS AND OUTLINE OF THE THESIS

This thesis explores if and how technical factors can improve the diagnostic outcome of EUS-guided tissue sampling, by

1. gaining insight in the current practice of the endosonographer community
2. searching for the optimal EUS-sampling device
3. exploring ways to improve EUS-specimen preparation and handling

Part one focuses on the current clinical practice. Although EUS-guided tissue sampling is globally established, little is known about intercontinental practice variations. It is also unknown how

practice guidelines are locally implemented, especially since they lack firm scientific evidence. Therefore, **chapter 2** describes the practice patterns of EUS-guided tissue sampling in today's endosonographic community. An online questionnaire was sent out to 400 endosonographers from the United States (US), Europe, and Asia to identify differences and concordances between practice patterns, and to assess how they match the recommendations expressed in the guidelines of the American and European Society of Gastroenterology (ASGE and ESGE).

Part II aims to identify the optimal EUS-sampling device by focusing on the diagnostic performance of FNA and FNB needles. **Chapter 3** compares the diagnostic performance of a new FNB needle, the 20G ProCore FNB needle, to a conventional FNA needle, the 25G EchoTip Ultra device, in terms of diagnostic accuracy, tissue core yield, sample quality, the number of needle passes, and the number of adverse events in patients with a solid gastrointestinal lesion. A randomized controlled trial, the ASPRO study (Aspiration versus PROcore), was performed in 13 EUS-centers in the US, Asia, Australia, Europe, and the Middle-East.

Ideally, the performance of a diagnostic device is reproducible in expert and non-expert hands. **Chapter 4** compares the diagnostic agreement on the samples obtained in the above-mentioned trial amongst academic and non-academic pathologists. In addition, we assess if, and to what extent, the experience of the pathologist and the characteristics of the specimen influence diagnostic accuracy.

Instead of choosing one EUS-needle over the other, some advocate the use of FNA and FNB consecutively (dual needle sampling). **Chapter 5** therefore explores the yield of combined use of the 20G ProCore FNB and the 25G FNA needle in patients with a suspicious solid gastrointestinal lesion, and assesses the indication, the optimal needle order, and safety of this strategy. **Chapter 6** aims to identify the optimal sampling device, by providing an updated meta-analysis on the diagnostic performance of FNA compared to the new generation of FNB needles, including the ProCore reversed and forward facing bevel, the SharkCore, and the Acquire needle.

The third and final part of this thesis focusses on the optimization of the tissue samples that are collected through EUS-guided tissue sampling. It is known that the traditional, so called, smear-technique, harbors a high artifact rate. Since most EUS-centers do not have the resources for a dedicated, on-site pathologist to handle and prepare the collected tissue (ROSE), **chapter 7** explores if a one-day-hands-on tissue preparation training for endoscopy staff can improve sample quality and thus diagnostic accuracy. **Chapter 8** continues to find a solution to the suboptimal FNA-sample quality in centers lacking ROSE, by assessing the diagnostic benefit of tissue collection using LBC, with the ThinPrep and cell block technique.

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Part I

Current practice in EUS-guided tissue sampling

Chapter 2

Mapping international practice patterns in EUS-guided tissue sampling: outcome of a global survey

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ABSTRACT

Background and Aims

Although Endoscopic Ultrasound (EUS)-guided tissue sampling is widely used, the optimal sampling strategy remains subject of debate. We evaluated practice patterns within the international endosonographic community.

Methods

An online questionnaire was sent to 400 endosonographers from the United States (US), Europe, and Asia.

Results

A total of 186 (47%) endosonographers participated: US 54 (29%), Europe 85 (46%), and Asia 47(25%). European (75%) and Asian (84%) respondents routinely check coagulation status, whereas US respondents only check on indication (64%, p=0.007). While propofol sedation is standard in the US (83%), conscious sedation is still widely used in Europe (52%) and Asia (84%, p<0.001). Overall, the 22G needle is most commonly used (52%). For FNA of solid pancreatic lesions, 22G (45%) and 25G (49%) needles are used equally. For FNB of solid masses, the 25G device is less favored than the 22G FNA device (49% versus 21%). The 19G needle is generally used for FNB of submucosal masses (62%). Rapid on-site pathological evaluation (ROSE) is utilized more often by US (98%) than by European and Asian respondents (51%, p<0.001). CytoLyt (52%), formalin (15%) and alcohol (15%) are used for FNA specimen preservation in the US and Europe, while saline (27%) and alcohol (38%) are widely used in Asia (p<0.001).

Conclusion

EUS-guided tissue sampling practices vary substantially within the international endosonographic community and differ considerably from recommendations expressed in guidelines. As the clinical relevance of these variations is largely unknown, the outcome of this survey prompts for further studies.

INTRODUCTION

Endoscopic ultrasound (EUS) guided tissue sampling is a safe and accurate modality to diagnose and stage lesions in and around the gastrointestinal tract [1]. It enables clinicians to obtain a tissue diagnosis during real-time imaging, using fine-needle aspiration (FNA) or fine-needle biopsy (FNB). The diagnostic accuracy of these sampling techniques ranges from 52 % – 98 % and is influenced by several factors including target lesion characteristics, operator skills, needle size and type, sampling techniques, presence of an on-site pathologist, and specimen handling and processing [2-9].

To provide endosonographers with some guidance, both the American and European Society of Gastrointestinal Endoscopy (ASGE and ESGE) issued a set of guidelines [10-16]. In 2011, the ESGE published practice guidelines on EUS-guided tissue sampling, covering its indications, learning phase, techniques, complications and results [11,12]. They were updated in 2013, adding two new techniques; elastography and contrast enhanced ultrasound [16]. The ASGE has issued practice guidelines concerning sedation, antibiotic prophylaxis, and prevention of adverse events. In addition, the Papanicolaou Society of Cytopathology, one of the leading societies in cancer cytopathology, published guidelines addressing EUS cytology techniques, terminology, ancillary studies, and post procedure management [17,18]. Table 1 compares their most important recommendations. Unfortunately, due to the limited number of well-conducted studies in this field, many of these recommendations lack firm scientific evidence. As a result, today's practice mainly relies on local hospital protocols, expert opinions, and personal preferences.

Although EUS-guided tissue sampling is globally established, little is known about intercontinental variations in clinical practice. It is also unknown how available practice guidelines are implemented in current local sampling routines. The purpose of this study was therefore: 1) to map the practice patterns in EUS-guided tissue sampling in today's endosonographic community, 2) to identify differences and concordances between endosonographers from the United States (US), Europe and Asia, and 3) to compare the current practice patterns to the guidelines of the ASGE and ESGE.

METHODS

Selection of study subjects

An online questionnaire was sent out per e-mail to endosonographers from the US, Europe, and Asia. Registered endosonographers were selected by 1) using the personal network of the research team, which consists of national and international experts in the field, and 2) performing a PubMed literature search to identify authors who have published on the topic of EUS-guided tissue sampling in the last 10 years. Not only first authors but all listed authors were approached. Consent to participate in the study was inferred from voluntary completion of the survey.

Table 1. Recommendations for EUS-guided tissue sampling from the ASGE, ESGE, and Papanicolaou Society of Cytopathology.

	ASGE	ESGE	Papanicolaou Society of Cytopathology
Anticoagulant use	• EUS-FNA of solid lesions can be performed in patients on aspirin or NSAIDS, but not in patients on Thienopyridines.	• Check coagulation status in patients with personal or family history suggesting bleeding disorder or with a clear clinical indication. • EUS-FNA of solid lesions can be performed in patients on aspirin or NSAIDS, but not in patients on Thienopyridines.	
Antibiotic prophylaxis	• Recommended before sampling of cystic lesions.	• Recommended before sampling of cystic lesions.	
Sedation	• Propofol provides more rapid onset of action and shorter recovery time. No proof of higher patient satisfaction or better safety. Cost-effectiveness for average-risk patients is not proven. • On site anesthesiologist suggested in presence of patient-related risk factors.	• Propofol provides higher post procedural patient satisfaction, decreases time to sedation and recovery. No proof of cost-effectiveness. • On site anesthesiologist suggested in presence of patient-related risk factors.	
Needle size		• 19G, 22G and 25G needles have similar diagnostic yields and safety profiles. • 19G should not be used for transduodenal puncturing.	• Generally: 22G or 25G • Vascular mass: 25G • Lymph nodes: 25G • Mucinous cyst: 22G • Fibrotic stromal rich mass: 19G
Number of passes		• Cysts: 1 • Solid pancreatic: ≥5 • Lymph nodes: 3	• Cysts: 1 • Solid pancreatic: 5-7 • Lymph nodes: <5 • Stromal cell tumor: 3-5
Suction		• Applying continuous suction with a syringe is recommended in solid masses but not in lymph nodes.	

Questionnaire

The survey consisted of a maximum of 65 multiple-choice questions and was designed to take less than 10 minutes to complete (Appendix 1). The survey was divided in four sections. The first part focussed on demographics including gender, age, country of residence, type and size of current practice, years of experience, training and familiarity with EUS and EUS-guided tissue sampling. The second part included questions regarding peri-procedural use of anticoagulants,

antibiotics, and sedation. The third part contained questions on preferred equipment and sampling techniques and whether these preferences depend upon target lesion type (pancreatic solid or cystic mass, lymph node or submucosal mass). The final part of the survey examined practice patterns regarding tissue processing and analysis.

Questionnaire administration

All endosonographers were approached by e-mail with a study invitation and were provided with a personal, direct link to the survey. This link was inactivated once the survey was completed. A reminder was sent by e-mail, after two, four, and six weeks. Without effect within the next 4 weeks, a subject was considered to be a non-respondent.

Statistical analysis

Only completed surveys were used for data analysis. For comparison between continents, the Chi-squared or Kruskal Wallis test was applied. All reported p-values are two-sided and a value < 0.05 was considered to be significant. Data was analyzed with SPSS 22, Statistical Package for the Social Sciences, SPSS Inc., Chicago, Illinois.

RESULTS

Demographics

A total of 400 endosonographers were approached, of which 197 responded (49%). Eleven responses were discarded because they were incomplete, which resulted in 186 participants (47%): 54 from the US (29%), 85 from Europe (46%), and 47 from Asia (25%, Table 2, Appendix 2). The majority of the respondents were male (90%) gastroenterologists (96%), working in an academic setting (79%), and performing >300 EUS (58%) and >100 EUS-FNA procedures per year (68%).

Preprocedural practice patterns

Coagulations status

In preparation of the procedure, most European (75%) and Asian (84%) respondents report to 'always check' coagulation status, while their US colleagues generally do so on indication (Table 3, p=0.007). continuing Acetylsalicylic acid is generally continued (77%), but this differed between continents. US respondents always continue acetylsalicylic acid, as compared to 87% of European and 50% of Asian respondents (Table 3, p<0.001). Regarding the use of heparin, coumarin, and New Oral Anticoagulants (NOACs), there is little consensus. While heparin is discontinued by all US and most Asian respondents (94%), it is stopped by 75% of the Europeans (p=0.022). The opposite is true for coumarins, which are stopped more often in Europe (86%)

Table 2. Demographics and practice details of survey respondents per continent.

Variables	All n = 186 (100%)	US n = 54 (29%)	Europe n = 85 (46%)	Asia n = 47 (25%)
Age, years [Median IQR]	46 (41-52)	44,5 (41-54)	47 (41-52)	43 (40-49)
Male gender [Median IQR]	168 (90)	48 (89)	77 (91)	43 (92)
Specialty				
Gastroenterology	178 (96)	54 (100)	78 (91)	46 (98)
Other	8 (4)		7 (9)	1 (2)
Type of hospital				
Academic	146 (78)	48 (89)	64 (76)	34 (72)
Community	24 (13)	2 (4)	17 (20)	5 (11)
Other	16 (9)	4 (8)	4 (4)	8 (17)
Years of experience [Median IQR]	13 (8-20)	13 (5-22.25)	14 (9-20)	12 (8-18)
EUS procedures/yr.				
<100	7 (4)	0 (0)	5 (6)	2 (4)
100-200	33 (18)	7 (13)	11 (13)	15 (32)
200-300	37 (20)	15 (28)	15 (18)	7 (15)
>300	109 (58)	32 (59)	54 (63)	23 (49)
EUS-FNA/yr.				
<50	16 (9)	2 (4)	6 (7)	8 (17)
50-100	44 (24)	11 (20)	20 (24)	13 (28)
100-200	53 (28)	17 (32)	20 (24)	16 (34)
>200	73 (39)	24 (44)	39 (45)	10 (21)
Formal EUS-training	114 (61)	37 (69)	48 (57)	29 (62)

EUS, endoscopic ultrasound; FNA, fine-needle aspiration; IQR, interquartile range; US, United States.

Table 3. Anticoagulation and antiplatelet management for EUS-guided tissue sampling per continent.

Variables	All n = 99 (%)	US n = 11 (%)	Europe n = 56 (%)	Asia n = 32 (%)	p-value*
Routine coagulation check					
Always	73 (74)	4 (36)	42 (75)	27 (84)	0.007
On indication	26 (26)	7 (64)	14 (25)	5 (16)	
Anticoagulant stopped					
Acetylsalicylic acid	23 (23)	0 (0)	7 (13)	16 (50)	<0.001
Thienopyridines	80 (81)	8 (73)	47 (84)	25 (78)	0.618
Heparin	83 (84)	11 (100)	42 (75)	30 (94)	0.022
Coumarins	72 (73)	5 (46)	48 (86)	19 (59)	0.003
NOACs	80 (81)	10 (91)	49 (88)	21 (66)	0.029

US, United States; NOACs, new oral anticoagulants.

**A chi square test was used to compare the three continents.*

than in the US (46%) and Asia (59%, p=0.003). In analogy, European respondents less often perform tissue sampling in patients with an INR >1.5 (11%), as compared to non-European respondents (33%, p=0.008). Lastly, NOACs are discontinued by virtually all US (91%) and European (88%) endosonographers, as compared to 66% of Asian respondents (p=0.029).

Antibiotic prophylaxes

In all continents, the majority of respondents use antibiotic prophylaxis for EUS-guided tissue sampling (77%); mostly depending on the indication (92%), but some use antibiotics routinely (8%). Of those endosonographers who report to prescribe antibiotics on indication, virtually all use it when sampling a cystic lesion (95%)[12]. A minority prescribes antibiotics for other indications, such as a prosthetic cardiac valve, vascular graft, previous infective endocarditis, or congenital heart disease (<39%, Table 4). US physicians reported the lowest use of antibiotic prophylaxis.

Table 4. Antibiotic prophylaxis for EUS-guided tissue sampling; the US, as compared to Europe and Asia.

	All n = 132 (%)	US n = 38 (%)	Europe + Asia n = 94 (%)	p-value*
Antibiotic prophylaxes				
Prosthetic valve	41 (31)	6 (16)	35 (37)	0.012
Vascular graft	17 (13)	1 (3)	16 (17)	0.018
History of IE	52 (39)	5 (13)	47 (50)	<0.001
History of CHD	19 (14)	2 (5)	17 (18)	0.045
Lesion lower GI tract	44 (33)	13 (34)	31 (33)	0.523

US, United States; IE, Infectious endocarditis; CHD, congenital heart disease; GI, gastrointestinal.
**A chi square test was used to compare Europe and Asia with the US.*

Sedation and anesthesia

Almost all endosonographers sedate their patients during EUS-guided tissue sampling (98%). Propofol is generally used in the US (83%), whereas conscious sedation is still used by 52% of European and 84% of Asian respondents (p<0.001). All US respondents who use propofol have anesthesia personnel in the endoscopy room (100%), compared to only 66% in Europe and 50% in Asia (p<0.001).

Sampling techniques and equipment

Target lesion size

While half of the respondents perform EUS-FNA, regardless of the lesion diameter, the other half has a preferred minimum size of 0.5 cm (32%), 1 cm (17%), or 2 cm (1%). For EUS-FNB, most respondents confine to a minimum size of 1 cm (59%). European respondents perform EUS-FNB in lesions <1 cm more often (51%) than non-European respondents (34%, p=0.014).

Needle size

The gross of respondents prefers a specific needle size for FNA (84%) and FNB (75%), depending on the position of the scope or the location of the target lesion (66%). Overall, the 22G needle is most popular (Table 5). However, for FNA of solid pancreatic lesions, 22G (45%) and 25G (49%) needles are used equally and for FNA of submucosal lesions, besides the 22G (44%), the 19G

needle (49%) is frequently used. For FNB of submucosal masses, most respondents use the 19G needle (62%). Responses did not differ between continents.

Table 5. Reported use of needle size for EUS-guided tissue sampling.

FNA	All n = 88 (%)	FNB	All n = 72 (%)
Overall		Overall	
25G	86 (24)	25G	34 (12)
22G	192 (55)	22G	150 (52)
19G	74 (21)	19G	104 (36)
Pancreatic cystic lesion		Pancreatic cystic lesion	
25G	4 (5)	25G	4 (6)
22G	61 (69)	22G	49 (68)
19G	33 (26)	19G	19 (26)
Pancreatic solid lesion		Pancreatic solid lesion	
25G	43 (49)	25G	15 (21)
22G	40 (46)	22G	35 (49)
19G	5 (5)	19G	22 (31)
Lymph node		Lymph node	
25G	33 (38)	25G	13 (18)
22G	48 (54)	22G	41 (57)
19G	7 (8)	19G	18 (25)
Submucosal mass		Submucosal mass	
25G	6 (7)	25G	2 (2)
22G	43 (49)	22G	25 (35)
19G	39 (44)	19G	45 (63)

FNA, fine needle aspiration; FNB, fine needle biopsy.

Number of passes

Generally, respondents perform 2-3 needle passes for FNA (49%) and FNB (57%). Most respondents adjust the number of passes according to the target lesion. In pancreatic cysts, a single pass is performed for FNA (81%) and FNB (76%). For FNA of solid pancreatic masses, 2-3 (46%) or >3 needle passes are performed (50%). For FNB of solid pancreatic masses most respondents report to carry out only 2-3 passes (70%). A minority reports to do more than 3 passes (26%). Asian respondents vary their number of needle passes less often (47%) than European (69%) and US respondents (63%, p=0.037).

Sampling technique

Fanning is the preferred needle motion technique for FNA (64%). For FNB, fanning (44%) and only moving 'to and fro' (46%) are favored equally. To increase the yield of EUS-FNA, most endosonographers apply suction with a syringe (47%) or use the slow-pull technique (42%). Most respondents use dry instead of wet suction (93%). Also, for FNB, most endosonographers use an additional technique to increase the yield (70%); slow pull (53%), suction (44%), or a combination (3%). Some respondents adjust the sampling technique according to the target lesion (38%). While the slow-pull technique is mostly used for solid pancreatic masses (58%)

and lymph nodes (62%), suction is generally applied for pancreatic cysts (82%) and submucosal lesions (48%).

Tissue processing and analysis

After FNA, a majority of the endosonographers prepares glass slides (65%), which they fixate in alcohol (45%) or leave to air dry (43%). As for liquid-based cytology, CytoLyt is generally used to preserve FNA specimens in the US (50%) and Europe (53%), while in Asia both saline (28%) and alcohol (38%) are used ($p<0.001$). Formalin is mostly used to preserve FNB or histologic tissue specimens (62%). In order to increase the yield of sampling, most respondents additionally prepare and analyze tissue cores after FNA (73%) or cytological material after FNB (73%). Asian respondents more often look for tissue cores after FNA (96%) than European (68%) and US respondents (61%, $p<0.001$).

ROSE

Rapid on-site pathological evaluation (ROSE) is available to 65% of endosonographers. Virtually all US respondents use ROSE (98%), compared to only half of respondents from Europe (48%) and Asia (55%, $p<0.001$). Reasons for omitting ROSE included 'limited pathology staffing' (74%), 'disbelieve in its additive value' (32%), 'high costs' (24%), and 'additional procedure time' (24%).

Ancillary techniques

The majority of respondents apply the cellblock technique (85%). In the US, almost all endosonographers use cellblock (96%), while it is used to a lesser extent in Europe (85%) and Asia (70%, $p=0.002$). Immunohistochemical analysis is also available for most respondents (96%), and generally used for diagnosing and staging of submucosal masses (91%), solid pancreatic lesions (75%) and lymph nodes (70%).

DISCUSSION

To the best of our knowledge, no study has investigated the practice trends in EUS-FNA guided tissue sampling with respect to the current ASGE and ESGE guidelines. This survey identified substantial intercontinental differences in EUS guided tissue sampling. Interestingly, some routines vary considerably from the recommendations expressed in existing guidelines.

We found that sedation with propofol is custom in the US, but not in Asia and Europe. In the past conscious sedation was standard of care, but procedures have become lengthier and more complex, requiring higher doses of sedatives. Propofol is appreciated as an alternative, because it provides a deep level of sedation with a short recovery time. However, costs may be higher, due to the need of aneasthesiological assistance in most countries [13,19,20]. Since cost-effectiveness of sedation with propofol has not been established, the American and European Society of Gastroenterology do not take a stand on this subject [11,13]. Although

we did not ask participants to motivate their choice, previous studies have suggested that the increased use of propofol in the US is caused by 1) the believe that it improves the diagnostic accuracy of EUS-guided tissue sampling, 2) efforts to offset falling procedure reimbursements, and 3) marketing strategies of anaesthesiologists [13,21,22].

The second interesting finding involves differences in anticoagulation and antiplatelet management. While respondents from the US generally check coagulation status on indication only, European and Asian respondents do this more routinely. Interestingly, the practice of the US respondents, rather than that of the Europeans, seems to follow the ESGE guidelines, which recommend that coagulation status is only checked in selected patients, that is in those using anticoagulant or antiplatelet therapy or with a (family) history of a bleeding disorder. Both the ASGE and ESGE recommend not to discontinue acetylsalicylic acid, while all other anticoagulation and antiplatelet therapy should be stopped [12,23]. In contrast to US respondents, not all European and Asian respondents adhere to this recommendation. One explanation might be that US physicians adhere to guidelines more promptly, possibly as a consequence of an increased chance for malpractice claims in the US. [24,25]. The relatively high number of Asian respondents who discontinue acetylsalicylic acid may reflect the fact that bleeding risks are weighted more heavily in Asia. It has been suggested that Asians are more susceptible to bleeding complications, while Caucasians are more at risk for thromboembolic events [26]. However, the Japan Gastroenterological Endoscopy Society has recently revised their guidelines, emphasizing the thromboembolism risks of discontinuation of antithrombotic agents [27]. Therefore, a shift towards continuance of acetylsalicylic acid is to be expected.

Another interesting finding of this survey is that for solid pancreatic masses, endosonographers report to perform fewer needle passes with FNB than with FNA. This finding is line with recently published data about using FNB to establish a diagnosis in solid pancreatic masses [28-31]. The ESGE recommends performing at least 5 passes for FNA of solid pancreatic masses, in the absence of ROSE. Neither the ASGE not the ESGE recommend a minimum number of passes for FNB.

Also noteworthy is that, overall, most respondents reported to use the 22G needle more often than the 25G needle. This finding is especially interesting, since two recent meta-analysis found no differences between the two needles, with regard to diagnostic accuracy, the number of needle passes, or complications [8,32]. In fact, a trend towards better performance of the 25G needle for FNA of solid pancreatic masses was observed in these studies. The ESGE guideline states that, although there is no difference in diagnostic yield and safety profiles, the 25G needle performs somewhat better with regard to number of required needle passes, presumably due to its higher flexibility [12]. One of the leading societies in cancer cytopathology, the Papanicolaou Society of Cytopathology, recommends adapting the needle size to the target lesion. For highly vascular lesions and lymph nodes they recommend a 25G needle, for mucinous cysts a 22G needle, and for fibrotic or stromal-rich lesions a 19G needle [17].

Another important outcome of this survey is the intercontinental variation in use of rapid on-site pathological evaluation. Whereas virtually all US respondents use ROSE, only half of the European and Asian respondents do. Respondents who refrain from using ROSE state that they consider it too time consuming and that reimbursement for pathology services is too low. However, more than two thirds of our respondents also mention that they have doubts with regard to the added benefit of ROSE, which might be influenced by the recommendations of the ESGE which state that ROSE should only be implemented at sites where specimen adequacy rates are below 90% or during the learning curve of EUS-FNA [12,33]. In contrast, the Papanicolaou Society of Cytopathology recommends the use of ROSE whenever possible [17].

The last, but certainly not least remarkable finding concerns the preservation of the tissue samples. After procurement, EUS-FNA specimens are susceptible to damage by colonizing bacteria and to autolysis by enzyme activity. To halt these processes, it must be placed in a fixative (e.g., formalin, CytoRich Red, CytoLyt) or physiologic solution (e.g., saline, Hanks' salt solution). Although most of the respondents use formalin to preserve histologic samples, there is no consensus regarding preservation of cytological samples. While a majority of the Asian respondents store cytology in alcohol or saline, their European and US colleagues store it in CytoLyt. Although there are currently no guidelines on this topic, we did not expect to find such striking differences between the three continents. It would be interesting to investigate the influence of preservation methods on the specimen's quality and diagnostic accuracy, as this aspect is under-investigated so far.

Our survey has some potential limitations. First, it seems conceivable that our results have been subject to a response bias, given our response rate of 47%. Although our response rate still falls at the high end of the spectrum of responses for online surveys amongst physicians [1-10], it might have caused a selection towards the more active, academic endosonographers. Although most respondents indeed reported to work in high volume academic centers, only 61% had participated in a formal EUS training program. This could have accounted for the low adherence to the practice guidelines. Currently, the ESGE and ASGE advise that a dedicated fellowship should last 6-24 months [12,34]. However, they also acknowledge that there is a lack of sufficient EUS-training and training capacity in Europe and the US [35,36]. Since most respondents in the present study are EUS experts, the number of formal trained endosonographers and the adherence to the guidelines is likely to be even lower in non-academic, low volume centers. Last, a reporting or goodwill bias is likely to exist, since this is inevitable for retrospective surveys that are based on self-reporting. If respondents indeed gave an expected answer rather than a true answer, this would only strengthen our main conclusion that practice patterns for EUS-guided tissue sampling differ and are not congruent with the guidelines. In conclusion, this survey shows that there is considerable intercontinental variation in the practice of EUS-guided tissue sampling. Despite of the growing number of studies in the field of EUS-guided tissue sampling, the optimal sampling strategy remains subject of debate. Moreover, some routines vary considerably from recommendations stated in existing guidelines.

Further studies are required to determine the relevance and impact of various practices on outcome and safety. Pending these outcomes, cost-effectiveness studies may be required to support the implementation of a certain sampling strategies.

APPENDICES 1 - 2

APPENDIX 1: The online survey

Background Information

1. **What is your gender?**
   - ☐ Female
   - ☐ Male

2. **What is your age?**
   Please write your answer here: _____

3. **What is your specialty?**
   - ☐ Gastroenterologist
   - ☐ Surgeon
   - ☐ Other

4. **In which year did you finish your training?**
   Please write your answer here: _____

5. **In what country are you currently working?**
   Please write your answer here: _____

6. **In what kind of hospital are you currently working? (More than one option possible)**
   Please choose all that apply:
   - ☐ Community hospital
   - ☐ Academic/University hospital
   - ☐ Private hospital or independent endoscopy unit
   - ☐ Other, please specify: _____

7. **How many EUS procedures do you perform each year?**
   Please choose only one of the following:
   - ☐ < 100
   - ☐ 100-200
   - ☐ 200-300
   - ☐ > 300

8. How many EUS-guided tissue-sampling procedures do you perform each year?

Please choose only one of the following:

□ < 50

□ 50-100

□ 100-200

□ > 200

9. Did you have formal training in performing EUS guided tissue sampling? *(Formal training is defined as a fellowship in a dedicated EUS training center for at least 3 months)*

Please choose only one of the following:

□ Yes

□ No

Preparation for EUS guided tissue sampling

10. Do you use any type of sedation when performing EUS-guided tissue sampling?

Please choose only one of the following:

□ Yes, conscious sedation, continue to 12

□ Yes, propofol

□ No, not as standard practice, continue to 12

11. Is anesthesia personnel routinely present during the procedure?

Please choose only one of the following:

□ Yes

□ No

12. Do you use antibiotic prophylaxis when performing EUS-guided tissue sampling?

Please choose only one of the following:

□ Yes, always, continue to 14

□ Yes, depending on the indication

□ No, continue to 14

13. Please specify for which indication you use AB prophylaxis? (More than 1 answer possible)

Please choose all that apply:

☐ Cystic lesions
☐ Prosthetic cardiac valve
☐ Vascular graft
☐ History of previous infective endocarditis
☐ Congenital heart disease
☐ Solid lesions of lower gastrointestinal tract
☐ Other, please specify: _____

14. Do you routinely check the coagulation parameters before EUS-guided tissue sampling?

Please choose only one of the following:

☐ Yes
☐ No, continue to 18

15. Please specify when you check coagulation status? (More than one answer possible)

Please choose only one of the following:

☐ Always
☐ In patients on anticoagulants
☐ In patients with a (family) history of bleeding disorder
☐ In both, patients on anticoagulants and patients with a (family) history of bleeding disorder

16. Which of the following anticoagulants do you generally discontinue, prior to a puncture procedure? (More than one answers possible)

Please choose all that apply:

☐ Acetylsalicylic acid (aspirin, carbasalate calcium (Ascal), dipyridamole (Persantin))
☐ Thienopyridines (Ccopidogrel (Plavix, Grepid, Iscover, Vatoud), prasugrel (Effient))
☐ Coumarin derivatives (acenocoumarol (Sintrom), phenprocoumon (Marcoumar, Marcumar, Falithrom))
☐ Heparin or derivatives (warfarin (Coumadin), dalteparin (Fragmin), nadroparin (Fraxiparin), tinzaparin (Innohep))
☐ New Oral Anticoagulant drugs (NOAC) (rivaroxaban (Xarelto), apixaban (Eliquis), dabigatran (Pradax))
☐ Other, please specify: _____

17. Up to which INR value would you consider it safe to perform EUS-guided tissue sampling?

Please choose only one of the following:

☐ INR 1.0

☐ INR 1.1 - 1.5

☐ INR 1.6-2.0

☐ INR > 2.0

This section contains questions about Fine Needle Aspiration

18. What is the minimum lesion diameter for you to consider FNA?

Please choose only one of the following:

☐ No minimum

☐ 0.5 cm

☐ 1 cm

☐ 2 cm

19. Do you have a preferred needle size for FNA?

Please choose only one of the following:

☐ Yes

☐ No, continue to 21

20. Does your preferred needle size depend on scope position and/or location of target lesion?

Please choose only one of the following:

☐ Yes, continue to 22

☐ No

21. Which needle size do you generally prefer?

Please choose only one of the following:

☐ 19G

☐ 22G

☐ 25G

22. Specify if your preferred needle size depends on: (More than one answer possible)

Please choose all that apply:

☐ Location of target lesion,

☐ Scope position, continue to 24

23. Please specify your preferred needle size for the following indications:

Please choose the appropriate response for each item:

	19G	22G	25G
Pancreatic solid mass	☐	☐	☐
Pancreatic cystic mass	☐	☐	☐
Lymph node	☐	☐	☐
Submucosal mass	☐	☐	☐

24. Please specify your preferred needle size for the following scope positions:

Please choose the appropriate response for each item:

	19G	22G	25G
Transgastric	☐	☐	☐
Transduodenal D1 (Superior part/ Duodenal bulb)	☐	☐	☐
Transduodenal D2 (Descending part)	☐	☐	☐
Transduodenal D3 (Horizontal part)	☐	☐	☐

25. Does your number of needle passes depend on the indication for FNA?

Please choose only one of the following:

☐ Yes

☐ No, continue to 27

26. Please specify the number of needle passes per indication.

Please choose the appropriate response for each item:

	1	2-3	> 3
Pancreatic solid mass	☐	☐	☐
Pancreatic cystic mass	☐	☐	☐
Lymph node	☐	☐	☐
Submucosal mass	☐	☐	☐

27. Please specify the number of needle passes you generally perform.

Please choose only one of the following:

☐ 1

☐ 2-3

☐ > 3

28. What is your preferred needle movement technique during FNA?

Please choose only one of the following:

☐ To & Fro

☐ Fanning

☐ No preferred technique

29. Which additional techniques do you employ to increase the yield of tissue sampling during FNA? Please choose only one of the following:

☐ Slow pull

☐ Syringe

☐ Wet suction

☐ Capillary technique

☐ None

☐ Other, please specify _____

30. How do you expel sampling material from the FNA needle? (More than one answer possible)

Please choose all that apply:

☐ Flushing with air

☐ Flushing with saline

☐ With stylet

31. Do you use on-site pathological evaluation of the specimen?

Please choose only one of the following:

☐ Yes, always

☐ Yes, sometimes

☐ No, continue to 33

32. Please specify who performs on-site pathological evaluation.

Please choose only one of the following:

☐ Pathologist

☐ Cytotechnician

☐ Myself

33. Why are you not using on-site pathological evaluation? (More than one answer possible)

Please choose all that apply:

☐ No added benefit with regard to yield

☐ Costs

☐ Time

☐ Expertise

☐ No pathological personnel available

☐ Other, please specify _____

34. Do you prepare glass slides after you performed FNA?

Please choose only one of the following:

☐ Yes

☐ No, continue to 37

35. How do you fixate these smears?

Please choose only one of the following:

☐ Air dry

☐ Direct fixation with alcohol

☐ Other, please specify _____

36. Which preservation medium do you use to collect cytology, obtained with FNA?

Please choose only one of the following:

☐ Saline

☐ CytoLyt

☐ A fixative (formalin)

☐ Hanks

☐ Alcohol

☐ Other, please specify _____

37. Is the cell block technique applied in your center?

Please choose only one of the following:

☐ Yes

☐ No

38. Do you or your pathologist routinely look for tissue cores after FNA?

Please choose only one of the following:

□ Yes, always, continue to 40

□ Yes, depending on the target lesion

□ No, continue to 44

39. Please specify for which indication(s) you look for tissue cores after FNA? (More than one answer possible)

Please choose all that apply:

□ Cystic pancreatic lesions (from solid components or cyst wall)

□ Solid pancreatic lesions

□ Lymph nodes

□ Submucosal lesion

40. Are these tissue cores processed differently compared to the cytological tissue sample?

Please choose only one of the following:

□ Yes

□ No, continue to 44

41. They are collected in a separate vial?

Please choose only one of the following:

□ Yes

□ No

42. They are collected in a different medium?

Please choose only one of the following:

□ Yes

□ No

43. In what medium?

Please choose only one of the following:

□ Saline

□ CytoLyt

□ A fixative (formalin)

□ Hanks

□ Alcohol

This section contains questions about Fine Needle Biopsy

44. What is the minimum lesion diameter for you to consider FNB?

Please choose only one of the following:

□ No minimum

□ 0.5 cm

□ 1 cm

□ 2 cm

45. Do you have a preferred needle size for FNB?

Please choose only one of the following:

□ Yes, continue to 47

□ No

46. Which needle size do you generally prefer?

Please choose only one of the following:

□ 19G

□ 22G

□ 25G

47. Does your preferred needle size depend on scope position and/or location of target lesion?

Please choose only one of the following:

□ Yes, continue to 49

□ No

48. Which needle size do you generally prefer?

Please choose only one of the following

□ 19G

□ 22G

□ 25G

49. Specify if your preferred needle size depends on: (More than one answer possible)

Please choose all that apply:

□ Location of target lesion

□ Scope position, continue to 51

50. Please specify your preferred needle size for the following indications:

Please choose the appropriate response for each item:

	19G	22G	25G
Pancreatic solid mass	☐	☐	☐
Pancreatic cystic mass	☐	☐	☐
Lymph node	☐	☐	☐
Submucosal mass	☐	☐	☐

51. Please specify your preferred needle size for the following scope positions:

Please choose the appropriate response for each item:

	19G	22G	25G
Transgastric	☐	☐	☐
Transduodenal D1 (Superior part/ Duodenal bulb)	☐	☐	☐
Transduodenal D2 (Descending part)	☐	☐	☐
Transduodenal D3 (Horizontal part)	☐	☐	☐

52. Does your number of needle passes depend on the indication for FNB?

Please choose only one of the following:

☐ Yes

☐ No, continue to 54

53. Please specify the number of needle passes per indication.

Please choose the appropriate response for each item:

	1	2-3	> 3
Pancreatic solid mass	☐	☐	☐
Pancreatic cystic mass	☐	☐	☐
Lymph node	☐	☐	☐
Submucosal mass	☐	☐	☐

54. Please specify the number of needle passes you generally perform.

Please choose only one of the following:

☐ 1

☐ 2-3

☐ > 3

55. What is your preferred needle movement technique during FNB?

Please choose only one of the following:

☐ To & Fro

☐ Fanning

☐ No preferred technique

56. Do you use a special technique (slow pull or syringe) to acquire tissue with the FNB needle?

Please choose only one of the following:

☐ Yes, this depends on the indication

☐ Yes, independent of the indication, continue to 58

☐ No, continue to 59

57. Please specify per indication

Please choose the appropriate response for each item:

	Slow pull	Syringe	Wet suction	Capillary technique	Other
Pancreatic solid mass	☐	☐	☐	☐	☐
Pancreatic cystic mass	☐	☐	☐	☐	☐
Lymph node	☐	☐	☐	☐	☐
Submucosal mass	☐	☐	☐	☐	☐

58. Please specify

Please choose only one of the following:

☐ Slow pull

☐ Syringe

☐ Wet suction

☐ Capillary technique

☐ Other, please specify _____

59. How do you expel sampling material from the FNB needle? (More than one answer possible) Please choose all that apply:

☐ Flushing with air

☐ Flushing with saline

☐ With stylet

60. Which preservation medium do you use to collect the FNB specimen?

Please choose only one of the following:

□ Saline

□ CytoLyt

□ A fixative (formalin)

□ Hanks

□ Alcohol

□ Other, please specify _____

61. Is immunohistochemical analysis performed in your center? (when sufficient sampling material is available)

Please choose only one of the following:

□ Yes, depending on the indication

□ Yes, independent of the indication, continue to 63

□ No, continue to 63

62. Please specify (More than one answer possible)

Please choose all that apply:

□ Solid pancreatic mass

□ Lymph node

□ Submucosal mass

63. Is a cytological sample also prepared and evaluated (i.e. glass slide, cyto spin), in addition to the histological tissue core specimen?

Please choose only one of the following:

□ Yes

□ No, end of survey

64. Does this depend on the needle size?

Please choose only one of the following:

□ Yes

□ No, end of survey

65. Please specify for which needle size you look for additional cytological sample?

Please choose all that apply:

□ 19G

□ 22G

□ 25G

APPENDIX 2: List of countries of respondents

	Number of respondents	Percentage of total (%)
Europe		
Finland	1	0.5
Israel	1	0.5
Latvia	1	0.5
Scotland	1	0.5
Belgium	2	1.1
Ireland	2	1.1
Norway	2	1.1
Switzerland	2	1.1
Sweden	3	1.6
Germany	7	3.8
Spain	9	4.8
France	10	5.4
England	13	7.0
Netherlands	13	7.0
Italy	18	9.7
Asia		
Korea	1	1.6
India	5	2.7
Malaysia	5	2.7
China	7	3.8
Singapore	8	4.3
Japan	19	10.2
North America		
United States	54	29
TOTAL	**186**	**100**

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Part II

The optimal EUS-sampling device

Chapter 3

A multicenter randomized trial comparing a 25-gauge EUS fine-needle aspiration device with a 20-gauge EUS fine-needle biopsy device

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ABSTRACT

OBJECTIVE

Several studies have compared EUS fine-needle aspiration (FNA) to biopsy (FNB) needles, but none has proven superiority. We performed a multicenter randomized controlled trial to compare the performance of a commonly used 25-gauge FNA needle to a newly designed 20-gauge FNB needle.

DESIGN

Consecutive patients with a solid lesion were randomized in this international multicenter study between a 25-gauge FNA (EchoTip Ultra) or a 20-gauge FNB needle (ProCore). Primary endpoint was diagnostic accuracy for malignancy and the Bethesda classification (non-diagnostic, benign, atypical, malignant). Technical success, safety, and sample quality were also assessed. Multivariable and supplementary analyses were performed to adjust for confounders.

RESULTS

608 patients were allocated to FNA (n=306) or FNB (n=302); 312 pancreatic lesions (51%), 147 lymph nodes (24%), and 149 other lesions (25%). Technical success rate was 100% for the 25-gauge FNA and 99% for the 20-gauge FNB needle (p=0.043), without differences in adverse events. The 20-gauge FNB needle outperformed 25-gauge FNA in terms of histological yield (77% vs 44%, p<0.001), accuracy for malignancy (87% vs 78%, p=0.002) and Bethesda classification (82% vs 72%, p=0.002). This was robust when corrected for indication, lesion size, number of passes, and presence of an on-site pathologist (OR 3.53, 95% CI 1.55-8.56, p=0.004), and did not differ between centers (p=0.836).

CONCLUSION

The 20-gauge FNB needle outperformed the 25-gauge FNA needle in terms of histological yield and diagnostic accuracy. This benefit was irrespective of the indication and consistent amongst participating centers, supporting the general applicability of our findings.

INTRODUCTION

Endoscopic Ultrasound (EUS)-guided fine-needle aspiration (FNA) is a well-established technique for tissue acquisition of lesions in and around the gastrointestinal tract. However, FNA needles generally provide cytological rather than histological specimens. To optimize the efficacy, efforts have been made to ensure collection of histological specimens, as intact tissue architecture enables a range of ancillary diagnostic tests, including immunochemical and biomolecular testing. As a result, dedicated EUS fine-needle biopsy (FNB) devices have been developed. Although these needles seem to generate good-quality specimens and provide a diagnosis in less passes than with FNA, some studies reported they did not so much improve the histological, but rather, the cytological yield [1-8]. Moreover, due to a more rigid design, their applicability is questioned in lesions that are difficult to sample from an angulated scope position, such as fibrotic pancreatic head masses [7, 9-11]. Lately, several novel FNB needles have been introduced, claiming to overcome this problem by having adapted their design to provide more flexibility.

Previous studies comparing FNA and FNB were retrospective, underpowered, did not include the whole range of indications, or were performed in a single center, which hampers their generalizability [3, 5, 7-9, 11-18]. So far, only one multicenter trial showed a benefit of FNB over FNA, but only in large pancreatic lesions [19]. Consequently, the authors of the latest 2017 ESGE guidelines on technical aspects of EUS-guided sampling in gastroenterology lacked scientific ground to favor a specific technique or needle design [20, 21].

As the role of EUS-guided tissue acquisition is expanding in this era of personalized medicine, identification of the optimal sampling technique bares even more relevance [22-25]. An EUS-needle device should be flexible, yet large enough to ensure ample representative tissue in as few passes as possible. Moreover, in the past FNA and FNB were regarded as separate entities, but this distinction seems less suitable nowadays. Although FNB needles incorporate specific design changes aimed to facilitate extraction of tissue cores, it has been shown that it is also possible to obtain tissue cores with FNA needles [7, 13, 19]. Moreover, the cell block technique allows for 'histology like' analysis of cytology material. Conversely, with FNB needles, besides true tissue cores, material for cytological analysis is also obtained.

We set up a multicenter randomized controlled trial to compare the performance and diagnostic accuracy of a newly designed flexible 20-gauge FNB needle with a forward facing bevel to a more conventional 25-gauge FNA needle with a standard bevel, which is widely used amongst endosonographers because of its flexibility and proven optimal diameter for FNA [26-30].

METHODS

Study design

This investigator initiated, prospective randomized multicenter study was conducted in 13 EUS-centers in the United States (Irvine, New Haven, New York), Europe (Leuven, Marseille, Milan, Rome, Rotterdam, Santiago de Compostela, and Stockholm), Australia (Adelaide), Asia (Osaka-Sayama), and the Middle-East (Tel Aviv). Data were collected using online case record forms, which were accessible through a designated study website (www.aspro-study.com). The study was approved by the Institutional Review Boards of all participating centers and registered online, at ClinicalTrials.gov: NCT02167074. Financial support was provided by Cook Medical, Ireland (www.cookmedical.com), in the form of an unrestricted grant.

Patient selection

Consecutive patients with an indication for EUS-guided tissue acquisition of a solid pancreatic lesion, lymph node or other solid or submucosal lesion were prospectively enrolled from February 2015 to September 2016. Inclusion criteria comprised patient age ≥18 years, visualization of the target lesion during EUS, a lesion diameter ≥1 cm and signed informed consent. Both, virgin and previously sampled target lesions were included. Exclusion criteria were; increased bleeding risk (a bleeding disorder that could not be corrected with co-fact or fresh frozen plasma) or anticoagulant use that could not be discontinued to guarantee an INR <1.5, a purely cystic lesion, previous inclusion in the current study, or pregnancy.

Allocation and blinding

Patients were randomized 1:1 by use of an online randomization tool assessable on-site, to tissue sampling with the 20-gauge ProCore® FNB needle (Figure 1) or the 25-gauge EchoTip® Ultra FNA needle (both Cook Medical, Ireland). Random block sizes were used for allocation concealment between groups. Patients were blinded as to which needle was used. Pathologists were only blinded if they were not present at the EUS-procedure.

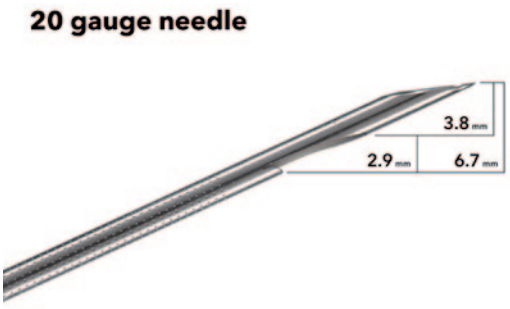

Figure 1. Needle tip design and dimensions of the 20-gauge FNB needle with a forward facing bevel and a Menghini tip-design.

EUS-procedure and tissue acquisition

All participating endosonographers were experienced with a life-time performance of >1000 EUS-guided tissue sampling procedures. They followed a standardized protocol, using a convex array echoendoscope (Pentax EG-3870UTK/3270UK, Olympus UTC 140/160/180/190/260 or UC140). If more than one lesion was identified, the most suspicious lesion was targeted. Each lesion was attempted to be punctured at least three times and tissue was obtained by a 'to and fro' movement. The number of 'to and fro' movements (gradual withdrawal of the stylet while moving the needle back and forth into the target), use of fanning, suction, or slow pull were left at the discretion of the endoscopist, as evidence on the superiority of these techniques is lacking [18, 20, 31]. Seven study sites had on-site pathological evaluation at their disposal (Irvine, Milan, New Haven, New York, Rotterdam, Santiago de Compostela, and Stockholm), and were allowed to use rapid on-site pathological evaluation (ROSE) according to their local protocols. After completion of the sampling protocol, the endoscopist was permitted to switch to another needle type and/or size, either during the same or a subsequent procedure, as long as specimens were analyzed separately.

Specimen processing

Specimens were collected in three vials to allow for analysis according to needle pass; one for the first pass, one for the second and third pass, and one for any subsequent passes. Samples were preserved according to local practice. Cytological samples from each vial were first smeared onto glass slides and stained with Diff Quick (Adelaide, Irvine, New Haven, Rotterdam, Santiago de Compostela, Stockholm, Tel Aviv), Hematoxylin and eosin staining (Milan, Osaka-Sayama, Rome), or PAP stain (New York). Two centers did not create glass slides (Leuven, Marseille). Remaining material was collected in CytoLyt (Adelaide, Marseille, New York, Rome, Rotterdam, Santiago de Compostela, Stockholm), saline (Osaka-Sayama), alcohol (Tel Aviv), formalin (Irvine, Milan), CytoRich Red (Leuven, New Haven). Cytological cell suspensions were further processed using the ThinPrep technique (Leuven, Marseille, New Haven, New York, Rome, Santiago de Compostela, Stockholm) or the cell block technique, either the Cellient™ automated cell block system (Hologic), the Agar technique, or Histogel (Irvine, Leuven, Marseille, Milan, New Haven, New York, Rotterdam, Santiago de Compostela, Stockholm, Tel Aviv). Adelaide and Osaka-Sayama did not further process cytology. Histology was collected in CytoLyt (Santiago de Compostela, Rotterdam) or formalin (Adelaide, Irvine, Leuven, Marseille, Milan, New Haven, New York, Osaka-Sayama, Rome, Rotterdam, Stockholm, Tel Aviv). Formalin samples were processed as paraffin blocks, sectioned at 3-4 microns and stained with Hematoxylin and eosin staining, PAP, or Giemsa for morphological evaluation.

Outcome measures and definitions

The collected vials were assessed according to the sampling order. The primary outcome measure was the diagnostic accuracy for malignancy and for the classification based on the

Bethesda nomenclature system (non-diagnostic, benign, atypical/suspect for malignancy, or malignant) [32]. Accuracy for malignancy was calculated from the correct number of cases that were defined as atypical/suspect for malignancy or malignant. Accuracy for the Bethesda classification was calculated from the number of cases that were correctly classified into the above-mentioned categories, according to the formula: (true positive + true negative) / all patients. The gold-standard diagnosis was either based on pathological evaluation of the surgical resection specimens or clinical follow-up for at least 9 months when surgical resection was not indicated because of a benign diagnosis or malignant advanced or metastasized disease. Consequently, alternative endpoints included a composite of outcomes including clinical follow-up, additional tissue collections, follow-up imaging investigations, and death. Gold standard diagnosis was recorded by the principal investigator of each of the participating centers. Serous cystadenoma (SCA) and leiomyomata were classified as benign. Lymphomas, solid-pseudopapillary neoplasms (SPN), and neuroendocrine (NET) and gastrointestinal stromal tumors (GISTs) grade 2 and 3 were classified as malignant [33, 34]. A sample was defined as non-diagnostic in case of absence or paucity of target cells.

Secondary outcome measures included the performance of the needles in terms of; 1) technical success rate (ability to obtain a sample), 2) procedural aspects (yield of the first pass, influence of on-site pathological assessment, safety), and 3) specimen specifics; i.e. sample quality (sufficiency for diagnosis or not), cellularity (</≥50% target cells present), and the presence of tissue cores. A tissue core was defined as a measurable microscopic cylinder, containing target organ cells with preserved histological architecture. As there are no uniform definitions to describe EUS-specimen quality and quantity, the definitions used in the current study were jointly created by the participating pathologists in this study.

Last, pathologists were asked to record if a sample diagnosis could be obtained from cytology, histology, or a combination. It was left at the discretion of the pathologist to assess cytology or histology first.

Sample size and statistical analysis

Sample size calculations for a two-side comparison of binominal proportions, with a power of 90% and a type-1 error of 5%, showed that with 600 inclusions an 8% difference in diagnostic accuracy between the two needles could be detected, which was considered by the group to be a clinically relevant difference (SAS 9.3, Proc POWER TwoSampleFreq). Frequencies and percentages were calculated for categorical data, while continuous data were displayed as medians with interquartile ranges (IQR). The chi-square test (with Yates' correction when appropriate) or the Fisher exact test was used to compare the two needle types. Diagnostic accuracy, sensitivity, and specificity were assessed by means of an intention-to-treat (ITT) analysis. In the calculation of sensitivity and specificity, non-diagnostic samples were considered to be benign. Multivariable logistic regression analysis was applied to assess differences in diagnostic accuracy for malignancy between the two sampling devices, adjusted for the sampling indication,

lesion size, number of needle passes, and the presence of an on-site pathologist. Furthermore, an interaction term between sampling device and indication was included in the model, as differences between devices might differ per sampling indication.

As the reported diagnostic accuracy rates of EUS-guided tissue sampling in the literature varies significantly [5, 8-12, 14, 17, 29, 35-37), we performed a supplementary analysis to assess the inter-center variation in diagnostic accuracy. For this, we used a logistic mixed model with the same fixed effect structure as our primary multivariable logistic regression model, but allowed for study center and needle specific effects by including random effects for these variables. An adapted likelihood ratio test was then used to determine if there was indeed significant variation in diagnostic accuracy between the centers, and to assess its effect on needle accuracy [38]. Results from the multivariable analyses were expressed as odds ratios (OR) with 95% confidence intervals. Statistical significance was defined as $p<0.05$ (two-tailed). Analyses were carried out using SAS version 9.3 (SAS Institute, Cary, NC, United States), SPSS version 22, Statistical Package for the Social Sciences, SPSS Inc., Chicago, Illinois, and R (version 3.4.2).

RESULTS

Patient and target lesion characteristics

A total of 612 consecutive patients were randomized, of which four were lost to follow-up; one FNA and three FNB cases. Of the 608 remaining cases, 306 were allocated to the 25-gauge FNA needle and 302 to the 20-gauge FNB needle. Targets comprised 312 pancreatic lesions (51%), 147 lymph nodes (24%), and 149 submucosal or other solid gastrointestinal tract lesions (25%). Baseline patient and target lesion characteristics are listed in table 1. After a median follow-up of 13 months (range 9-26), 463 malignancies were diagnosed (76%, table 2). There was no difference in final diagnoses between the needles (p=0.564). The gold standard diagnosis was obtained from surgical resection specimens in 135 cases (22%).

Diagnostic performance

Technical feasibility and safety

Sampling was technically feasible in all FNA cases and all but four FNB cases (99%, p=0.043, table 3). Five minor adverse events occurred, three in the 25-gauge FNA group and two in the 20-gauge FNB group. In the 25-gauge FNA group, a case of mild pancreatitis and a case of post-procedural pain were managed conservatively. Also, one patient developed fever and positive blood cultures, for which antibiotics were given, after which the patient quickly recovered. In the 20-gauge FNB group, a minor bleeding was clipped during the same procedure and a case of mild pancreatitis was treated conservatively.

Table 1. Patient and target lesion characteristics

Variables	Total n=608	20-gauge FNB n=302	25-gauge FNA n=306
Male, n (%)	344 (57)	162 (54)	182 (60)
Age in years, mean ± SD	66 ± 0.5	66 ± 0.7	66 ± 0.7
Lesion size, median mm (P25-P75**)**	28 (20-40)	29 (20-40)	27 (20-40)
Lesion type and location, n (%)			
Pancreas	**312 (51)**	**154 (51)**	**158 (52)**
Head	165 (27)	88 (29)	77 (25)
Non-head	144 (24)	64 (21)	80 (27)
Lymph node	**147 (24)**	**73 (24)**	**74 (24)**
Abdominal	108 (18)	52 (17)	56 (18)
Mediastinal	39 (6)	21 (7)	18 (6)
Submucosal and other solid lesions	**149 (25)**	**75 (25)**	**74 (24)**
Gastric	57 (9)	28 (9)	30 (10)
Esophagus	22 (4)	11 (4)	11 (4)
Small intestines	17 (3)	7 (2)	10 (3)
Colorectal	7 (1)	3 (1)	4 (1)
Other	48 (8)	28 (9)	20 (7)

SD: standard deviation.

Table 2. Final gold standard diagnosis

Variables	Overall (n=608)	20-gauge FNB (n=302)	25-gauge FNA (n=306)
Malignant lesions, n (%)	**463 (76)**	**233 (77)**	**229 (75)**
Adenocarcinoma	292	153	139
Metastatic carcinoma	74	35	39
GIST	27	10	16
NET	25	11	14
Malignant lymphoma	25	13	12
Squamous cell carcinoma	8	5	3
IPMN	6	2	4
Non-small cell carcinoma	2	1	1
Small cell carcinoma	2	1	1
Leiomyosarcoma	2	2	0
Benign lesions, n (%)	**145 (24)**	**68 (23)**	**77 (25)**
Lymph adenopathy	42	22	20
Leiomyoma	13	5	8
Chronic pancreatitis	11	4	7
GIST	27	10	17
NET	7	4	3
Sarcoidosis	7	5	2
SCA	3	1	2
Schwannoma	2	0	2
Other	33	17	16

GIST: gastrointestinal stromal tumor, NET: neuroendocrine tumor, IPMN: intraductal papillary mucinous neoplasm, SCA: serous cyst adenoma

Tissue acquisition techniques

The slow pull technique was performed more often in the 20-gauge FNB than 25-gauge FNA group (27% versus 13%, p=0.001, table 3). Vice versa, fanning was applied more often with 25-gauge FNA (85%) than 20-gauge FNB (68%, p<0.001). As for the number of needle passes, >3 passes were more frequently undertaken in the FNA group (p=0.002). On-site pathological assessment was performed in a minority of procedures (17%), also more often in the 25-gauge FNA group (24% versus 9%, p<0.001).

Table 3. Sampling specifications

Variables	Total n=608	20-gauge FNB n=302	25-gauge FNA n=306	p-value
Technical success rate, n (%)	604 (99)	298 (99)	306 (100)	0.043
Number of passes				
1-3, n (%)	514 (85)	268 (90)	246 (81)	0.002
>3, n (%)	88 (15)	30 (10)	58 (19)	
Stylet use, n (%)				
In place	345 (57)	209 (71)	136 (44)	<0.001
Withdrawn several cm	121 (20)	39 (13)	82 (27)	
Removed after needle insertion	72 (12)	33 (11)	39 (13)	
Removed before needle insertion	63 (11)	14 (5)	49 (16)	
Use of Fanning, n (%)	462 (77)	204 (68)	258 (85)	<0.001
Additional techniques (per case), n (%)				
Suction with syringe	387 (63)	177 (58)	210 (69)	0.001
Slow Pull	169 (28)	103 (34)	66 (22)	
Combination	23 (4)	10 (4)	13 (4)	
None	29 (5)	12 (4)	17 (5)	
ROSE applied, n (%)	100 (17)	26 (9)	74 (24)	<0.001

ROSE: rapid on-site pathological evaluation

Specimens specifics

Although sample sufficiency and cellularity were equally good for the two needles, procurement of histologically intact tissue cores was accomplished more often with 20-gauge FNB than 25-gauge FNA (77% versus 44%, p<0.001, table 4, figure 2-5). In the same line, with 20-gauge FNB, the diagnosis was more often based on histology (29%) or histology and cytology combined (30%), whereas with 25-gauge FNA, it was mostly based on cytology, processed as cell blocks (47%). Immunohistochemical staining was performed in similar percentages with a trend in favor of 20-gauge FNB (20-gauge FNB 46%, 25-gauge FNA 39%, p=0.090). The actual contribution of immunohistochemical staining in establishing the final diagnosis was not assessed.

Table 4. Pathology outcome of EUS-guided tissue sampling

Variables	Overall n=608	20-gauge FNB n=302	25-gauge FNA n=306	p-value
Tissue quality sufficient, n (%)				
1st pass	415 (68)	209 (69)	206 (68)	0.659
1-3 passes	506 (83)	261 (86)	245 (80)	0.044
Overall	509 (84)	263 (87)	248 (82)	0.062
Sample cellularity >50%, n (%)				
1st pass	243 (59)	131 (63)	112 (54)	0.086
1-3 passes	307 (61)	160 (61)	147 (60)	0.764
Overall	315 (62)	164 (62)	151 (61)	0.733
Tissue cores present, n (%)				
1st pass	284 (47)	183 (61)	101 (33)	<0.001
1-3 passes	361 (61)	229 (77)	132 (44)	<0.001
Overall	368 (61)	232 (77)	136 (45)	<0.001
Immunohistochemistry performed, n (%)				
1st pass	172 (28)	94 (31)	78 (26)	0.316
1-3 passes	244 (40)	135 (45)	109 (36)	0.034
Overall	257 (43)	139 (46)	118 (39)	0.090
PA diagnosis based on, n (%)*				
Cytology (slides/LBC)	74 (15)	14 (5)	60 (24)	<0.001
Cell block	197 (39)	82 (32)	115 (47)	
Histology	82 (16)	74 (29)	8 (3)	
Combination	150 (30)	87 (34)	63 (26)	

LBC: liquid-based cytology. *Missing data is explained by the lack of sufficient pathological anatomical (PA) material for that particular diagnostic purpose.

Diagnostic accuracy, sensitivity and specificity

Overall, 20-gauge FNB had a higher diagnostic accuracy for malignancy (87% versus 78%, p=0.002), with a higher sensitivity (90% versus 82%, p=0.008) and comparable specificity (96% versus 91%, p=0.229). After the first pass, the yield of 20-gauge FNB and 25G FNA was not statistically different but showed a trend in favor of FNB (72% versus 65%, p=0.069, table 5). The accuracy for classification according to Bethesda was better for 20-gauge FNB than 25-gauge FNA (82% versus 72%, p=0.002). Multivariable logistic regression analysis demonstrated this to be independent of indication, lesion size, number of needle passes, and presence of an on-site pathologist (OR 3.53, 95% CI 1.55-8.56, p=0.004, table 6). Besides needle type, diagnostic accuracy was influenced by lesion type and number of needle passes. Lesions of pancreatic or lymphatic origin, and the performance of >3 passes were predictive of a correct final diagnosis (Table 6). Although the diagnostic accuracy varied between centers, for FNA between 56% and 100%, and for FNB between 70% and 100%, this did not affect the difference in diagnostic accuracy between the two study needles (p=0.835) (Figure 6).

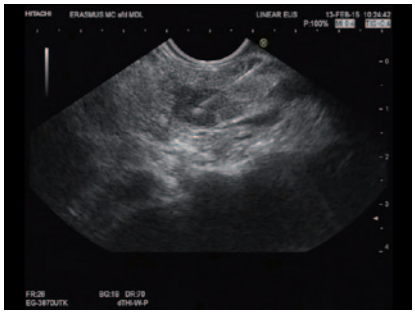

Figure 2. Endoscopic ultrasound image of a hypodense lesion of the pancreatic head, 2 cm in size, irregular borders.

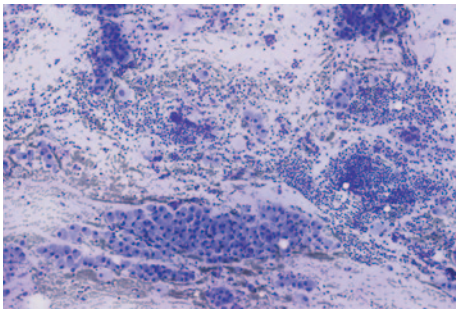

Figure 3. Cytology collected with the 25-gauge FNA needle, showing a monotonous cell population, with enlarged nucleoli, and mucus producing cells (May Grunwald Giemsa stain).

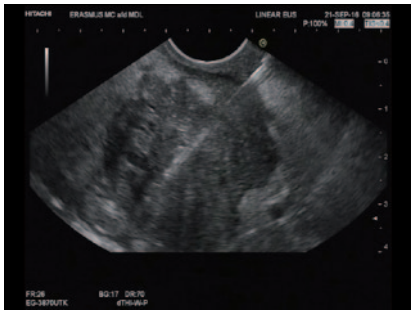

Figure 4. Endoscopic ultrasound image of a hypodense pancreatic head lesion, 4cm in size, irregular borders, and a close relation with SMA.

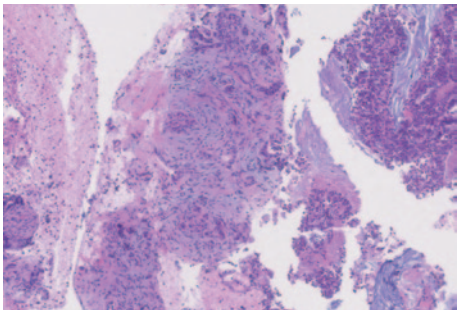

Figure 5. Histology obtained with the 20 FNB needle shows a well-differentiated adenocarcinoma with clear invasive groups of tumor cells, (hematoxylin-eosin stain).

Table 5. Sample diagnosis and performance characteristics for final diagnosis

Outcome parameters for malignancy	20-gauge FNB n=302	25-gauge FNA n=306	p-value
Sample diagnosis, n (%)			
Non-diagnostic	25 (8)	38 (12)	0.078
Benign	48 (16)	51 (17)	
Atypical	55 (18)	70 (23)	
Malignant	174 (58)	147 (48)	
Sensitivity for malignancy, % (95% CI)			
1st pass	75 (70-81)	69 (63-75)	0.119
1-3 passes	89 (85-93)	80 (75-86)	0.007
Overall	90 (86-94)	82 (77-87)	0.008
Specificity for malignancy, (95% CI)			
1st pass	99 (97-100)	93 (87-99)	0.072
1-3 passes	96 (91-100)	91 (85-97)	0.229
Overall	96 (91-100)	91 (85-97)	0.229
Accuracy for malignancy, n (%)			
1st pass	218 (72)	200 (65)	0.069
1-3 passes	261 (86)	232 (76)	0.001
Overall	263 (87)	237 (78)	0.002
Accuracy for Bethesda classification, n (%)			
1st pass	197 (65)	182 (60)	0.143
1-3 passes	245 (81)	215 (70)	0.002
Overall	248 (82)	219 (72)	0.002

CI: confidence interval.

Table 6. Multivariable analysis of factors influencing diagnostic accuracy for malignancy

Variables	Correct diagnosis, % (n/n)	Odds ratio (95% CI)	p-value
Needle type			
20-gauge FNB	87 (263/302)	3.53 (1.55-8.56)	0.004
25-gauge FNA	78 (233/306)	*	
Target lesion			
Pancreas	86 (268/312)	2.89 (1.47-5.70)	0.002
Lymph node	82 (121/147)	2.20 (1.03-4.82)	0.044
Submucosal/other solid lesions	75 (111/149)	*	
Lesion size			
1-3 cm	79 (237/299)	*	0.106
≥3 cm	85 (232/273)	1.47 (0.92-2.37)	
Number needle passes			
1-3	82 (419/514)	*	
>3	89 (78/88)	2.41 (1.11-6.06)	0.039
Application of ROSE			
Yes	83 (83/100)	0.97 (0.51-1.76)	0.917
No	83 (415/502)	*	

ROSE: rapid on-site pathological evaluation, CI: confidence interval.

*Reference category. Interaction terms for needle type and target lesion were included in our model, but not displayed in this table.

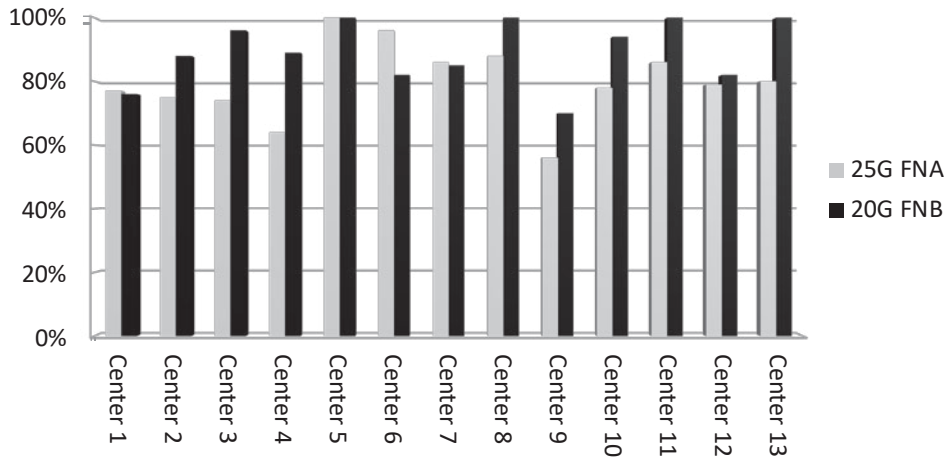

Figure 6. Diagnostic accuracy per center for the 25G FNA and 20G FNB needle.

DISCUSSION

The results of this multicenter randomized controlled trial demonstrate that a novel 20-gauge FNB needle (ProCore design) outperforms a conventional 25-gauge FNA needle, in terms of diagnostic accuracy and histological yield. This equally applies for pancreatic and non-pancreatic lesions and is irrespective of lesion size, number of needle passes, and presence of an on-site pathologist. Moreover, despite inter-center differences, the benefit of the 20-gauge FNB needle was consistent amongst participating centers. Despite the notion amongst endosonographists that larger size needles are not as flexible and might fail to procure tissue in more difficult anatomical scope positions, this conception is contradicted by the results of the current study; the larger-bore 20-gauge FNB needle yielded better results than a small-bore 25-gauge FNA needle, which seems a relevant observation for EUS practice.

FNA needles are designed to procure cytological samples, which lack information on tissue architecture. This may hamper the diagnostic process, for instance in case of neuroendocrine tumors, well-differentiated carcinomas, or lymphomas [2, 39-42]. FNB can overcome this limitation. An intact cellular arrangement facilitates establishing a diagnosis and allows for application of a wide-range of diagnostic tests, including genetic profiling, needed for a personalized medicine approach. Furthermore, studies suggest that FNB facilitates interpretation by less experienced pathologists and obviates the need for ROSE [43, 44]. All this is achieved in less needle passes than FNA, thereby limiting traumatic injury and procedure time [7, 20]. However, if the larger diameter and subsequent stiffness of an FNB needle hinder maneuverability and hence impede procurement of tissue, all these benefits may be annulled.

Several studies have compared the performance of EUS-FNA to FNB, but they did not establish superiority of one needle over the other [7, 20]. These studies were either underpowered,

did not entail the whole range of potential indications, or were performed in a single center or confined geographical region [5, 8, 9, 11-17, 19]. Although in the present study the diagnostic accuracy varied between the 13 centers, this did not affect the difference in diagnostic accuracy between both needles, affirming the general applicability of our results.

Recently, a randomised trial compared procurement of histological tissue core by 22-gauge FNB and FNA in 46 patients with a pancreatic mass. The yield of total and tumor tissue was quantified by specialized software and showed a benefit of FNB over FNA [45]. However, this study was not powered to establish the impact of needle type on diagnostic accuracy. Also, the software used for tissue quantification has not been validated. So far, only one multicenter trial from China included an adequate number of patients to compare the diagnostic accuracy of FNA and FNB [19]. This trial found a diagnostic benefit of FNB over an equally sized FNA needle (accuracy for diagnosis of 93% versus 82%), but this benefit was limited to pancreatic lesions.

The 20-gauge FNB needle used in the present study was designed to combine a large lumen with enhanced flexibility, to facilitate tissue acquisition, even from an angulated scope position. According to the manufacturers design specifications this was achieved by coating the sheet of the needle with a smooth and flexible material (Polytetrafluoroethylene). Also, the cutting edges of the needle were changed from a reversed to a forward-facing bevel, and from a Lancet to a Menghini tip-design, to decrease resistance during tissue traversing (Figure 1). With these design modifications, the technical success rate of the 20-gauge FNB needle reached 99%, despite the fact that a significant number of lesions were sampled from an angulated scope position, including pancreatic head masses. In addition, with the 20-gauge FNB needle, diagnoses were more often based on histology, as compared to FNA, while the cytological yield of the two needles was comparable.

In accordance with existing literature, multivariable analysis demonstrated that overall accuracy of both needles was higher for pancreatic lesions and lymph nodes than for submucosal and other solid lesions [46, 47]. Notably, this did not annul the diagnostic superiority of FNB over FNA. Whereas previous FNB needles particularly improved the diagnostic accuracy for large and submucosal lesions, the currently tested needle showed to be the better choice for all types of solid GI-lesions. Multiple needle passes increased the diagnostic accuracy, which is in accordance with previous reports [20]. However, with the 20-gauge FNB, needle a higher diagnostic accuracy was achieved in less passes. Beyond three passes, hardly any performance improvement was observed.

The present study has some limitations. First, inherent to a lack of evidence-based practice guidelines, there was a diversity in EUS-practices of the participating centers which were not all controlled for the purpose of the study [46, 48]. It is still unknown which method for EUS guided tissue acquisition and processing is superior [18, 20, 31, 49]. For example, the attributive value of ROSE has not been proven and seems to depend on user's experience and the sampling indication [43, 44, 49, 50]. Particular sampling techniques such as 'slow pull or suction' recently was shown to have no impact on the diagnostic outcome [31]. Second, as ROSE was allowed the

pathologist could not be blinded for needle type in these cases. However, as ROSE was performed in a minority of the cases this effect is expected to be limited. Third, for 22% of patients who underwent surgery a pathological gold standard diagnosis from a surgical resection specimen was available while for the remainder of patients the gold standard diagnosis was based on clinical follow-up with a median time of 13 months. This is in line with other studies and inherent to the clinical application of EUS-FNA/FNB. Fourth, this study was performed in high volume expert centers. Ideally, our results should be affirmed and found equally good in lower volume centers and community practices. In the current multicenter set-up including 13 international centers, we found a somewhat lower accuracy rates compared to other, mostly single center, studies [51-55]. Although the diagnostic accuracy varied between centers, this however did not affect the difference in diagnostic accuracy between the two study needles affirming the general applicability of our results for clinical practice. Lastly, we investigated one specific FNB needle, *in casu* the 20-gauge ProCore needle of Cook Medical. It should be noted that there is a continuously growing number of EUS FNB needles, designed to procure histological rather than cytological specimens, including the SharkCore (Medtronic-Covidien) and Acquire biopsy needle (Boston Scientific) [51-55]. Future studies should evaluate and compare FNB needles with distinguished design features.

In conclusion, the 20-gauge FNB needle (ProCore design) consistently out-performed one of the most widely used 25-gauge FNA needles, in terms of histological yield and diagnostic accuracy, in pancreatic as well as non-pancreatic lesions and independent of the number of passes performed. The consistency of its diagnostic benefit amongst the 13 participating centers supports the general applicability of these findings.

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Chapter 4

Agreement on eus-guided tissue specimens: comparing a 20-gauge fnb to a 25-gauge fna needle amongst academic and non-academic pathologists

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ABSTRACT

OBJECTIVE

A recently performed randomized controlled trial demonstrated the benefit of a novel 20G fine needle biopsy (FNB) over a 25G fine needle aspiration (FNA) needle. The current study evaluated the reproducibility of these findings among expert academic and non-academic pathologists.

DESIGN

This study was a side-study of the ASPRO (Aspiration vs PROcore) study. Five centers retrieved 74 (59%) consecutive FNB and 51 (41%) FNA samples from the ASPRO study according to randomization; 64 (51%) pancreatic and 61 (49%) lymph node specimens. Samples were re-reviewed by five expert academic and five non-academic pathologists and rated in terms of sample quality and diagnosis. Ratings were compared between needles, expert academic and non-academic pathologists, target lesions, and cytology versus histological specimens.

RESULTS

Besides a higher diagnostic accuracy, FNB also provided for a better agreement on diagnosing malignancy (κ=0.59 vs κ=0.76, $p<0.001$) and classification according to Bethesda (κ=0.45 vs κ=0.61, $p<0.001$). This equally applied for expert academic and non-academic pathologists and for pancreatic and lymph node specimens. Sample quality was also higher rated for FNB, but agreement ranged from poor (κ=0.04) to fair (κ=0.55). Histology provided better agreement than cytology, but only when a core specimen was obtained with FNB ($p=0.004$ vs $p=0.432$).

CONCLUSION

This study demonstrates that the 20G FNB outperforms the 25G FNA needle in terms of diagnostic agreement, independent of the background and experience of the pathologist. This endorses the use of the 20G FNB needle in both expert and lower volume EUS centers.

KEYWORDS

FNA, FNB, interobserver agreement, pathology

INTRODUCTION

Traditionally, endoscopic ultrasound (EUS)-guided tissue sampling has been carried out using a thin and flexible fine-needle aspiration (FNA) needle, which mainly yields individual cells (cytology) rather than histologically intact tissue fragments. Although diagnostic accuracy rates of FNA are fair, intact tissue fragments are preferred to enable identification of tumor invasion and allow for ancillary immunological and molecular testing, for example in submucosal and neuro-endocrine tumors [1-9]. Furthermore, histology enables genetic profiling and a patient tailored approach, which is becoming increasingly relevant in this era of personalized medicine [10-14]. The growing need for histology resulted in the introduction of the fine needle biopsy (FNB) needles.

So far, most studies reported an equal performance of FNA and FNB needles [6-9, 15], but recently, two large randomized trials showed a significant diagnostic benefit of FNB [16, 17]. One of these studies, the randomized controlled ASPRO (ASpiration *vs* PROcore) trial, was carried out in 13 EUS-clinics, worldwide [17]. This study showed a diagnostic benefit of a novel 20G FNB needle (ProCore, Cook Medical, Bloomington, IN, USA) over a widely used 25G FNA needle (EchoTip Ultra, Cook Medical), irrespective of lesion type, size, and the number of passes performed. However, general applicability of these findings cannot be warranted, as study participation was confined to expert centers only.

Ideally, the superiority of a diagnostic device is reproducible in expert and non-expert hands. Therefore, the present study compares the diagnostic agreement on samples obtained with the novel 20G FNB to the 25G FNA needle amongst expert academic pathologists and non-academic pathologists.

METHODS

Study design

In the course of the ASPRO trial (ClinicalTrials.gov: NCT02167074), 13 EUS centers randomized 608 consecutive patients with a solid pancreatic lesion, lymph node, or submucosal or other solid lesion to sampling with a 20G FNB (ProCore, Cook Medical) or 25G FNA needle (EchoTip Ultra, Cook Medical), between February 2015 and September 2016. Parameters regarding specimen characteristics and diagnostic accuracy were compared. Gold standard diagnosis was based on the prior ASPRO study [17] either on pathological evaluation of the surgical resection specimens or clinical follow up for at least 9 months when surgical resection was not indicated. Gold standard diagnosis was recorded by the principal investigator of each of the participating centers.

For the present side-study, the first 125 pancreatic and lymph node cases that were enrolled in the ASPRO study were included. The samples of these cases were reassessed by five expert

academic and five non-academic pathologists. Diagnosis of malignancy and quality scores were assessed, and agreement on these outcome measures was compared between the two needles and between academic and non-academic pathologists.

As our study was a clinical trial, all authors could access the study data and have reviewed and approved the final manuscript.

Center, pathologist and case selection

Aspiration versus PROcore study centers were invited to contribute to this study if they had collected at least 20 solid pancreatic and lymph node samples by April 2016, and their pathologist was trained to read both cytology and histology. Five ASPRO study centers fulfilled these criteria (Milan, Osaka-Sayama, Rome, Rotterdam, and Santiago de Compostela). Each center was represented by the specialized 'academic' pathologist, who was also involved in the original ASPRO study. This academic pathologist invited a 'non-academic' colleague from a local community practice hospital with a general clinical profile to participate. Expert academic pathologists had reviewed between 3000 and 40 000 EUS samples, including both, FNA and FNB, during their career, whereas the non-academics had a sample review track record between 50 and 1000. Per case, the academic pathologists selected the minimum number of slides required to obtain a tissue diagnosis, including immunohistochemically stained slides, if available.

EUS-guided tissue sampling

Endoscopic ultrasonography procedures were carried out with a convex array echoendoscope (either Pentax EG-3870 UTK or EG-3270UK; Pentax, Tokyo, Japan, or Olympus UTC 140/180/26; Olympus, Tokyo, Japan) as is described in the ASPRO study [17]. Three study sites had on-site pathological evaluation at their disposal (Milan, Rotterdam, and Santiago de Compostela).

Specimen processing

Tissue samples were preserved according to local practice. Cytological tissue samples were smeared on to glass slides and stained with Diff Quick (RAL diagnostics) (Rotterdam and Santiago de Compostela) or Hematoxylin and eosin staining (HE) (Milan, Osaka-Sayama, Rome). Remainder of the cytological specimens were collected in CytoLyt (CytoLyt Solution, Marlborough, MA, USA) (Rome, Rotterdam, Santiago de Compostela), saline (Osaka-Sayama), or formalin (Milan). Cell suspensions were processed into cell blocks, using the Cellient™ automated cell block system (Hologic, Toronto, Canada) (Rotterdam) or Agar technique (Milan, Rome, Santiago de Compostela). Osaka-Sayama did not further process cytology. Histology was collected in CytoLyt (Santiago de Compostela and Rotterdam) or formalin (Milan, Rome, Rotterdam, Osaka-Sayama). Samples collected in formalin were processed as paraffin blocks, sectioned at 3-4 microns, and stained with HE for morphological evaluation.

Review session

Cases were reviewed during a 2-day session at the Erasmus MC University Medical Center Rotterdam, the Netherlands in April 2016. Each expert academic pathologist presented the selected cases providing information on the patient's gender, age and relevant medical history, type of target lesion (lymph node or solid pancreatic lesion) and a summary of the EUS report. Pathologists were blinded for the final clinical and pathological outcome. Slides were viewed simultaneously, using a multi-headed light microscope, but assessed individually. Slides, representative of a case, were presented, including immunohistochemically stained slides, if available. Each pathologist reviewed all cases, including their own.

Outcome measures and definitions

The primary outcome measure was to compare the diagnostic agreement on samples obtained with the two needles. First, samples were assessed for malignancy (yes/no) and classified according to Bethesda (non-diagnostic, benign, atypical/suspect of malignancy, and malignant) [18]. Solid-pseudopapillary neoplasms were classified as malignant. Neuroendocrine and spindle cell tumors were classified malignant only if they harbored high-grade dysplasia or an invasive component. Secondly, we evaluated if diagnostic agreement for the two needles differed between expert academic and non-academic pathologists, between pancreatic and lymphatic lesions, and between specimens containing cytology and histology.

Furthermore, agreement on specimen quality parameters was assessed and compared between the two needles, and between expert academic and non-academic pathologists. The following quality parameters were scored: presence of artifacts, sample sufficiency, presence of target cells and tissue cores and suitability for additional analysis. Artifacts were subdivided in five categories; poor fixation or drying artifacts, thick smears, blood clots, contamination with other cells (mesothelial, liver, gastric or intestinal epithelium), and other. Sample sufficiency was defined as the presence of sufficient target cells to obtain or exclude a certain diagnosis. Target cells were classified as less or more than 50%. Presence of tissue core was defined as the presence of a measurable microscopic cylinder containing target organ cells with preserved histologic structure.

Last, we assessed if and to what extent, pathologist's experience or specimen characteristics influenced diagnostic accuracy.

Statistics

The sample size for this study was derived from Walter et al [19]. Given the availability of 10 observers (5 academic and 5 non-academic pathologists), 50 samples are needed to be analyzed per needle type (50 x 2 = 100 in total), given a one-sided alpha of 0.05, a power of 80%, a minimally acceptable interrater reliability of 0.6 for agreement on the presence of malignancy, and a minimal deviation from the interrater reliability of 0.2 between the two needles, n=10. Inter-observer agreement was calculated by the use of kappa statistics Fleiss'κ-

statistic and 95% confidence intervals (CIs). Kappa- statistics were interpreted according to the convention of Landis and Koch; <0, no agreement; 0-0.20, slight agreement; 0.21-0.40, fair agreement; 0.41-0.60, moderate agreement; 0.61-0.80, substantial agreement; and 0.81-1.0; almost perfect agreement. The chi-squared test was used to compare the diagnostic agreement between the two study needles, academic and non-academic pathologists, target lesion types, and cytological and histological samples. Although all 10 observers assessed the samples for each of the outcome parameters, we only report the average outcome per parameter. Last, univariate logistic regression analysis was applied to assess if a pathologist's expertise and sample quality influenced diagnostic accuracy. Outcomes of this analysis were expressed as odds ratio (OR) with 95% CI. Statistical significance was defined as $p<0.05$ (two-tailed). Analyses were carried out using SAS version 9.4 (SAS Institute, Cary, NC, USA), and SPSS version 22, Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA).

RESULTS

Target lesion and procedure characteristics

A total of 125 samples were reviewed, of which 74 were collected by FNB (59%) and 51 by FNA (41%), with a mean of 2.8 needle passes. Sixty-four were solid pancreatic lesions (51%) and 61 lymph nodes (49%), with a mean size of 30.4 ± 1.3 mm. Table 1 shows the case and sampling specifics. Techniques intended to increase the sample yield were applied in 94% of cases; suction with a syringe in 74 (63%), the slow-pull technique in 50 (37%), and a combination of the two in five (4%). The gold standard diagnosis comprised 26 (21%) non-malignant cases, and 99 (79%) malignant cases (Table 1). The gold-standard diagnosis was based on surgical resection specimens in 31 cases (25%).

Diagnostic accuracy and agreement

In line with the ASPRO study results, FNB samples provided higher accuracy than FNA for malignancy (88% vs 77%, p=0.002) and classification according to Bethesda (76% vs 61%, p=0.002). Regarding the primary question of diagnostic agreement, FNB samples provided better agreement on the presence of malignancy (κ=0.76 vs 0.59, p<0.001) and classification according to Bethesda (k=0.61 vs 0.45, p<0.001, table 2). This was true for both, expert academic and non-academic pathologists (Table 2).

Assessment per target lesion showed that for lymph nodes, FNB provided higher agreement on the presence of malignancy and classification according to Bethesda. However, in pancreatic lesions FNB only outperformed FNA for agreement on the Bethesda classification, not for the presence of malignancy (Table 3). When comparing histology to cytology, agreement on the presence of malignancy was better for histological samples, but agreement on the Bethesda classification was better for histological samples if they had been obtained with the FNB needle (Table 4).

Table 1. Case and sampling specifics.

Variables, n (%)	All (n=125)	FNB (n=74)	FNA (n=51)
Center of origin			
Rotterdam	33 (26)	23 (31)	10 (20)
Rome	30 (24)	20 (26)	10 (20)
Milan	22 (18)	11 (15)	11 (20)
Santiago De Compostela	20 (16)	10 (14)	10 (20)
Osaka-Sayama	20 (16)	10 (14)	10 (20)
Target lesion,			
Solid pancreatic lesion	64 (51)	39 (53)	25 (49)
Lymph node	61 (49)	35 (47)	26 (51)
Size (mm), mean ± SD	30.4 ± 1.3	31.5 ± 1.8	28.8 ± 1.8
Location pancreatic lesions			
Head	40 (62)	28 (72)	12 (48)
Neck	5 (8)	2 (5)	3 (12)
Corpus	12 (19)	7 (18)	5 (20)
Tail	7 (11)	2 (5)	5 (20)
Location lymph nodes			
Mediastinal	21 (34)	14 (40)	7 (27)
Abdominal	40 (66)	21 (60)	19 (73)
Gold Standard diagnosis			
Benign, normal tissue	18 (14)	14 (19)	4 (8)
Sarcoidosis	1 (1)	0 (0)	1 (2)
Pancreatitis	2 (2)	1 (1)	1 (2)
Leiomyoma	1 (1)	0 (0)	1 (2)
GIST, low grade	2 (2)	0 (0)	2 (4)
NET low grade	2 (2)	1 (1)	1 (2)
NET high grade	4 (3)	3 (5)	1 (2)
Leiomyosarcoma	1 (1)	1 (1)	0 (0)
Solid pseudopapillary neoplasm	3 (2)	2 (3)	1 (2)
Metastatic disease	13 (10)	6 (8)	7 (13)
Malignant lymphoma	11 (9)	5 (7)	6 (11)
Adenocarcinoma	67 (53)	41 (55)	26 (50)

FNB; fine needle biopsy, FNA; fine needle aspiration, mm; millimeter, SD; standard deviation, GIST; gastrointestinal stromal lesion, NET; neuroendocrine tumor.

Specimen quality and agreement

Compared to FNA, FNB samples contained fewer artifacts (52% vs 45%, p=0.007, table 2), but agreement was low for both FNB (κ=0.10; 95% CI 0.07-0.14) and FNA samples (κ=0.17; 95% CI 0.13-0.21). Agreement did not differ between expert academic and non-academic pathologists for FNA (p=0.132) or FNB (p=0.212). Sample sufficiency for diagnosis, percentage of target cells, presence of tissue cores, and suitability for additional analysis were all better for FNB than FNA, but again, agreement on these parameters was poor (κ=0.04) to fair (κ=0.55, table 2). As for the collection of histology, FNB obtained histological samples more often than FNA (70% vs 36%, p<0.001, table 2). Agreement on all of the above-mentioned quality parameters was highest for the expert academic pathologists. Furthermore, agreement amongst the expert academic

pathologists was higher for FNB than FNA specimens. In non-academic pathologist however, FNB only provided for better agreement than FNA for the identification of tissue cores (κ=0.26 vs 0.04, p<0.001).

Table 2. Agreement on sample diagnosis and quality amongst the pathologist groups per needle type

Cases scored as	FNB (n=74)	FNA (n=51)	p-value
Malignant– no. (%)	47 (63)	27 (52)	<0.001
Agreement - κ (95% CI)			
All	0.76 (0.73-0.79)	0.59 (0.55-0.63)	<0.001
Expert academic	0.74 (0.66-0.81)	0.54 (0.45-0.62)	<0.001
Non-academic	0.78 (0.71-0.85)	0.64 (0.55-0.72)	<0.001
Bethesda classification – no. (%)			
Non-diagnostic	6 (9)	8 (16)	<0.001
Benign	9 (12)	3 (6)	
Neoplastic	12 (16)	13 (26)	
Malignant	47 (63)	27 (52)	
Agreement - κ (95% CI)			
All	0.61 (0.60-0.64)	0.45 (0.43-0.48)	<0.001
Expert academic	0.62 (0.57-0.67)	0.43 (0.37-0.49)	<0.001
Non-academic	0.59 (0.55-0.64)	0.46 (0.40-0.52)	<0.001
Sufficient quality – no. (%)	67 (91)	40 (79)	<0.001
Agreement - κ (95% CI)			
All	0.49 (0.46-0.53)	0.48 (0.44-0.52)	0.366
Expert academic	0.50 (0.43-0.58)	0.33 (0.28-0.37)	<0.001
Non-academic	0.42 (0.35-0.49)	0.46 (0.37-0.54)	0.358
Target cells ≥50% – no. (%)	50 (68)	29 (56)	<0.001
Agreement - κ (95% CI)			
All	0.31 (0.28-0.34)	0.38 (0.33-0.41)	<0.001
Expert academic	0.33 (0.26-0.40)	0.55 (0.47-0.64)	<0.001
Non-academic	0.27 (0.20-0.34)	0.33 (0.24-0.42)	0.127
Tissue core present – no. (%)	52 (70)	18 (36)	<0.001
Agreement - κ (95% CI)			
All	0.37 (0.34-0.41)	0.14 (0.10-0.18)	<0.001
Expert academic	0.41 (0.34-0.48)	0.08 (0.00-0.16)	<0.001
Non-academic	0.26 (0.19-0.33)	0.04 (-0.04-0.13)	<0.001
Additional analysis possible – no. (%)	56 (76)	28 (54)	<0.001
Agreement - κ (95% CI)			
All	0.47 (0.43-0.50)	0.42 (0.38-0.46)	0.016
Expert academic	0.51 (0.44-0.58)	0.43 (0.34-0.51)	0.042
Non-academic	0.38 (0.30-0.45)	0.38 (0.29-0.47)	0.593

FNB; fine needle biopsy, FNA; fine needle aspiration, no.; number, κ; kappa statistic, CI; confidence interval.

Table 3. Diagnostic agreement of FNA and FNB per target lesion.

Scored variables Agreement κ (95% CI)	FNB (n=74)	FNA (n=51)	p-value
Bethesda classification			
Pancreas	0.54 (0.51-0.58)	0.47 (0.43-0.52)	<0.001
Lymph node	0.64 (0.61-0.67)	0.43 (0.39-0.47)	<0.001
Presence of Malignancy			
Pancreas	0.64 (0.59-0.69)	0.60 (0.54-0.66)	0.114
Lymph node	0.84 (0.79-0.89)	0.58 (0.52-0.63)	<0.001

FNB; fine needle biopsy, FNA; fine needle aspiration, CI; confidence interval.

Table 4: Diagnostic agreement on cytological and histological specimens per needle type.

Agreement κ (95% CI)	Cytology	Histology	p-value
Bethesda classification			
All samples (n=121)	0.51 (0.49-0.52)	0.60 (0.59-0.61)	<0.001
FNA (n=47)	0.49 (0.46-0.50)	0.52 (0.49-0.55)	0.432
FNB (n=74)	0.52 (0.49-0.54)	0.62 (0.61-0.63)	<0.001
Presence of Malignancy			
All samples (n=121)	0.76 (0.74 – 0.78)	0.97 (0.95-0.99)	<0.001
FNA (n=47)	0.73 (0.71-0.76)	0.89 (0.86-0.92)	0.002
FNB (n=74)	0.78 (0.75-0.81)	0.99 (0.79-1.00)	<0.001

FNB; fine needle biopsy, FNA; fine needle aspiration, CI; confidence interval.

Factors affecting diagnostic accuracy

Besides the type of needle, other factors affecting EUS-sample diagnosis are shown in table 5. A pathologist's background (expert academic or non-academic) did not influence the diagnostic accuracy of either needle (p=0.250). The presence of artifacts did have an effect, as this resulted in a lower diagnostic accuracy (p=0.030). Last, the presence of tissue cores significantly improved diagnostic accuracy (p=0.003).

Table 5. Factors affecting diagnostic accuracy, univariable analysis**.**

Diagnostic accuracy Bethesda classification	Univariate OR (95%CI)	p-value	Diagnostic accuracy for malignancy	Univariate OR (95%CI)	p-value
Pathologist experience			**Pathologist experience**		
Expert academic	0.96 (0.82-1.12)	0.587	Academic	0.88 (0.70-1.10)	0.250
Non-academic			Non-academic		
Presence of artifacts			**Presence of artifacts**		
No	1.45 (1.22-1.74)	<0.001	No	1.34 (1.03-1.75)	0.030
Yes			Yes		
Type of tissue			**Type of tissue**		
Histology	0.55 (0.32-0.94)	0.030	Histology	0.39 (0.21-0.72)	0.003
Cytology			Cytology		

OR; Odds ratio, CI; confidence interval.

DISCUSSION

In addition to the previously reported diagnostic benefit of a novel 20G FNB over a commonly used 25G FNA needle, the present study shows that diagnostic agreement is also higher for the FNB than FNA samples. More importantly, agreement on FNB samples was higher amongst pathologists from different backgrounds (academic vs community practice) and with different levels of experience (high vs lower volume). The benefit of FNB equally applies to pancreatic and lymphatic target lesions. The finding that FNB samples were of better quality and harbored histology more often, likely contributed to their superior diagnostic performance.

Most studies on EUS-needle devices have been carried out in expert high-volume centers. However, EUS-guided tissue sampling is increasingly applied in lower-volume centers. So far, few studies have evaluated the reproducibility of EUS-FNA/FNB results. Moreover, most of these studies had a limited number of observers, concerned one type of target lesion, or were carried out in an academic practice only [20-24]. Previous studies reported diagnostic agreement rates ranging from moderate to excellent for FNA (κ=0.45-0.89) and FNB (κ=0.61-0.94). Recently, a promising study aimed to validate a novel scoring system to further optimize diagnostic agreement amongst cytopathologists [24]. Unfortunately, despite the fact that observers were selected from tertiary centers, diagnostic agreement for pancreatic FNA specimens was still suboptimal (κ=0.56). Compared to these agreement rates, the 20G FNB needle performed well, especially when taken into account pathologists from all over the world were included, academics and non-academics alike. The 20G FNB needle may thus contribute to improve reproducibility of EUS-FNA/B diagnosis.

The first explanation for a better agreement on FNB samples is its high tissue core rate, as the collection of histology rather than cytology was positively associated with a higher agreement. This is supported by the finding that the cytological yield of FNB was also higher than for FNA, but only availability of tissue cores for histology, and not cytology, contributed to a better diagnostic accuracy. The importance of tissue core samples over cytological ones to reach a correct diagnosis when using an FNB needle has been previously described by others [20]. Compared to other FNB needles, the cytological yield of the current 20G FNB needle was high as well [9, 20, 22, 25-31]. Whereas previous studies reported sufficient cellularity in 19% to 52%, in the current study this was 68%. The only device that provides higher histology and cytology rates is the 19G needle [22, 25], which obtains cores in 88% of samples and an adequate amount of loose target cells in 91%. However, the reported clinical applicability of being able to obtain tissue with the 19G FNB needle (81%) is much lower than the 20G FNB needle (99%). Although the increased flexibility of the 20G FNB needle is likely a major contributor to its better performance, other needle design adjustments may have improved the tissue acquisition rate too[32, 33].

Another quality parameter that may have contributed to the high diagnostic agreement on samples obtained with FNB is a low artifact rate. Although artifacts not necessarily decrease ac-

curacy when abundant tissue is collected, previous studies have shown that they may hamper for example advanced genetic testing [34]. Interestingly, agreement on the presence of artifacts was low for both needles (although slightly better for FNB than FNA). This is in line with the fact that agreement on all sample quality parameters was rather low, similar to reports from others [21, 24]. This may result from a lack of EUS-sample quality definitions. In the current study, we tried to minimize this limitation by using the predefined scoring system, as proposed by the Papanicolaou Society of Cytopathology in 2014 [18].

There are several limitations to our study. First, each academic pathologist brought and presented his or her own slides. Although they too were blinded for the final outcome, we cannot exclude recall bias. However, this only applied to a few cases per pathologist. Secondly, pathologists assessed samples individually, while in daily practice, difficult cases are often discussed amongst colleagues. Therefore, interobserver agreements reported in the current study may underestimate real-life reproducibility. Thirdly, our study involved pathologists from 10 centers from around the world, while previous studies were confined to no more than five centers from the same geographical region. In the absence of uniform guidelines for EUS-guided tissue sampling and processing, it is inevitable that there are geographical and institutional differences in the work-up of specimens. These differences may have resulted in slight differences in the appearance of specimens, which may have hampered interpretation by pathologists not familiar with certain preparation techniques. Lastly, it must be considered that all samples were collected by expert endosonographers. For an ideal assessment of the reproducibility of the outcome of the ASPRO study, the study should be repeated in low volume centers, with less experienced endosonographers.

In conclusion, this study demonstrates that the novel 20G FNB needle outperforms the 25G FNA needle in terms of diagnostic agreement, as its diagnostic superiority is not limited by the expertise and experience of the reviewing pathologist. Better sample quality and presence of histology seem to be the responsible determinants for the better diagnostic performance of the 20G FNB needle. Together with the favorable accuracy rates from the previous ASPRO study, current findings advocate the use of the novel 20G FNB needle in high as well as lower volume EUS centers.

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Chapter 5

Combined versus single use of the 20-gauge FNB and the 25-gauge FNA needle for EUS-guided tissue sampling of solid gastrointestinal lesions

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ABSTRACT

Background

Instead of choosing one endoscopic ultrasound (EUS) needle over the other, some advocate the use of fine-needle aspiration (FNA) and fine-needle biopsy (FNB) consecutively. We explored the yield of combined use of 20-gauge (G) FNB and 25G FNA needles in patients with a suspicious solid gastrointestinal lesion.

Methods

Patients from the ASPRO (Aspiration vs PROcore) study who were sampled with both needles during the same procedure were included. The incremental yield of dual sampling compared with the yield of single needle use on the diagnostic accuracy for malignancy was assessed for both dual sampling approaches – FNA followed by FNB, and vice versa.

Results

73 patients were included. There were 39 (53%) pancreatic lesions, 18 (25%) submucosal masses, and 16 (22%) lymph nodes. FNA was used first in 24 patients (33%) and FNB was used first in 49 (67%). Generally, FNB was performed after FNA to collect tissue for ancillary testing (75%), whereas FNA was used after FNB to allow for on-site pathological assessment (76%). Diagnostic accuracy for malignancy of single needle use increased from 78% to 92% with dual sampling (p = 0.002). FNA followed by FNB improved the diagnostic accuracy for malignancy (p = 0.03), whereas FNB followed by FNA did not (p = 0.13).

Conclusion

Dual sampling only improved diagnostic accuracy when 25G FNA was followed by 20G FNB and not vice versa. As the diagnostic benefit of the 20G FNB over the 25G FNA needle has recently been proven, sampling with the FNB needle seems a logical first choice.

INTRODUCTION

Over the past decades, endoscopic ultrasound (EUS)-guided tissue sampling has become an important tool in the diagnosis and staging of lesions around the gastrointestinal tract. For this purpose, both fine-needle biopsy (FNB) and aspiration (FNA) devices are used. Whereas FNA needles are generally more flexible and easier to use from an angulated scope position, FNB needles are designed to collect histologically intact tissue samples, which enable ancillary testing. As histology is indispensable in this era of personalized medicine, collection of samples for histological analysis has become more important. Recently, two randomized trials demonstrated that FNB needles produce a better diagnostic yield than FNA needles in this context [1,2].

Instead of choosing one needle over the other, some advocate the selective use of both needle types consecutively (dual needle sampling) [3–7]. As the number of passes only seems to improve diagnostic accuracy up to a certain number of passes, the benefit of dual sampling probably lies within the combination of two different needle designs [8]. For example, FNA can be applied to obtain an on-site diagnosis through rapid on-site tissue evaluation (ROSE) and may then be followed by FNB to harvest tissue cores. This sequence prevents costly ancillary testing of unrepresentative tissue. Others start with FNB and switch to FNA in case they are faced with a small or fibrotic lesion that is anatomically difficult to reach. Although endosonographers are confronted with this needle choice dilemma on a daily basis, few studies have addressed the yield of dual needle sampling, and those that have report inconclusive results [8–14].

Owing to scant data, there is no clarity on the indication for dual sampling, the optimal needle order, safety, or costs. The current study aimed to explore the incremental yield of combined needle use in patients requiring EUS-guided tissue sampling of a solid gastrointestinal lesion. This was assessed for two sampling orders: sampling with a conventional 25G FNA needle followed by a 20G FNB needle, and vice versa.

METHODS

Study design and case selection

Patients from the ASpiration vs PROcore needle (ASPRO) study, which was approved by the institutional review boards of the participating centers, were included in the current study. This multicenter trial compared the diagnostic value of a 20G FNB needle (ProCore; Cook Medical, Limerick, Ireland) with a 25G FNA needle (EchoTip Ultra; Cook Medical) in patients undergoing EUS-guided tissue sampling of a solid pancreatic lesion, lymph node, or subepithelial or other solid lesion (ClinicalTrials.gov: NCT02167074). Patients were allocated to one needle, but the protocol allowed additional sampling with the other needle, as long as specimens were ana-

lyzed separately. All patients who were sampled with both needles during the same procedure were included in the present side study (Figure 1).

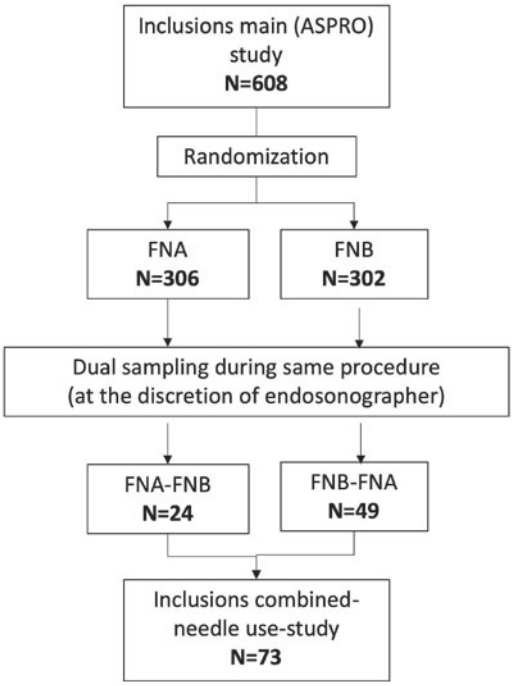

Figure 1. Flow chart of patient selection from the main ASPRO study.

EUS-procedure and tissue acquisition

The participating endosonographers were experts who performed at least 250 EUS-guided sampling procedures per year. They used Pentax (EG-3870UTK; Pentax, Tokyo, Japan) or Olympus echoendoscopes (UTC 140/160/180/190/260 or UC140; Olympus, Tokyo, Japan) with color Doppler. In cases of multiple lesions, the most suspicious lesion was targeted. Lesions were punctured at least three times with the allocated needle, followed by one or more punctures with the alternative needle during the same procedure. For each needle, the number of passes was recorded. ROSE was available at four of the study sites (Irvine, Milan, New Haven, Rotterdam).

Specimen processing protocol

Cytological samples were smeared onto glass slides and stained with Diff-Quik (Adelaide, Irvine, New Haven, Rotterdam), or hematoxylin and eosin staining (HE) (Milan, Osaka-Sayama). The remaining material was collected in CytoLyt (Cytyc Corp., Marlborough, Massachusetts, USA) (Adelaide, Rotterdam), saline (Osaka-Sayama), formalin (Irvine, Milan), or CytoRich Red

(Thermo Fischer Scientific, Kalamazoo, Michigan, USA) (New Haven). Cytological cell suspensions were further processed using the ThinPrep technique (Hologic Inc., Marlborough, Massachusetts, USA) (New Haven) or cell block technique using the Cellient automated cell block system (Hologic Inc.), the agar technique or Histogel (Richard-Allan Scientific Co., Kalamazoo, Michigan, USA) (Irvine, Milan, New Haven, Rotterdam). Histological samples were collected in formalin, processed as paraffin blocks, and stained with HE or Giemsa. In Rotterdam, histological specimens were also collected in CytoLyt and processed according to the cell block technique.

Outcome parameters and definitions

The primary outcome was the incremental yield of dual sampling, compared with single needle use, on the diagnostic accuracy for malignancy for both sampling orders – FNA followed by FNB, and vice versa. A diagnosis of malignancy was confirmed (gold standard) by surgical resection specimens, when available, or, in nonsurgical patients, by a compatible clinical disease course during a 9-month follow-up period. To further specify the type of cases that were selected for the current study, we compared case characteristics of the current cohort with those of patients in the main ASPRO study.

Statistics

Categorical data were recorded as frequencies and percentages. Continuous data were displayed as means with standard error as medians with interquartile range. The student's *t* test was used to compare normally distributed continuous variables, and the Fisher's exact test or chi-squared test was used to compare categorical variables. An exact McNemar test was used to compare the diagnostic accuracy of single vs. combined needle use within the same patient. A chi-squared test was used to compare the accuracy between the two sampling orders, and between cases from the current study and the ASPRO study cohort (patients from the current study were excluded from the ASPRO cohort). Statistical significance was established as $P < 0.05$ (two tailed). Analyses were carried out using SPSS version 22 (IBM Corp., Armonk, New York, USA).

RESULTS

Sampling order and reason for dual sampling

Six of the 13 ASPRO centers used dual sampling (Adelaide, Irvine, Milan, New Haven, Osaka-Sayama, Rotterdam). In total, 73 of the 608 ASPRO patients (12%) were punctured by both needles; FNA was used first in 24 patients (33%) and FNB was used first in 49 patients (67%). According to the endosonographers, the rationale for performing additional sampling differed, depending on the allocated needle ($P < 0.001$, table 1); generally, after FNA, FNB was performed to collect tissue for ancillary testing (75%), whereas after FNB, FNA was used to allow for ROSE (76%).

Table 1. Baseline characteristics.

Variables	All cases (n=73)	FNA – FNB regime (n=24)	FNB-FNA regime (n=49)	p-value
Center of origin, n (%)				
Rotterdam, the Netherlands	2 (3)	0 (0)	2 (4)	0.002
Osaka-Sayama, Japan	6 (8)	5 (21)	1 (2)	
Adelaide, Australia	8 (11)	5 (21)	3 (6)	
Irvine, USA	5 (7)	1 (4)	4 (8)	
New Haven, USA	32 (44)	12 (50)	20 (41)	
Milan, Italy	20 (27)	1 (4)	19 (39)	
Reason sampling alternative needle, n (%)				
To allow for ROSE	38 (52)	1 (4)	37 (76)	<0.001
Obtain tissue for ancillary studies	22 (30)	18 (75)	4 (8)	
Insufficient sample	7 (10)	4 (17)	3 (6)	
Sampling failure	2 (3)	0 (0)	2 (4)	
Other	4 (5)	1 (4)	3 (6)	
Target lesion, n (%)				
Pancreatic	39 (53)	12 (50)	27 (55)	0.563
Lymph node	16 (22)	7 (29)	9 (18)	
Submucosal	18 (25)	5 (21)	13 (27)	
Location, n (%)				
Pancreas				
Head	25 (64)	6 (55)	19 (68)	0.500
Non-head	14 (36)	5 (45)	9 (32)	
Lymph node				
Mediastinal	1 (6)	0 (0)	1 (11)	0.563
Abdominal	15 (94)	7 (100)	8 (89)	
Submucosal				
Esophageal	2 (11)	1 (20)	1 (8)	0.847
Gastric	8 (44)	2 (40)	6 (47)	
Small intestine	3 (17)	1 (20)	2 (15)	
Rectum	3 (17)	1 (20)	2 (15)	
Missing	2 (11)	0 (0)	2 (15)	
Lesion size (mm), median (IQR)	35.0 (20-41)	26 (21-40)	35 (25-43)	0.786
Total number of needle passes, mean ± SE				
Overall	4.93 ± 0.17	5.38 ± 0.26	4.71 ± 0.20	0.059
With randomized needle	2.87 ± 0.08	3.13 ± 0.14	2.87 ± 0.10	0.028
With alternative needle	2.23 ± 0.12	2.46 ± 0.21	2.12 ± 0.14	0.183
Pathologist present, n (%)				
With randomized needle	30 (41)	13 (54)	17 (35)	0.146
With alternative needle	57 (78)	14 (58)	43 (88)	0.004

FNA: fine needle aspiration; FNB: fine needle biopsy; USA: United States of America; ROSE: rapid on-site pathological evaluation; n: number of cases; mm: millimeter; IQR: interquartile range; SE: standard error.

Case characteristics

Target lesions included 39 (53%) solid pancreatic lesions, 18 (25%) submucosal masses, and 16 (22%) lymph nodes. Figure 2 shows endoscopic images of EUS-guided tissue sampling of two cases with solid pancreatic lesions. Most pancreatic lesions were located in the head (64%),

most lymph nodes were located in the abdomen (94%), and submucosal lesions were mostly of gastric origin (44%). There were no significant differences in patient or target lesion characteristics between cases sampled with FNA or FNB first (Table 1 and 2).

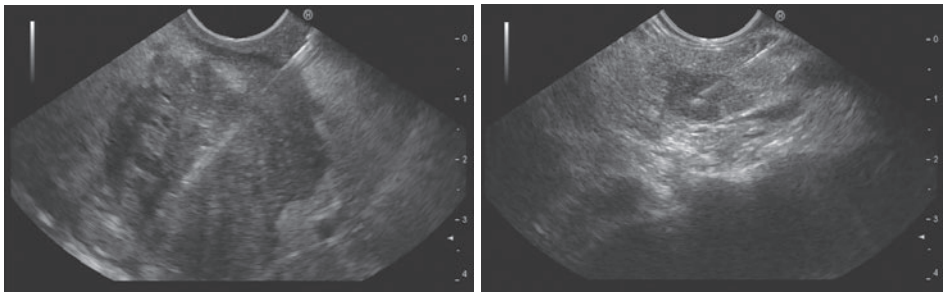

Figure 2. Endoscopic ultrasound images of hypodense lesions of the pancreatic head.
a Lesion, 4 cm in size, irregular borders, and in close proximity to superior mesenteric artery, sampled with the 20G fine-needle biopsy needle.
b Lesion, 2 cm in size, irregular borders, sampled with the 25G fine-needle aspiration needle.

Table 2. final diagnosis

Variables	Overall (n=73)	FNA-FNB (n=24)	FNB-FNA (n=49)	p-value
Malignant lesions, n (%)	59 (81)	18 (75)	43 (88)	0.16
Adenocarcinoma	39	10	29	
GIST	7	2	6	
NET	3	2	2	
Malignant lymphoma	4	1	3	
Squamous cell carcinoma	1	1	0	
Malignant cyst	2	1	1	
Other malignant lesions	3	1	2	
Benign lesions, n (%)	14 (19)	6 (25)	8 (16)	
Lymph adenopathy	4	2	2	
Leiomyoma	1	0	1	
GIST	1	0	1	
NET	2	0	2	
Duplication cyst esophagus	1	1	0	
Benign schwannoma	1	1	0	
Other benign lesions	4	2	2	

FNA: fine needle aspiration; FNB: fine needle biopsy; n: number of cases; GIST: gastrointestinal stromal tumor; NET: neuroendocrine tumor.

Compared with the main ASPRO cohort, patients who were selected for combined needle use had larger target lesions (37.1 ± 2.54 vs. 32.0 ± 0.83 mm; p = 0.04, table 3), were more often sampled from a duodenal scope position (p = 0.04), and more often had a pathologist in the room during the procedure (p < 0.001). As expected, more needle passes were performed per case (p < 0.001). Interestingly, the diagnostic accuracy for single use of either randomized needle (FNA or FNB) was comparable for the cases from the main ASPRO study and the current cohort (Table 3).

Table 3. Patient and target lesion characteristics of combined needle cases compared to other ASPRO study cases.

Variables	Combined cases (n=73)	ASPRO cohort (n=535)	p-value
Male, n (%)	37 (51)	307 (57)	0.279
Age (years), mean ± SE	65.2 ± 1.69	65.8 ± 0.51	0.731
Indication, n (%)			
Pancreatic	39 (53)	273 (51)	0.883
Lymph node	16 (22)	131 (25)	
Submucosal	18 (25)	131 (25)	
Lesion size (mm), mean ± SE	37.1 ± 2.54	32.0 ± 0.83	0.042
Sampling location randomized needle, n (%)			
Duodenum	41 (56)	232 (43)	0.039
Other	32 (44)	303 (57)	
Total number of needle passes randomized needle			
1-3 passes, n (%)	61 (86)	453 (85)	0.892
>3 passes, n (%)	10 (14)	78 (15)	
Total number of needle passes overall			
1-3 passes, n (%)	8 (11)	453 (85)	<0.001
>3 passes, n (%)	65 (89)	78 (15)	
Pathologist present, n (%)			
Randomized needle	30 (43)	70 (13)	<0.001
Overall	57 (78)	70 (13)	<0.001
Final diagnosis			
Benign	14 (19)	131 (24)	0.318
Malignant	59 (81)	404 (76)	
Diagnostic accuracy, n (%)			
Randomized needle overall	57 (78)	430 (81)	0.514
Randomized needle FNA	18 (75)	211 (76)	0.897
Randomized needle FNB	39 (80)	219 (87)	0.181

ASPRO: aspiration versus procore needle; FNA: fine needle aspiration; FNB: fine needle biopsy; n: number of cases; SE: standard error; GIST: gastrointestinal stromal tumor; NET: neuroendocrine tumor

Incremental yield of dual sampling on diagnostic accuracy

Gold standard diagnosis demonstrated 59 malignancies (81%), of which 18 (25%) were diagnosed based on resection specimens (Table 2). Overall diagnostic accuracy for malignancy of single needle use was 78% (57/73), which increased to 92% (67/73) when both needles were

used (p = 0.002, table 4). The incremental yield of dual sampling over single needle use differed depending on which needle was used first: FNA followed by FNB resulted in a significant increase in accuracy (p = 0.03), whereas FNB followed by FNA did not (p = 0.13, table 4).

Table 4. No. of correctly diagnosed cases for single versus combined needle use per sampling regime.

Sampling regime	Single use of randomized needle, n (%)	Combined needle use, n (%)	p-value
Overall, n=73	57 (78)	67 (90)	0.002
FNA-FNB, n=24	18 (75)	24 (100)	0.031
FNB-FNA, n=49	39 (80)	43 (88)	0.125

FNA: fine needle aspiration; FNB: fine needle biopsy; n: number of cases, No: number.

Of all 16 cases (22%) that were incorrectly diagnosed after single needle sampling, 10 (59%) benefited from the alternative needle (Table 5). Cases that benefitted comprised 6 of the 24 cases in which FNB was used after FNA (25%), and 4 of the 49 in which FNA was applied after FNB (8%). FNA cases that benefitted from subsequent FNB sampling comprised a pancreatic adenocarcinoma, a metastatic lymph node, a benign schwannoma of the rectum, and three cases of benign lymphadenopathy. The four FNB cases that benefitted from subsequent FNA sampling included three pancreatic adenocarcinomas and a lymph node metastasis.

Table 5. Specification of all cases that were incorrectly diagnosed after sampling with initial needle/could potentially benefit from combined needle use.

FNB-FNA case no.	Indication	Lesion size (mm)	Puncture location	Use of ROSE	Diagnosis 1st attempt	Diagnosis 2nd attempt	Final Diagnosis
1	Mesenteric mass near stomach and pancreas	35	Gastric corpus	Yes	Atypical	Non-diagnostic	Malignant fibromatosis
2	Lymph node	20	D2, then D1	Yes	Non-diagnostic	Non-diagnostic	Benign lymph adenopathy, resection performed
3	Pancreas	11	Antrum	No	Non-diagnostic	Non-diagnostic	NET, Octreoscan confirmed
4	Pancreas	35	Corpus	Yes	Bile duct tissue only	HG IPMN	HG IPMN
5	Submucosal	140	D2	Yes	Benign	Non-diagnostic	Leiomyosarcoma
6	Lymph node	27	Rectum	Yes	Non-diagnostic	Malignant	Lymph node metastasis
7	Pancreatic head	45	D2	Yes	Non-diagnostic	Malignant	Adenocarcinoma

Table 5. Specification of all cases that were incorrectly diagnosed after sampling with initial needle/could potentially benefit from combined needle use. (*continued*)

FNB-FNA case no.	Indication	Lesion size (mm)	Puncture location	Use of ROSE	Diagnosis 1st attempt	Diagnosis 2nd attempt	Final Diagnosis
8	Pancreatic corpus	10	D1	No	Non-diagnostic	Non-diagnostic	NET on CT
9	Pancreatic head	20	D1	Yes	Benign	Non-diagnostic	Adenocarcinoma
10	Pancreatic head	25	D1	Yes	Benign	Malignant	Adenocarcinoma
11	Lymph node	26	Gastric corpus	No	Insufficient	Adenocarcinoma	Metastasis
12	Pancreatic head	25	D2	No	Few atypical cells	Adenocarcinoma	Adenocarcinoma
13	Lymph node	25	D1	Yes	Suspicious for malignancy	Benign lymph adenopathy	Benign lymph adenopathy
14	Submucosal	29	Rectum	No	Insufficient	Benign	Schwannoma
15	Lymph node	15	Gastric corpus	No	Insufficient	Benign lymph node swelling	Benign lymph node swelling
16	Lymph node	20	D1	Yes	Insufficient	Benign lymph node swelling	Benign lymph node swelling

FNA: fine needle aspiration; FNB: fine needle biopsy; n: number of cases; mm: millimeter; ROSE: rapid on-site pathological evaluation; NET: neuroendocrine tumor; HG: high grade; IPMN: intraductal papillary mucinous neoplasm; D1: superior duodenal part; D2: descending duodenal part; CT: computed tomography

DISCUSSION

Two recent large randomized controlled trials showed that FNB outperforms FNA in terms of histological yield and diagnostic accuracy [1,2]. The current study demonstrated that combined needle sampling only improves diagnostic accuracy when FNA is followed by FNB, but not vice versa. As stated above, the theory behind the benefit of a needle switch, or so called "dual sampling," is that FNA and FNB are complementary. FNA needles collect cytological samples rather than material for histological analysis; their strength lies in their flexibility, which enables them to reach and traverse difficult target lesions, and the fact that they allow for on-site pathological evaluation. FNB devices, however, collect intact histological tissue cores, allowing for a wide range of diagnostic tests. Regarding the use of ROSE, the so-called "sample crush technique" may also allow for on-site specimen assessment of FNB samples [15,16]. However, the optimal method of performing ROSE on FNB samples has yet to be determined.

The explanation as to why FNB following FNA has incremental value and FNA following FNB does not is multifactorial. First, the 20G FNB needle was proven to be diagnostically superior to the 25G FNA needle in our previous study. Thus, this also explains why FNA followed by FNB results in a higher diagnostic accuracy than FNA alone and the limited value of the reversed

approach. Second, puncture with the more traumatic FNB needle poses a higher risk of blood contamination of subsequent specimens. In addition, the larger FNB needle may cause "tracking," impeding the FNA needle from finding its own diagnostic route. Third, secondary FNA was mostly used to allow for ROSE. However, the incremental yield of ROSE is questionable [2,17]. Its impact on diagnostic accuracy has only been demonstrated for endosonographers in training or in centers with low accuracy rates [11]. The current study included only high-volume expert centers, in which the benefit of ROSE is expected to be limited.

The finding that one sampling order benefitted from dual sampling, whereas the other did not, cannot be explained by differences in case or procedure characteristics, as these did not differ between groups. However, we did observe that non-pancreatic lesions mainly benefitted from FNB following FNA. This may be explained by the fact that diagnosing and staging of lymphomas, smooth muscle tumors, and metastases require abundant histological tissue for ancillary testing [8,18–22]. In contrast, three out of four cases that benefitted from additional FNA concerned pancreatic lesions. As these lesions tend to be hard and fibrotic, they may be more easily sampled using a smaller FNA needle.

When comparing the current subgroup with the main ASPRO study cohort, endosonographers selected cases for dual sampling that were bigger in size and more often punctured from the duodenum. Although it was not reported, a larger lesion may have created a desire to harvest more tissue, in order to secure a diagnosis. The high frequency of duodenal punctures seems to indicate that target lesions that were difficult to reach were selected for dual sampling. Interestingly, the diagnostic accuracy of combined needle use did not differ from our previously reported single use [2].

So far, only seven studies have reported on the incremental yield of dual sampling [8–14]. Four studies found a significant increase in diagnostic accuracy when both FNA and FNB were used during the same procedure. However, two studies used the TruCut needle and two used a reversed bevel ProCore device, thus hampering comparison with our results. Furthermore, no study assessed the needle order, and only reported the incremental yield of additional FNB or the combination of the two devices. Our study is the only available study that has looked at the "needle order," and is hypothesis generating, especially with respect to studying the cost-effectiveness of different sampling strategies. Of the previous dual sampling studies, so far only one reported on cost-effectiveness; FNB alone was cost saving compared with FNA alone or FNA followed by FNB [12]. As only one study has reported on this, it would be interesting for future studies to assess the costs of the different sampling strategies, such as FNA with or without ROSE, FNA followed by FNB or FNB alone.

Obviously, this study has limitations. As mentioned earlier, cases were selected based on the endosonographers' choice and not on predefined criteria. This may have introduced selection bias. A second limitation is that dual sampling was not compared with continuous sampling with the allocated needle. As harvesting more tissue alone may be responsible for a diagnostic benefit, this is of interest. Third, the diagnosis of the first needle may have encouraged the

pathologist to find the same diagnosis in the second sample. However, as needle sequence was randomly determined, the effect of this bias is negligible. Finally, we did not assess the impact of dual sampling on procedure time and costs.

In conclusion, for EUS-guided tissue sampling, the 20G FNB needle seems a logical first choice, as it was proven to be superior to the 25G FNA needle, and an incremental value of FNA following FNB was not demonstrated. FNA after FNB may be considered in fibrotic pancreatic lesions, or lesions that are too difficult to reach from an angulated score position. Some may still prefer to start with FNA to assess the accessibility and cellularity of a lesion and to prevent costly ancillary testing of unrepresentative tissue. There seems to be a role for FNB after a non-diagnostic attempt with FNA, especially for lesions of non-pancreatic origin.

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Chapter 6

The optimal EUS sampling-strategy: a meta-analysis of FNA and new generation FNB devices

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Submitted

ABSTRACT

BACKGROUND

EUS guided tissue acquisition is extensively used, but the optimal sampling device is still a matter of debate. Since the last meta-analysis on this subject, a substantial number of studies have been published. Thus, an update was required.

METHODS

EMBASE, MEDLINE/PubMed, Web of Science, the Cochrane Library, and Google Scholar were systematically searched. We included randomized controlled trials that involved at least 50 cases with a suspected solid gastrointestinal lesion and that compared one of the novel FNB needles (ProCore, SharkCore, and Acquire) to FNA. Outcome measures included diagnostic accuracy, adequacy, number of passes, presence of tissue cores, and adverse events. Quality was assessed based on the QUADAS-2 tool.

RESULTS

We identified 18 RCT that compared 1046 FNA to 1004 FNB cases, and 648 cases that were sampled with both needles. All studies involved ProCore as FNB needle. The pooled diagnostic accuracy was higher for FNB (OR1.70 95%CI 1.19 to 2.41, p=0.003), as was the tissue core rate after sensitivity analysis (OR2.17, 95%CI 1.21 to 3.91, p=0.01). In addition, less passes were performed with FNB (MD -0.54, 95%CI -1.03 to -0.04, p=0.03). Complication rate was low and did not differ between FNA and FNB (p=0.80). These findings equally applied to solid gastrointestinal lesions and pancreatic lesions. Studies were sufficiently powered, well designed, and harbored a low risk of bias.

CONCLUSION

The ProCore FNB needles outperform FNA in establishing a diagnosis of any type of solid gastrointestinal lesion. Moreover, they secure a higher tissue core rate, with comparable complications.

INTRODUCTION

Endoscopic ultrasound (EUS) guided tissue sampling is a well-established technique to provide a pathology-based diagnosis of lesions in and around the gastrointestinal tract [1, 2]. Also, it is increasingly used to enable pre-therapeutic tissue analysis for targeted treatment [3]. Traditionally, EUS-guided tissue sampling was performed with a fine-needle aspiration (FNA) needle, which mainly harvests loose cells or cytology. Apart from its limited ability to establish tumor invasion and diagnose certain conditions (i.e. auto-immune pancreatitis, submucosal or stromal lesions, and neuro-endocrine tumors [4-6], it strongly depends on rapid on-site tissue evaluation (ROSE) by a dedicated pathologist, which is not always available [7-10]. Also, it may not provide enough material for ancillary testing.

Fine-needle biopsy (FNB) devices were introduced to overcome these limitations, by harvesting histologically intact tissue fragments. The first devices, the TruCut^{tm} (Travenol Laboratories) and Quick-Core$^{®}$ (Cook Medical) needles, were hampered by a rigid design and difficult deployment. Since then, several novel FNB devices have been introduced, including the ProCore reversed and forward facing bevel needles (Cook Medical, Ireland), the Fork-tip (SharkCore, Medtronic), and Franseen needle (Acquire, Boston Scientific).

Despite growing evidence on the diagnostic benefits of FNB over FNA, so far, just one meta-analysis found a higher diagnostic accuracy for FNB, but only for pancreatic masses [11]. As several large studies have been published since then, we aimed to provide an updated review and meta-analysis of studies comparing FNA to the novel FNB needles, including ProCore, SharkCore and Acquire (Figure 1A-D).

METHODS

Study selection

EMBASE, MEDLINE/PubMed, Web of Science, the Cochrane Library, and Google Scholar were systematically searched to identify studies that had compared FNA to the new generation of FNB needles, including the ProCore reversed and forward facing bevel needles (Cook Medical, Ireland), and the Fork-tip (SharkCore, Medtronic) and Franseen needles (Acquire, Boston Scientific) for sampling of a solid lesion reachable from the gastrointestinal tract. A combination of subject headings and text words were used. The key search words were 'endoscopic ultrasound', 'fine needle aspiration', 'fine needle biopsy', 'core biopsy', 'procore', 'sharkcore', 'franseen', 'acquire', 'fork-tip', 'histology' and 'cytology'. The EMBASE search strategy was adapted for use in the other databases. The search focused on human studies without language restrictions. All searches were performed on April 8, 2019. Two independent authors (PvR and DC) systematically reviewed the title and abstract of every retrieved record. If this information suggested that inclusion criteria were met, available full text articles were read and evaluated.

Any disagreement between the reviewers was resolved by discussion with the third author (MB).

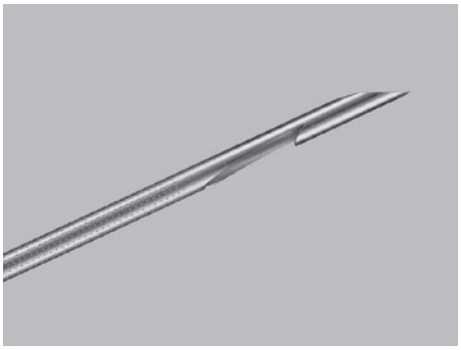


Figure 1A. 22G ProCore FNB reversed bevel needle

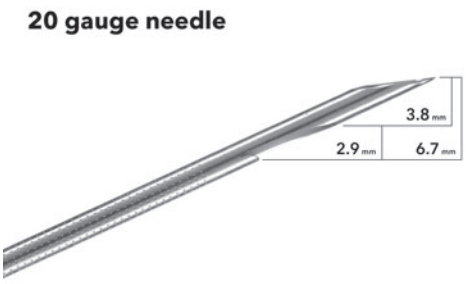


Figure 1B. 20G ProCore FNB forward facing bevel needle

Figure 1C. SharkCore FNB needle

Figure 1D. Acquire FNB needle

In- and exclusion criteria

We included randomized controlled trials (RCTs) that involved at least 50 patients and reported on at least two outcome measures. Studies were excluded if they lacked data to reliably extract the outcome measures. In case of multiple publications on the same population, the most recent publication was included. Conference abstracts were included from the year 2017, as they are still likely to be published as a manuscript.

Outcome measures: definitions and data extraction

Data was extracted on; 1) diagnostic accuracy, based on the final diagnosis (or confirmation of malignancy) obtained from a resection specimen or clinical follow-up period; 2) diagnostic adequacy, defined as the macroscopic sample sufficiency for diagnosis (yes/no) ; 3) presence of tissue cores, defined as a measurable microscopic cylinder, containing target organ cells with preserved histological architecture preserved tissue architecture, adequate for histologic

evaluation (yes/no); 4) the number of needles passes required for final diagnosis or diagnostic adequacy; and 5) procedure-related adverse events. In addition, general study, patient, lesion, sampling and tissue handling characteristics were recorded, as well as the definition of the gold standard diagnosis.

Assessment of methodological quality

Study quality and risk of bias were assessed using a scoring system based on the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool with the Cochrane Collaboration's tool for assessing risk of bias [12]. Risk of bias was assessed based on; random sequence generation (selection bias), allocation concealment (selection bias), comparable study arms (patient and EUS-characteristics), statistical design (power analysis and data interpretation), blinding of participants and personnel (performance bias), blinding of the data analyst to the final outcome (detection bias), coping with incomplete outcome data (attrition bias), selective reporting (reporting bias), the gold standard used (resection specimens, FNA/B or clinical follow-up), and whether the study design was single or multicenter. Any disagreement was resolved by discussion with the third author (MB).

Statistical analysis

Categorical outcome measures were summarized as weighted proportions and 95% Confidence Interval (CI) for both needle types. The number of needle passes was summarized as the standardized mean difference (SMD) and 95% CIs. The meta-analysis was performed by pooling the estimates of effect of the included studies using the random effect Manzel-Haenszel method. P-values of < 0.05 were considered to be statistically significant. The degree of heterogeneity was calculated using the I^2 index, and the presence of publication bias was assessed by examination of funnel plot asymmetry. Statistical analyses were executed by Review manager 5.3 (The Cochrane Collaboration, Oxford, UK) and R (version 3.4.2).

To assess the influence of lower quality studies, those with a 'high risk of bias' in more than four QUADAS-2 categories were in turn removed from the analysis. In addition, to rule out small study effects and correct disparity of the underlying data, studies with a wide CI or a sample size of less than 100 were excluded in a sensitivity analysis. A wide CI was defined as more than five times the Odds ratio. Sensitivity analyses were only performed if at least three studies remained for analysis.

RESULTS

Results of the search

Our search identified 2841 titles, of which 1770 potential eligible studies were reviewed (Figure 2). 1697 studies were excluded based on their title and/or abstract. Reviewing the full text

of the remaining 73 articles resulted in an additional 55 exclusions, as these studies met our exclusion criteria. This resulted in 18 full text articles on FNA versus FNB.

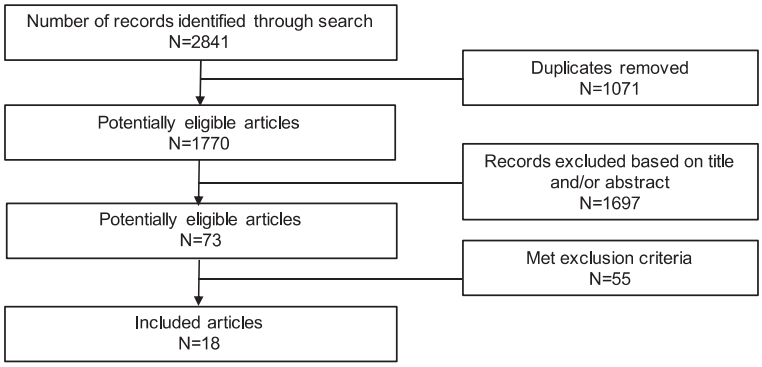


Figure 2. Flowchart of search and included studies.

FNA versus FNB

Study and case characteristics

The 18 studies comparing FNA to an FNB device comprised 2698 patients [13-30]; 1046 allocated to FNA and 1004 to FNB. The remaining 648 patients were alternately sampled with both needles. Study characteristics are presented in table 1-3. FNA was generally performed using the EchoTip Ultra needle (Cook Medical), mostly 22-gauge (G), followed by 25G and 19G. For FNB, all trials used a ProCore needle; 17 reversed and one forward-facing bevel. 12 studies assessed comparable needle sizes [14-16, 19-22, 24-27, 29] and three studies compared needles of different sizes [17, 28, 30]. One study varied needle size according to the target lesion location [23] and two left the decision up to the endosonographer [13, 18].

Eight studies concerned solid pancreatic masses [14, 15, 17, 21, 23, 25, 29, 30] and one subepithelial lesions [18]. The remainder 10 included any solid gastrointestinal lesion (Table 1). The number of needle passes varied considerably, ranging from a single to more than six passes per target lesion (Table 2). ROSE was available in 11 of the 18 studies. Tissue handling differed greatly for FNA samples, ranging from processing as smears or liquid based cytology (LBC, comprising ThinPrep or cell block) to collection in formalin. FNB specimens were generally collected in formalin (Table 2).

Quality and risk of bias

The quality and risk of bias of the trials is summarized in figures 3A and B. Overall, more than 75% of the studies harbored a low risk of selection, performance, detection, attrition, and reporting bias. Also, most studies were sufficiently powered, well designed and had comparable study arms for FNA and FNB. However, FNA or FNB diagnosis was used as the gold standard

Table 1: Characteristics of patients and studies included, FNA versus FNB

Study details	Full text	N	Target lesions	Needle sizes	Dual needle use**	Lesion size, mean ±SD, or median (range) mm		Primary endpoint	Gold standard
						FNA	FNB		
Aadam et al. 2016, USA	Yes	140	Solid lesions in the GI-tract	**FNA**: EchoTip, Expect 22, 25G **FNB:** ProCore 19, 22, 25G	No	30.2 ±18.7	29.2 ±14.1	Diagnostic accuracy	FNA/B diagnosis
Alatawi et al. 2015, France	Yes	100	Solid pancreatic lesions	**FNA**: EchoTip 22G **FNB:** ProCore 22G	No	33 ±2.7	32 ±5.1	No. of passes to obtain a diagnosis	Resection or 1 yr FU
Bang et al. 2012, USA	Yes	56	Solid pancreatic lesions	**FNA**: Expect 22G **FNB:** ProCore 22G	No	33.7 ±7.2	32.5 ±9.0	No. of passes to obtain a diagnosis	Resection or >6 mo. FU
Cheng 2018, China	Yes	377	Solid GI lesions	**FNA**: EchoTip 22G **FNB:** ProCore 22G	No	29.5 (4.5-90)	29.1 (6.0-85)	Diagnostic accuracy after 4 passes	Resection or 48 weeks FU
Hedenström 2017, Sweden	Yes	68	Solid pancreatic lesions	**FNA:** Various brands 25G **FNB:** ProCore 22G	Yes	30 (8-150)	30 (8-150)	Diagnostic accuracy	Resection or 12 mo. FU
Hedenström 2017, Sweden	Yes	70	Subepithelial lesions	**FNA:** Various brands 22, 25G **FNB**: ProCore 22, 19G	Yes	30 (6-220)	30 (6-220)	Diagnostic accuracy	Resection or 12 mo. FU
Hucl et al. 2013, India	Yes	139	Solid pancreatic lesions and lymph nodes	**FNA**: EchoTip 22G **FNB:** ProCore 22G	Yes	38.7 ±15.0	38.7 ±15.0	Diagnostic accuracy	Resection or 6 mo. FU
Iwashita 2017, Japan	Yes	110	Solid lesions around upper intestine	**FNA:** EchoTip 19G **FNB:** ProCore 19G	Yes	36 (27-45)	35 (27-43)	Diagnostic accuracy of a histological diagnosis	Resection or 6 mo. FU
Kamata et al. 2016, Japan	Yes	214	Solid pancreatic lesions	**FNA:** EchoTip 25G **FNB:** ProCore 25G	No	27.9 ±14.4	29.3 ±15.6	Diagnostic accuracy	Resection or >1 yr FU
Lee et al. 2014, Korea	Yes	116	Solid pancreatic lesions	**FNA:** EchoTip 22, 25G **FNB:** ProCore 22G, 25G	No	36.5 (17-74)	36.5 (15-100)	Diagnostic accuracy	Resection or >6 mo. FU
Lee 2017, Korea	Yes	58	Solid GI lesions	**FNA:** EchoTip 22G **FNB**: ProCore 22G	No	44.3 ±32.3	37.5±20.6	Area of overall specimen and core tissue	Resection, EUS-FNA/B or >1 yr FU
Nagula 2018, USA	Yes	274	Solid GI lesions	**FNA:** EchoTip, Expect 22G **FNB**: ProCore 22G	No	33.0 ±16.6* 23.9 ±16* 29.2 ±6.7*	35.1 ±23.8* 21.9 ±8.1* 32.7 ±10.8*	Diagnostic yield	EUS-FNA/B or 3-6 mo. FU

Table 1: Characteristics of patients and studies included, FNA versus FNB (*continued*)

Study details	Full text	N	Target lesions	Needle sizes	Dual needle use**	Lesion size, mean ±SD, or median (range) mm		Primary endpoint	Gold standard
Noh 2018, Korea	Yes	60	Unresectable pancreatic cancer	**FNA:** EZ shot 22G **FNB**: ProCore 22G	Yes	31 ±8.0	31 ±8.0	Diagnostic accuracy for malignancy	Repeated EUS-guided sampling
Othman 2017, USA	Yes	107	Pancreatic, peripancreatic and submucosal lesions	**FNA:** EZ shot, Expect 22G **FNB**: 22G ProCore	No	NR	NR	Specimens adequacy	Resection or 6 mo. FU
Sterlacci 2016, Germany	Yes	56	Solid GI lesions	**FNA:** EchoTip 22G **FNB**: ProCore 22G	Yes	33 ±12	33 ±12	Diagnostic accuracy	Resection, FU until end of study
Van Riet, 2019, The Netherlands	Yes	608	Solid GI lesions	**FNA:** EchoTip 25G **FNB:** ProCore 20G	No	27 (20-40)	29 (20-40)	Diagnostic accuracy	Resection or FU > 9 mo.
Vanbiervliet et al. 2014, France	Yes	80	Solid pancreatic lesions	**FNA**: EchoTip 22G **FNB**: ProCore 22G	Yes	33.9 ±10.8 (11-60)	34 ±11 (11-60)	Diagnostic accuracy	FNA/B diagnosis
Weston, 2017, USA	Yes	60	Solid pancreatic lesions	**FNA**: EchoTip 25G **FNB**: ProCore 22G	Yes	NR	NR	Diagnostic adequacy	NR

*NR: not reported; FNA: fine-needle aspiration; FNB; fine-needle biopsy; SD: standard deviation; FU: follow-up; mo: months; yr: year, no.: number. *Lesion size of solid mass lesions (pancreas, mediastinum, liver, and other), Lymph nodes, and submucosal tumors respectively. **Dual needle use: both needles used for the same target lesion during the same EUS-procedure.*

Table 2: Summary of EUS-procedure details in included studies, FNA versus FNB

Study details	No. of passes according to protocol	No. of to-and fro movements	Fanning	Sampling technique	ROSE	Preparation of cytology	Preparation of histology
Aadam et al. 2016, USA	≤3	10-15	NR	**FNA**: suction or slow pull **FNB**: slow pull	Yes	- air dry, DiffQuick and PAP stain - cell block of remaining specimens	- smash preparation on slides - rest specimens in formalin
Alatawi et al. 2015, France	NA	10-20	Yes	suction	No	- formalin, cell block, HE and PAP stain	- formalin, HE stain
Bang et al. 2012, USA	NA	**FNA**: 12-16 **FNB**: 4	NR	**FNA**: none **FNB**: suction	Yes	- air dry, DiffQuick, alcohol dry, PAP stain	- Hank buffered salt solution, formalin, HE stain
Cheng 2018, China	4	**20**	No	Pass 1 and 2: slow pull Pass 3 and 4: suction	Yes	- smear preparation	- macroscopic cores in formalin
Hedenström 2017, Sweden	2-6	**8-10**	Yes	suction	Yes	-air dry, Giemsa stain -ThinPrep of remaining specimens or when ROSE was not available	- formalin
Hedenström 2017, Sweden	2-6	NR	Yes	suction	Yes	-air dry, Giemsa stain -ThinPrep of remaining specimens or when ROSE was not available	- formalin
Hucl et al. 2013, India	NA	10	NR	suction	No	- formalin	
Iwashita 2017, Japan	1	3-5	NR	suction	No	-alcohol fixation, PAP stain	-whitish parts in formalin
Kamata et al. 2016, Japan	1	20	Yes	slow Pull	No	- formalin, mercurochrome and HE stain	
Lee et al. 2014, Korea	≤3	10-20	NR	suction	Yes	- air and alcohol dry, DiffQuick and PAP stain - rest material in formalin, HE and PAS stain	
Lee 2017, Korea	1-3	NR	NR	slow pull, if unsuccessful suction	No	-formalin	

Table 2: Summary of EUS-procedure details in included studies, FNA versus FNB (*continued*)

Study details	No. of passes according to protocol	No. of to-and fro movements	Fanning	Sampling technique	ROSE	Preparation of cytology	Preparation of histology
Nagula 2018, USA	1-4	NR	NR	**FNA**: suction **FNB**: slow pull	Yes	-air dry, DiffQuick stain on-site -Alcohol fixed off site, PAP stain -remaining aspirate or in absence of ROSE, specimen in standard solution for cell block	
Noh 2018, Korea	2	15-20	NR	**FNA**: suction **FNB**: slow pull	No	-alcohol fixed smears, PAP stain	- formalin
Othman 2017, USA	1-7	10	NR	suction	Yes	-air dry or fixation in Carnoy's solution and staining with DiffQuick or PAP	
Sterlacci 2016, Germany	1-3	10-20	NR	suction	No	-air dry, Giemsa staining	-macroscopic cores in formalin -remaining material alcohol fixed on smear and stained with PAP
Van Riet, 2019, The Netherlands	≥3	NR	Yes	suction, slow pull, a combination, or none	Yes	-smears DiffQuick, HE, PAP stain -remaining material CytoLyt, alcohol, formalin or CytoRich Red -cell suspension processed using ThinPrep or cell block technique	- CytoLyt or formalin
Vanbiervliet et al. 2014, France	**FNA**: 2 **FNB**: 1	10	Yes	suction	Yes	- collected in CytoLyt, ThinPrep[1], fixed in 95% ethanol, PAP stain	- collected in CytoLyt, fixed in formalin, cell block and HE stain
Weston, 2017, USA	2 with each needle	5-10	NR	**FNA**: suction **FNB**: suction or capillary suction	Yes	-air dry or alcohol fixed and DiffQuick or PAP stain -cell block specimens in formalin	-formalin

FNA: fine-needle aspiration; HE: Hematoxylin and eosin; LBM: liquid based medium; NA: not applicable; NR: not reported; PAP: Papanicolaou; PAS: periodic acid-Schiff; ROSE: rapid on-site pathological examination, No.: number. [1]ThinPrep: Hologic Inc., Marlborough, Massachusetts, USA.

Table 3: Outcome measures for individual studies (all lesion types) of meta-analysis comparing FNA to FNB

Study details	Diagnostic accuracy n/n (%)		No. of passes performed, mean±SD, or median (range)		Diagnostic adequacy n/n (%)		Tissue cores n/n (%)		Adverse events n/n (%)	
	FNA	FNB	FNA	FNB	FNA	FNB	FNA	FNB	FNA	FNB
Aadam et al. 2016, USA	47/70 (67.1)	63/70 (90)	3.0±1.0	2.8±1.0	42/70 (60)	58/70 (83)	NR	NR	0/70 (0)	0/70 (0)
Alatawi et al. 2015, France	45/50 (90)	50/50 (100)	3.28±1.0	2.59±0.49	NR	NR	16/50 (32)	38/50 (76)	0/50 (0)	0/50 (0)
Bang et al. 2012, USA	28/28 (100)	25/28 (89)	1.61±0.88	1.28±0.54	28/28 (100)	26/28 (93)	19/28 (68)	23/28 (82)	1/28 (4)	1/28 (4)
Cheng 2018, China	152/190 (80)	171/187 (91)	4	4.	NR	NR	152/190 (80)	171/187 (91)	3/190 (2)	1/187 (1)
Hedenström 2017, Sweden	53/68 (78)	47/68 (69)	3 (2-4)	2 (2-3)	NR	NR	NR	NR	1/68 (1)	0/68 (0)
Hedenström 2017, Sweden	34/70 (49)	58/70 (83)	3 (1-4)	2 (1-4)	NR	NR	NR	NR	0/70 (0)	1/70 (1)
Hucl et al. 2013, India	112/139 (80.6)	110/139 (79)	2.5±0.9	1.2±0.5	127/144 (88)	125/144 (87)	84/127 (66)	86/125 (69)	0/144 (0)	0/144 (0)
Iwashita 2017, Japan	87/110 (79)	99/110 (90)	1	1	NR	NR	89/110 (81)	93/110 (85)	4/110 (4)	4/110 (4)
Kamata et al. 2016, Japan	82/108 (76)	90/106 (85)	1	1	NR	NR	75/108 (69)	86/106 (81)	0/108 (0)	0/106 (0)
Lee et al. 2014, Korea	55/58 (95)	57/58 (98)	2.0 (1-5)	1.0 (1-5)	NR	NR	45/58 (78)	48/58 (83)	1/58 (2)	3/58 (5)
Lee 2017, Korea	22/27 (82)	25/31 (81)	2 (1-3)	1 (1-3)	NR	NR	27/29 (93)	29/29 (100)	0/29	0/29
Nagula 2018, USA	123/135 (91)	123/139 (89)	1.83 ±1.17	1.65 ±0.93	NR	NR	NR	NR	1/135 (1)	2/139 (1)
Noh 2018, Korea	57/60 (95)	56/60 (93)	2	2	NR	NR	NR	NR	0/60 (0)	0/60 (0)
Othman 2017, USA	NR	NR	2.74±1.32	2.67±1.37	50/73 (68)	27/36 (75)	NR	NR	3/72 (4)	1/35 (3)
Sterlacci 2016, Germany	48/54 (89)	49/51 (96)	1.5±0.6	1.7±0.6	54/56 (96)	51/56 (91)	35/56 (63)	36/56 (64)	0/56 (0)	0/56 (0)
Van Riet, 2019, The Netherlands	237/306 (78)	263/302 (87)	NR	NR	248/306 (82)	263/302 (87)	136/306 (45)	232/302 (77)	3/306 (1)	2/302 (1)
Vanbiervliet et al. 2014, France	74/80 (93)	72/80 (90)	1	2	75/80 (94)	71/80 (89)	70/80 (88)	56/80 (70)	1/80 (1)	0/80 (0)
Weston, 2017, USA	NR	NR	2	2	49/60 (82)	48/59 (81)	NR	NR	0/60 (0)	0/60 (0)

NR: not reported.

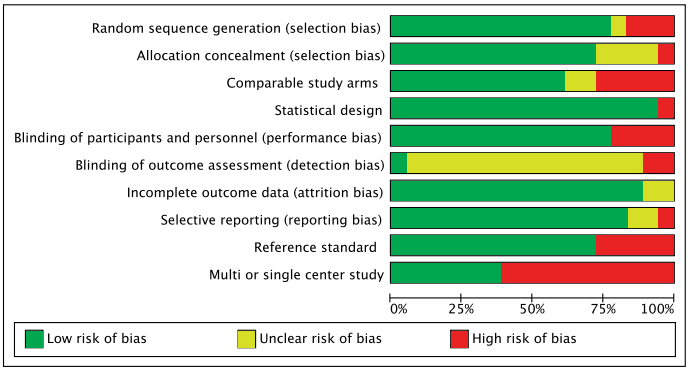


Figure 3A. Quality and risk of bias of studies comparing FNA to FNB based on the QUADAS-II and Cochrane Collaboration's tool.

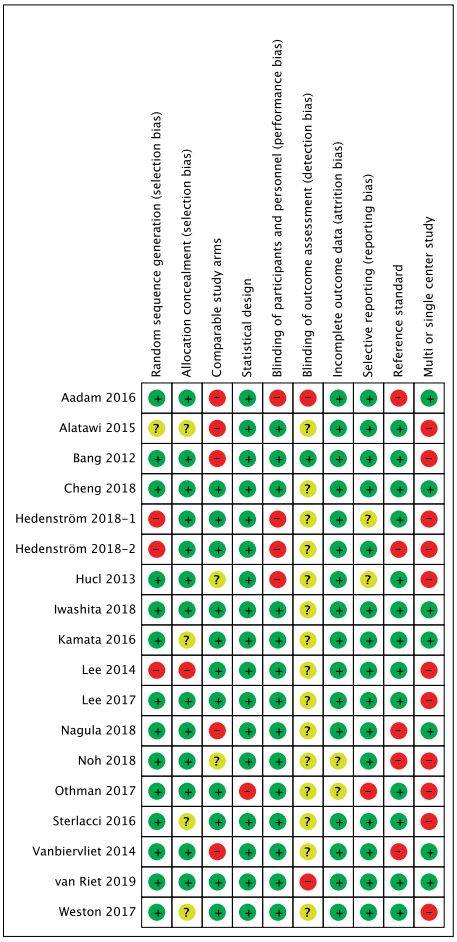


Figure 3B. Summary of quality and risk of bias in all studies comparing FNA to FNB based on the QUADAS-II and Cochrane Collaboration's tool.

diagnosis for malignancy in approximately 30% of the studies [13, 18, 24, 25, 29], which we considered to be suboptimal. Lastly, only seven out of 18 studies were multicenter trials [13, 16, 20, 21, 24, 28, 29].

Outcome parameters of the included studies

The outcome measures of all individual studies from this meta-analysis are presented in table 3 and will be discussed separately below.

Diagnostic accuracy

16 studies, involving 2528 patients, reported on the diagnostic accuracy [13-25, 27-29]. The pooled accuracy for sampling any solid lesion was significantly better for the ProCore FNB of than for the FNA needles (OR1.70, 95%CI 1.19 to 2.41, p=0.003, table 4 and figure 4A). Heterogeneity amongst the studies was moderate (I^2=59%) and the funnel plot did not demonstrate signs of publication bias (Figure 4E). Sensitivity analyses did not change outcomes (Table 4 and figure 4B). Subgroup analyses for solid pancreatic target lesions demonstrated a similar outcome, with a higher pooled diagnostic accuracy for FNB than FNA (Table 4, figure 4C), without change after sensitivity analysis (Table 4, figure 4D).

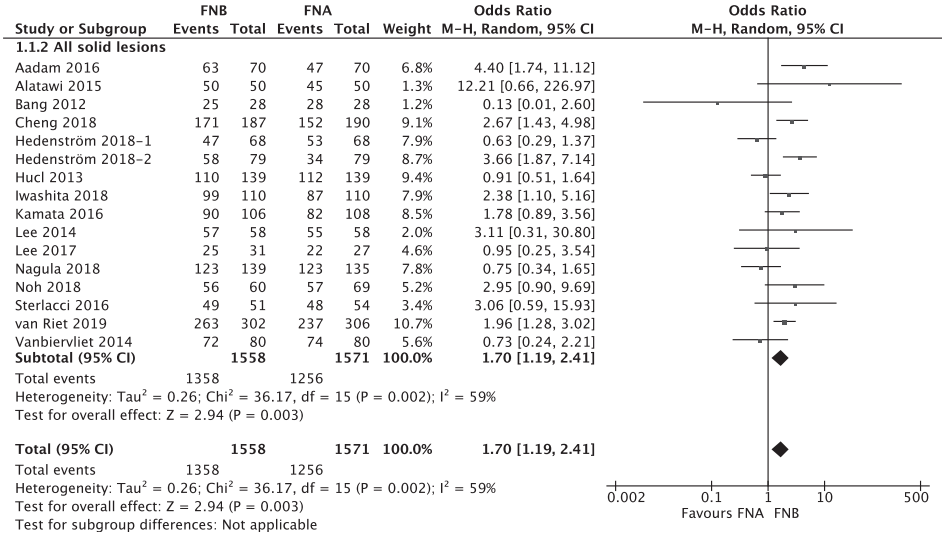
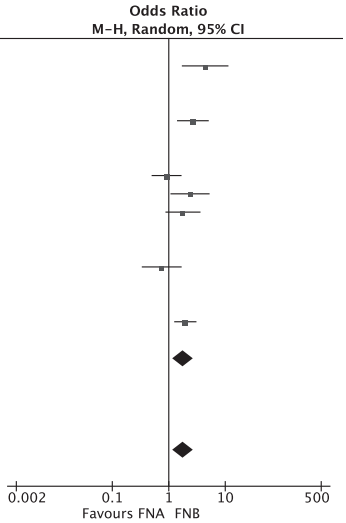


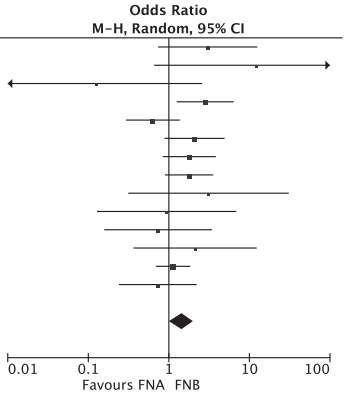
Figure 4A-D. Forest plots comparing diagnostic accuracy between FNA and FNB.
A: All studies

Study or Subgroup	FNB Events	FNB Total	FNA Events	FNA Total	Weight	Odds Ratio M-H, Random, 95% CI
1.1.2 All solid lesions						
Aadam 2016	63	70	47	70	10.6%	4.40 [1.74, 11.12]
Alatawi 2015	50	50	45	50	0.0%	12.21 [0.66, 226.97]
Bang 2012	25	28	28	28	0.0%	0.13 [0.01, 2.60]
Cheng 2018	171	187	152	190	15.3%	2.67 [1.43, 4.98]
Hedenström 2018-1	47	68	53	68	0.0%	0.63 [0.29, 1.37]
Hedenström 2018-2	58	79	34	79	0.0%	3.66 [1.87, 7.14]
Hucl 2013	110	139	112	139	15.9%	0.91 [0.51, 1.64]
Iwashita 2018	99	110	87	110	12.7%	2.38 [1.10, 5.16]
Kamata 2016	90	106	82	108	14.1%	1.78 [0.89, 3.56]
Lee 2014	57	58	55	58	0.0%	3.11 [0.31, 30.80]
Lee 2017	25	31	22	27	0.0%	0.95 [0.25, 3.54]
Nagula 2018	123	139	123	135	12.5%	0.75 [0.34, 1.65]
Noh 2018	56	60	57	69	0.0%	2.95 [0.90, 9.69]
Sterlacci 2016	49	51	48	54	0.0%	3.06 [0.59, 15.93]
van Riet 2019	263	302	237	306	18.9%	1.96 [1.28, 3.02]
Vanbiervliet 2014	72	80	74	80	0.0%	0.73 [0.24, 2.21]
Subtotal (95% CI)		**1053**		**1058**	**100.0%**	**1.78 [1.19, 2.66]**
Total events	919		840			
Heterogeneity: $Tau^2 = 0.18$; $Chi^2 = 15.60$, df = 6 (P = 0.02); $I^2 = 62\%$						
Test for overall effect: Z = 2.80 (P = 0.005)						
Total (95% CI)		**1053**		**1058**	**100.0%**	**1.78 [1.19, 2.66]**
Total events	919		840			
Heterogeneity: $Tau^2 = 0.18$; $Chi^2 = 15.60$, df = 6 (P = 0.02); $I^2 = 62\%$						
Test for overall effect: Z = 2.80 (P = 0.005)						
Test for subgroup differences: Not applicable						



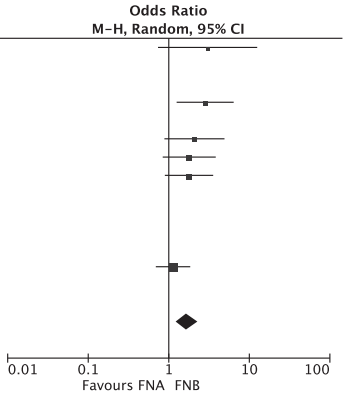
B: Sensitivity analysis

Study or Subgroup	FNB Events	FNB Total	FNA Events	FNA Total	Weight	Odds Ratio M-H, Random, 95% CI
Aadam 2016	33	36	29	37	4.6%	3.03 [0.74, 12.52]
Alatawi 2015	50	50	45	50	1.2%	12.21 [0.66, 226.97]
Bang 2012	25	28	28	28	1.2%	0.13 [0.01, 2.60]
Cheng 2018	114	123	103	126	10.6%	2.83 [1.25, 6.39]
Hedenström 2018-1	47	68	53	68	11.4%	0.63 [0.29, 1.37]
Hucl 2013	59	69	51	69	9.9%	2.08 [0.88, 4.92]
Iwashita 2018	55	70	47	70	11.6%	1.79 [0.84, 3.83]
Kamata 2016	90	106	82	108	13.0%	1.78 [0.89, 3.56]
Lee 2014	57	58	55	58	2.0%	3.11 [0.31, 30.80]
Lee 2017	5	9	4	7	2.5%	0.94 [0.13, 6.87]
Noh 2018	56	60	57	60	4.0%	0.74 [0.16, 3.44]
Sterlacci 2016	36	38	34	38	3.2%	2.12 [0.36, 12.32]
van Riet 2019	112	154	111	158	17.9%	1.13 [0.69, 1.85]
Vanbiervliet 2014	72	80	74	80	6.9%	0.73 [0.24, 2.21]
Total (95% CI)		**949**		**957**	**100.0%**	**1.44 [1.03, 2.01]**
Total events	811		773			
Heterogeneity: $Tau^2 = 0.10$; $Chi^2 = 17.89$, df = 13 (P = 0.16); $I^2 = 27\%$						
Test for overall effect: Z = 2.15 (P = 0.03)						



C: Pancreatic lesions only

Study or Subgroup	FNB Events	FNB Total	FNA Events	FNA Total	Weight	Odds Ratio M-H, Random, 95% CI
Aadam 2016	33	36	29	37	4.4%	3.03 [0.74, 12.52]
Alatawi 2015	50	50	45	50		Not estimable
Bang 2012	25	28	28	28		Not estimable
Cheng 2018	114	123	103	126	13.4%	2.83 [1.25, 6.39]
Hedenström 2018-1	47	68	53	68		Not estimable
Hucl 2013	59	69	51	69	12.0%	2.08 [0.88, 4.92]
Iwashita 2018	55	70	47	70	15.4%	1.79 [0.84, 3.83]
Kamata 2016	90	106	82	108	18.5%	1.78 [0.89, 3.56]
Lee 2014	57	58	55	58		Not estimable
Lee 2017	5	9	4	7		Not estimable
Noh 2018	56	60	57	60		Not estimable
Sterlacci 2016	36	38	34	38		Not estimable
van Riet 2019	112	154	111	158	36.2%	1.13 [0.69, 1.85]
Vanbiervliet 2014	72	80	74	80		Not estimable
Total (95% CI)		**558**		**568**	**100.0%**	**1.68 [1.24, 2.26]**
Total events	463		423			
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 5.04$, df = 5 (P = 0.41); $I^2 = 1\%$						
Test for overall effect: Z = 3.39 (P = 0.0007)						



D: Sensitivity analysis of pancreatic lesions

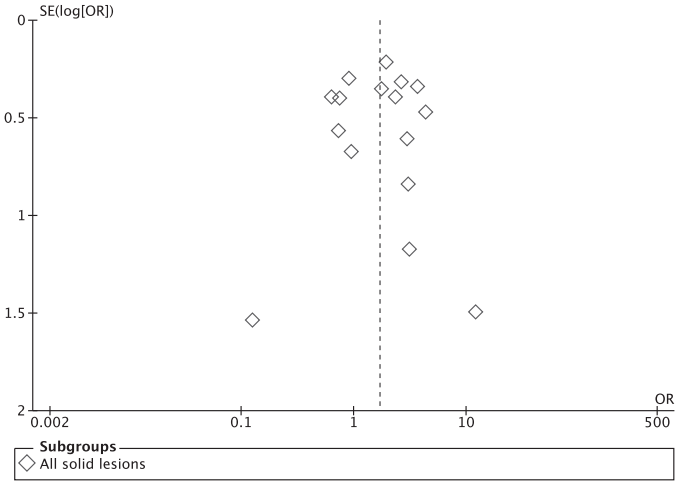


Figure 4E. Funnel plot of the diagnostic accuracy for all included studies.

Diagnostic adequacy

Seven studies concerning 1186 patients reported on diagnostic adequacy [13, 15, 19, 26-29], with moderate heterogeneity (I^2=54%). There was no difference in sample adequacy between FNA and FNB (OR1.17, 95%CI 0.70 to 1.96, p=0.55, table 4, figure 5A), even after sensitivity analysis (Table 4, figure 5B). The same was true for pancreatic lesions (Table 4, figure 5C). Sensitivity analysis on this subgroup was not performed as this would leave less than three studies for analysis.

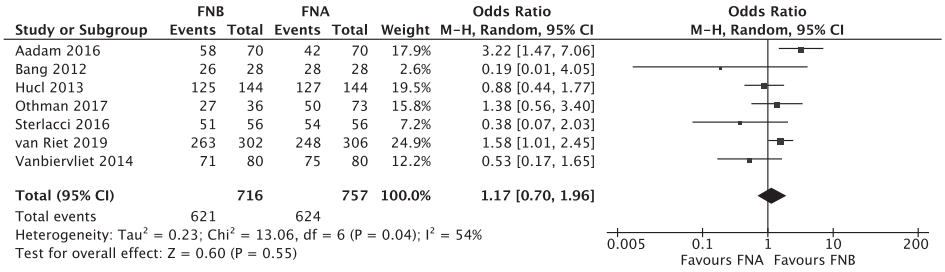
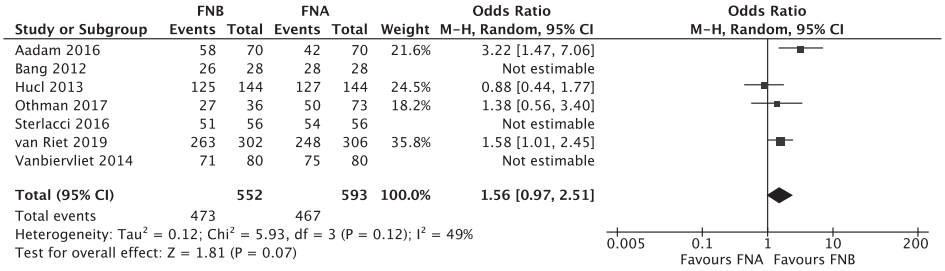
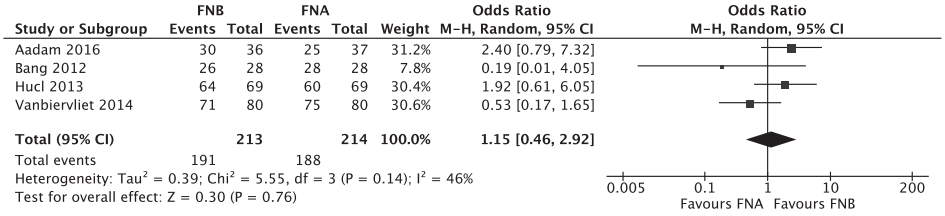


Figure 5A-C. Forest plots comparing sample adequacy between FNA and FNB.
A: All studies



B: Sensitivity analysis



C: Pancreatic lesions only

Number of passes performed

Five studies reported on the mean or average number of passes performed to establish a diagnosis [14, 15, 24] or obtain an adequate sample according to the on-site pathologist [13, 19]. The mean number of passes was lower for FNB than FNA needles (MD -0.54, 95%CI -1.03 to -0.04, p=0.03, table 4, figure 6A). Heterogeneity was high amongst the studies (I^2=94%). Sensitivity analysis could not be performed, as this would involve less than three studies. Subgroup analysis for pancreatic lesions showed similar results, requiring fewer passes with FNB (Table 4, figure 6B).

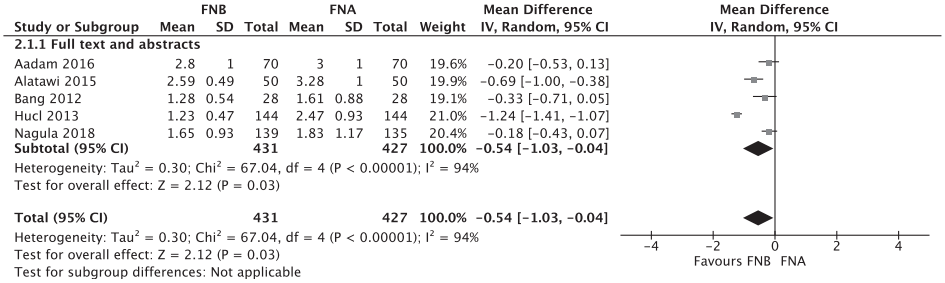
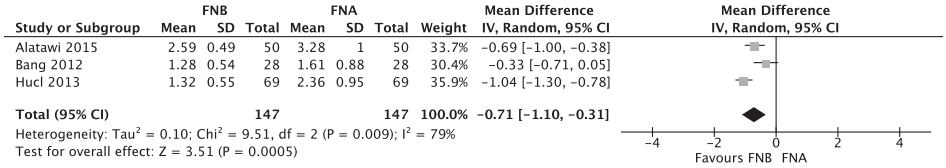


Figure 6A-D. Forest plots comparing the mean number of needle passes to obtain a diagnosis between FNA and FNB.

A: All studies



B: Pancreatic lesions only

Presence of tissue cores

Ten studies reported on presence of tissue cores in 1537 patients [14, 15, 19-23, 27-29]. Heterogeneity amongst the studies was high (I^2=83%). Before sensitivity analysis, a trend towards better tissue core yield for ProCore FNB was observed, with a pooled estimate rate of 77% (95%CI 75 to 80) for ProCore, and 63% (95%CI 61 to 68, table 4, figure 7A) for FNA. After sensitivity analysis, this became a significant benefit (OR2.34, 95%CI 1.21 to 4.51, p=0.01, table 4, figure 7B). Similarly, subgroup analysis for pancreatic lesions demonstrated a trend towards a higher tissue core rate for FNB (Table 4, figure 7C), and a significantly higher yield after studies of suboptimal quality were removed (OR3.64 95%CI 1.85 to 7.13, p<0.001, table 4, figure 7D).

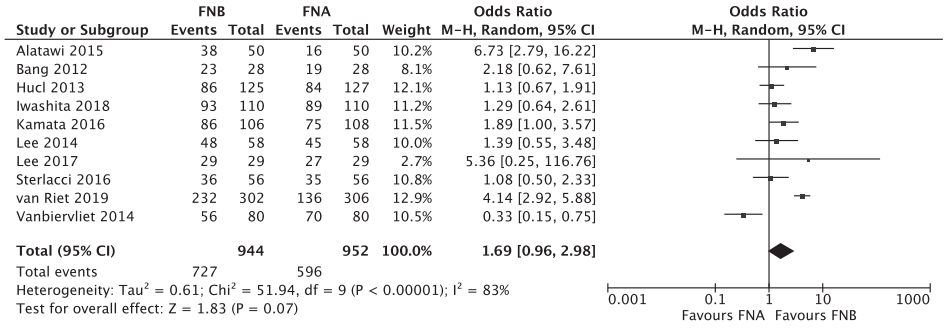
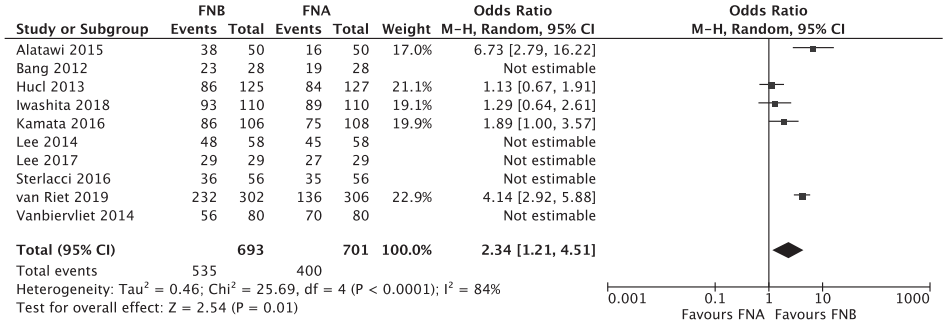
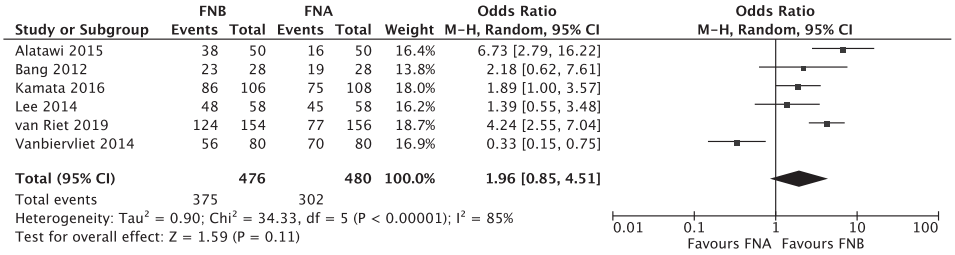


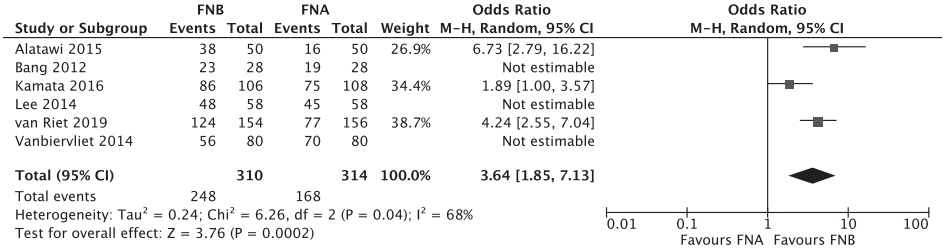
Figure 7A-D. Forest plots comparing the presence of tissue cores between FNA and FNB samples.
A: All studies



B: Sensitivity analysis



C: Pancreatic lesions only



D: Sensitivity analysis of pancreatic lesions

Adverse events

All studies reported on the occurrence of procedure-related adverse events, which did not differ between FNA and FNB (OR 0.91, 95%CI 0.45 to 1.86, p=0.80, table 4, figure 8). The pooled estimate for complications was 0.9% (95%CI 0.5 to 1.4) for the ProCore and 1.1% (95%CI 0.6 to 1.6) for the FNA devices. There was no heterogeneity amongst the studies for this endpoint (I^2=0%).

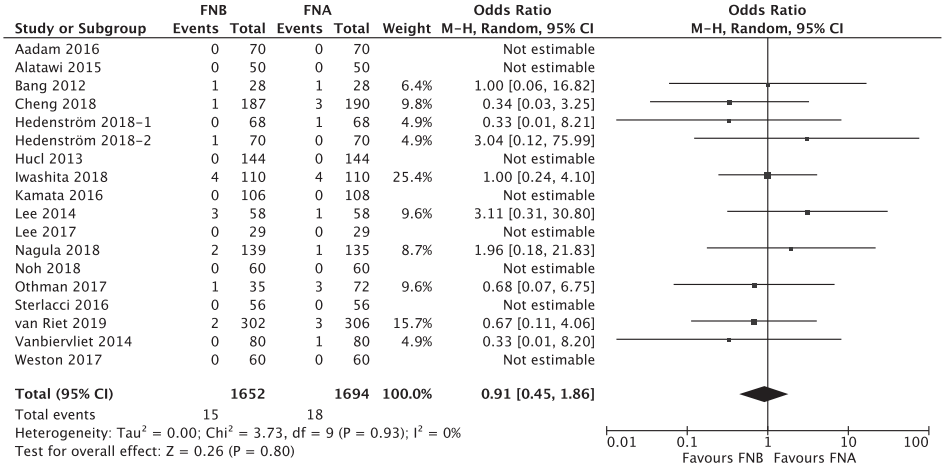


Figure 8. Forest plot comparing the adverse event rate between FNA and FNB.

Table 4: Summary of findings: pooled estimates of all outcome measures comparing FNA to FNB.

Outcome measure	Pooled estimate % (95%CI)		Pooled OR (95%CI)	p-value
	FNA	FNB		
Diagnostic accuracy				
All studies	81 (79 - 82)	87 (86 - 89)	1.70 (1.19 - 2.41)	0.003
Sensitivity analysis	79 (76 – 82)	87 (85 - 89)	1.78 (1.19 - 2.66)	0.005
Pancreas only	81 (78 – 83)	85 (83 – 88)	1.44 (1.03 – 2.21)	0.030
Sensitivity analysis	74 (71 – 78)	83 (80 – 86)	1.68 (1.24 – 2.26)	<0.001
Diagnostic adequacy				
All studies	82 (80 – 85)	87 (84 – 89)	1.17 (0.70 – 1.96)	0.550
Sensitivity analysis	79 (75 – 82)	86 (83 – 88)	1.56 (0.97 – 2.51)	0.070
Pancreas only	88 (83 – 92)	90 (85 – 93)	1.15 (0.46 – 2.92)	0.760
Sensitivity analysis	Not performed	Not performed	Not performed	
Mean no. of passes performed[1]				
All studies			-0.54 (-1.03 to – 0.04)	0.030
Pancreas only			-0.71 (-1.10 to -0.31)	0.009
Presence of tissue cores				
All studies	64 (61 – 67)	78 (75 – 80)	1.69 (0.96 – 2.98)	0.070
Sensitivity analysis	57 (53 – 61)	77 (74 – 80)	2.34 (1.21 – 4.51)	0.010
Pancreas only	63 (59 – 67)	79 (75 – 82)	1.96 (0.85 – 4.51)	0.110
Sensitivity analysis	54 (50 – 59)	80 (75 – 84)	3.64 (1.85 – 7.13)	<0.001
Adverse events				
All studies	1.0 (0.6 – 1.6)	0.8 (0.5 – 1.4)	0.91 (0.45 – 1.86)	0.800

OR: Odds ratio, No. : number, NP: not performed.

[1]*Standardized mean difference.*

DISCUSSION

The current meta-analysis is the first to demonstrate a convincing diagnostic benefit of the new generation FNB needles, specifically of the ProCore design, over FNA. The recent wave of publications enabled us to include a considerable number of new randomized controlled trials and limit our evaluation to high quality studies [11, 31-34]. Compared to FNA, FNB needles achieved a higher diagnostic accuracy and a higher tissue core rate, both in pancreatic and non-pancreatic lesions, with less needle passes. Complication rates were low and comparable for FNA and FNB. Since all of the included studies comprised ProCore needles, these results may not be straightly extrapolated to the other new FNB needles.

FNB outperformed FNA in several ways. First, the current meta-analysis confirmed their superior tissue core rate. Interestingly, sample adequacy did not differ between FNA and FNB. New preparation techniques such as ThinPrep and cell block enable pathologists to perform comparable tests on cytology and histology. However, these preparation techniques were widely applied in the current study population, yet diagnostic accuracy was still better for the ProCore FNB needles. The same applied for ROSE. The fact that most EUS-centers lack such

additional techniques and services is another reason to endorse FNB sampling, especially in low-volume or non-academic centers.

Furthermore, FNB required less passes to obtain a diagnosis. Although the complication rate was comparable for FNA and ProCore, fewer passes will minimize the risk of traumatic tissue traversing. Furthermore, less passes will limit procedure time and costs. The single study that assessed procedure time and costs [13] found FNB cost saving, which is in line with two other reports [35, 36].

Despite the better diagnostic performance and other above-mentioned benefits of FNB over FNA needles, the significant heterogeneity amongst studies should not be overlooked. This has already been described in previous meta-analyses to results from a diversity in EUS-sampling protocols and inconsistent use of outcome definitions. Regarding diverse practice patterns, it is well known that EUS-guided tissue sampling and processing techniques vary substantially [10]. Although present EUS-guidelines offer some recommendations, solid evidence is scarce [7, 37-40]. On top of this, many recommendations are dated, and keep running behind on the latest innovations [7, 40]. The subsequent lack of uniformity in EUS-practice renders EUS-device studies difficult to compare.

In addition, the lack of universal definitions for diagnostic outcome measures plays a role. The inconsistent use of terms for diagnostic accuracy, adequacy, and yield create confusion and hamper comparison of results. For example, diagnostic accuracy can refer to the accuracy in establishing a diagnosis or just accuracy in establishing presence of malignancy. Also, certain studies equate the diagnostic yield and diagnostic accuracy, whereas others use 'diagnostic yield' as a more subjective term, describing the presence of sufficient tissue for ROSE or pathological analyses. In addition, presence of malignancy (as gold standard) is variably established from resection specimens or FNA/B specimens. Another confusing definition is that of 'tissue cores'. It may be described as the presence of intact histologically tissue fragments or 'whitish material', the proportion or ratio of intact tissue fragments compared to the entire sample, or an intact 'tissue core' of a certain length and/or size.

Despite the fact that we selected large randomized controlled trials and removed studies of inferior quality in our sensitivity analyses, heterogeneity remained substantial. To improve the precision of our pooled estimates, we chose to perform a random rather than a fixed effect model for our analyses, as this model includes the variance within and between studies. The fact that this approach resulted in the inclusion of ProCore needles only (mainly with the reversed bevel design) results from the lack of trials that assessed SharkCore, Acquire, or the novel ProCore forward facing bevel design. As these needles are relatively new on the market, data on their performance is limited to small sized, single center, retrospective studies.

According to ClinicalTrials.gov, multiple studies comparing different design FNB needles are currently ongoing including two randomized controlled trials comparing FNA to Acquire (NTC02911974 and NTC0 3109639), two studies comparing FNA to SharkCore (NCT02678442 and NTC0 3532347), one comparing SharkCore to FNA with ROSE (NTC03485924), one compar-

ing a side-fenestrated needle to an Acquire needle (NTC03622229), one comparing SharkCore to Acquire (NTC03672032), and lastly one study that compares an unspecified core needle to FNA (NTC03435588). So far, no randomized controlled trials have compared ProCore to SharkCore. Unfortunately, two studies that aimed to do so were withdrawn, one because ProCore was no longer used in that clinic (NTC02766842), and another one because of a slow inclusion rate (NTC03011229).

In conclusion, this study demonstrates that the FNB ProCore needles outperform conventional FNA needles in diagnosing any solid lesion surrounding the gastrointestinal tract. This observation is of paramount importance in light of the increased need of EUS-guided tissue acquisition for personalized medicine and targeted therapy. However, head to head comparison studies between each of the new generation of FNB devices are needed to further establish the optimal needle design, preferably in an (international) multicenter randomized controlled setting.

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Part III

Improving EUS-specimen preparation and handling

Chapter 7

Optimizing tissue handling of EUS-FNA of solid pancreatic lesions: a pilot study to the effect of a tissue preparation training for endoscopy personnel on sample quality and diagnostic accuracy

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ABSTRACT

BACKGROUND

In the absence of rapid on-side pathological evaluation (ROSE), endoscopy staff generally 'smears' endoscopic ultrasound guided (EUS) fine-needle aspiration (FNA) specimens on a glass slide. As this technique is vulnerable to preparation artifacts, we assessed if its quality could be improved through a tissue-preparation-training for endoscopy staff.

METHODS

In this prospective pilot study, 10 endosonographers and 12 endoscopy nurses from 7 regional EUS-centers in the Netherlands were invited to participate in a EUS-FNA smear-preparation-training. Subsequently, post training slides derived from solid pancreatic lesions were compared to pre-training 'control' slides. Primary outcome was to assess if the training positively affects smear quality and, consequently, diagnostic accuracy of EUS-FNA of solid pancreatic lesions.

RESULTS

Participants collected and prepared 71 cases, mostly pancreatic head lesions (48%). 68 controls were selected from the pre-training period. The presence of artifacts was comparable for smears performed before and after training (76% versus 82%, p=0.363). Likewise, smear cellularity (≥50% target cells) before and after training did not differ (44% (30/68) versus 49% (35/71), p=0.480). Similar, no difference in diagnostic accuracy for malignancy was detected (p=0.998).

CONCLUSION

In this pilot EUS-FNA smear-preparation-training for endoscopy personnel, smear quality and diagnostic accuracy were not improved after the training. Based on these results, we plan to further study other training programs and possibilities.

INTRODUCTION

Since its introduction in 1992, endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) is increasingly popular, due to its ability to sample difficult-to-reach target lesions at a low complication rate. Although the technique has gained global ground, diagnostic accuracy rates still vary from 68% to 98% [1-5], depending on patient characteristics, sampling techniques, and tissue handling and processing [6-14].

Historically, EUS-FNA tissue has been collected by spreading material on a glass slide, the so called 'smear technique'. Although this technique is fast and cheap, its diagnostic value is easily hampered by contamination and preparation artifacts [15, 16]. In the absence of (cyto) pathological assistance in the room (ROSE), specimens are prepared by the endoscopy staff, generally without formal training. There is limited data on their performance as compared to a specialized (cyto)pathologist. Although it seems that endoscopy staff is capable of assessing smear adequacy for diagnostic purposes [17-21], reports on their ability to prepare the smears themselves are conflicting [22-25]. We hypothesized that a tissue-preparation-training for endoscopy staff can improve smear quality and, thus, diagnostic accuracy of EUS-FNA.

METHODS

Study design

In this prospective pilot study, endosonographers and endoscopy nurses of seven regional EUS-centers in the Netherlands were invited to participate in a one-day EUS-FNA-specimen preparation training, if they had not undergone formal tissue preparation training before. To assess the impact of the training, quality and diagnostic accuracy of smears were compared before and after the training. For this, all study samples were sent to the Erasmus MC University Medical Center Rotterdam for expert review. As the study did not intervene with routine patient care, the Medical Ethics Committee of the Erasmus University Medical Center of Rotterdam waived the need to comply to the Medical Research Involving Human Subjects Act (MEC-2016-022). This committee also specifically approved for the use of any tissue and fluid samples as a model, as the training location was restricted to a controlled area (biohazard) at the department of Pathology in the Erasmus University Medical Center in Rotterdam.

Training program

The specifically designed training program comprised of a 2-hour theoretical and 2-hour practical 'hands-on' part. The training was provided by an expert pathologist and a group of cytotechnicians from the Erasmus University Medical Center in Rotterdam. During the theoretical part, participants were educated on pancreas pathology, including solid and cystic pancreatic neoplasms, chronic pancreatitis, and focal inflammation. Furthermore, several examples of

normal pancreas cytology and histology were discussed, as was the Bethesda classification and common diagnostic pitfalls in pancreas (cyto)pathology. Next, participants were lectured on the different FNA tissue preparation techniques, including smears, and commonly encountered pitfalls. The main focus of the training was optimal smear preparation. To prepare a good smear, participants were taught to apply the collected specimens 1cm from the edge of the glass slide. Then, they were told to place a second glass slide on top of the first glass slide that contained the drop of FNA material, and try to evenly distribute the tissue using the so-called sandwhich method. In addition, participants were explained to limit the amount of tissue per glass slide (only 1 drop!) to prevent thick cells layers or overlapping cells, and to avoid crushing artefacts by pressing the two glass slides too firmly. Last, they were instructed on the importance and timing of on-site fixation, staining and drying of the material. During the hands-on workshop, participants learned how to optimally smear and stain FNA-specimens, and how to avoid common pitfalls during preparation. Porcine pancreatic tissue was used as training specimens.

FNA-sample selection

After the training day, each participating center prospectively included all consecutive cases, scheduled for EUS-FNA of solid pancreatic lesions between April 2016 and September 2017. Subsequently, an equal number of historical controls (prior to the training date) was selected for each center. We did not match our controls based on needle type or size or the sampling technique used, as there is there is limited evidence on the impact of these variables on diagnostic accuracy of EUS-FNA. Samples that were prepared by (cyto)pathologists and/or cytotechnicians were excluded.

EUS-guided tissue sampling and specimens handling

EUS-guided tissue sampling was performed according to a standard protocol, using a convex array echoendoscope (Pentax EG-3870 UTK, Pentax EG-3270 UK; Pentax, Tokyo, Japan, Olympus UTC 140/180, Olympus linear GF-UCT180; Olympus, Tokyo, Japan). Tissue sampling was done by endosonographers, who performed between 25 and 100 EUS-guided tissue sampling procedures annually. The optimal sampling position was determined by scanning the target lesion and its environment with color and pulsed Doppler. Patients were punctured using a 19, 22- or 25-gauge FNA needle (EchoTip; Cook Medical, or Expect; Boston Scientific). Per target lesion, the trainees performed two smears from a single pass. All residual material was processed according to the standard protocols of the laboratories involved (Table 1). Furthermore, the number of passes, sampling strategy, and use of additional sampling techniques (e.g. applying negative suction with a syringe) was left at the discretion of the endosonographers. If available, ROSE, but only after the trainee had performed the study smears. In that case, the on-site (cyto)pathologist was not allowed to comment on in the glass slide preparation of the trainee.

Table 1. EUS-guided tissue sampling and tissue processing specifics per center

Center	EUS-scope type	Annual EUS-FNA per endosonographer	ROSE available	Additional techniques	SMEAR preparation	Liquid cytology medium	Thin-layer cytology technique	cell block technique
Albert Schweitzer Hospital, Dordrecht	Olympus linear GF-UCT180	25	Yes	Slow pull or Suction	Air dry, Hemocolor	CytoLyt	ThinPrep	Cellient Hologic
Reinier de Graaf Hosptial, Delft	Olympus linear GF-UCT180	30	No	Slow pull	Air dry No stain	CytoLyt, or Polytransportbuffer*	ThinPrep	Agar
Erasmus MC University Medical Center Rotterdam	Pentax EG-3870 UTK Olympus UTC 140/180	50	Yes	Slow pull or Suction	Air dry Diffquick	CytoLyt	ThinPrep	Cellient Hologic
Haga Hospital, The Hague	Olympus linear GF-UCT180	25	Yes	Slow pull	Air dry Diffquick	Formalin	None	Paraffin cell block
Ijsselland Hospital, Rotterdam	Olympus linear GF-UCT180	25	Yes	Slow pull	Air dry Diffquick Giemsa	CytoRichRed	None	Agar
Maasstad Hospital, Rotterdam	Pentax EG-3270 UK Olympus linear GF-UCT180	30	No	Slow pull	Air dry Diffquick	CytoRichRed	None	Aalfix cell block*
Sint Franciscus Hospital, Rotterdam	Pentax EUS-scope	20	No	Slow pull or Suction	Air dry No stain	CytoRichRed	None	Agar

FNA: fine needle aspiration, ROSE: rapid on-site pathological evaluation,
**medium/technique developed locally.*

Outcome measures and definitions

The primary outcome measure was to assess if this one day 'hands-on' EUS-specimens-preparation-training improved the diagnostic accuracy of smears, in the absence of an on-site (cyto) pathologist. Diagnostic accuracy for malignancy was calculated from the correct number of cases that were defined as atypical/suspect for malignancy or malignant. In addition, accuracy for the Bethesda classification was calculated from the number of cases that were correctly classified into the categories; non-diagnostic, benign, atypical/suspect for malignancy or malignancy, according to the formula: (true positive + true negative) / all patients. Gold-standard diagnosis was based on surgical resection specimens, or a clinical follow-up period of at least 1 year for non-operated patients.

Secondly, we assessed if the training improved sample quality, which was defined as sample artifacts (fixation, thick smear/clots, obscuring blood or inflammation, cytolysis, contamination, other) and cellularity (presence of </≥50% target cells).

Statistics

Outcome measures were expressed as means ± standard deviations (SD) or as medians with interquartile ranges (IQR). Statistical significance was assessed with the use of Student's t-test for normally distributed continuous data; either the chi-square test for categorical data (with Yates' correction when appropriate) or Fisher exact test for categorical data; and the median test for non-normally distributed continuous data. Sample quality and diagnostic accuracy were compared between cases and controls using a logistic mixed effect model with a random intercept for participating center [26]. The latter has been done to take into account the clustering structure of this multi-center trial, i.e., that observations from the same site may be correlated. Statistical significance was established as $p<0.05$ (two-tailed). Analyses were carried out using SPSS version 21, Statistical Package for the Social Sciences, SPSS Inc., Chicago, Illinois, and R (version 3.4.2).

Power calculation

To determine the power needed for this study, we assessed the impact of the introduction of ROSE in one of the participating centers as a substitute intervention for our smear-preparation-training. To determine if smear accuracy had improved, an expert pathologist reviewed 20 smears from the period before and 18 smears from the period after ROSE was introduced in that center. Smear accuracy improved with 30% since the implementation of ROSE. Based on this assumption, a two-group continuity corrected chi-squared test with a 0.05 two-sided significance level will have 80% power to detect the difference between a Group 1 proportion (results before training), $\pi 1$ of 0.400 and a Group 2 proportion (results after training), $\pi 2$, of 0.670 (odds ratio of 3.045) when the sample size in each group is 60 cases [27].

Table 2. Characteristics of EUS-tissue training participants

Hospital	Profession	Age (years)	Female	Experience with EUS-FNA (years)	No. of EUS-FNA procedures performed annually
1	Doctor	42	No	12	100
1	Doctor	39	Yes	4	30
2	Nurse	24	Yes	2	300
2	Nurse	33	Yes	6	300
2	Nurse	22	Yes	2	300
2	Nurse	23	Yes	0	25
2	Nurse	30	Yes	0	30
3	Doctor	38	No	3	10
3	Doctor	35	Yes	1	25
3	Nurse	48	Yes	3	25
4	Doctor	44	Yes	10	50
4	Doctor	42	Yes	8	50
4	Nurse	48	Yes	11	92
5	Nurse	37	Yes	8	60
5	Doctor	49	No	7	50
5	Nurse	31	Yes	7	50
6	Nurse	29	Yes	5	40
6	Nurse	29	Yes	5	40
6	Nurse	47	Yes	0	45
6	Doctor	36	Yes	2	50
7	Doctor	39	No	1	60
7	Doctor	44	Yes	10	25

FNA: fine needle aspiration, no.: number.

RESULTS

Endoscopy staff characteristics

A total of 10 endosonographers and 12 endoscopy nurses attended the EUS-specimens-preparation-training. Participants were selected by the principal investigators of the participating centers, during a meeting in February 2016. If they had not received a formal EUS-sample-preparation-training previously, the study coordinator invited the participants by e-mail. Table 2 demonstrates the participants' characteristics. Majority of the trainees was female, with a median age of 38 (range 22-49). As only one of the centers was an academic hospital, most were working at a community hospital (77%). Experience with EUS-FNA ranged from several months to years. We consider our study population to be representative for, at least, the other regions in the Netherlands, since most regions in the Netherlands comprise an academic and several smaller hospitals. Furthermore, majority of today's medical staff comprises young to

Table 3. Characteristics of included cases and controls.

Variables	Controls (n=68)	Cases (n=71)	p-value
Center of inclusion, n (%)			
Albert Schweitzer	6 (9)	6 (9)	n.s.
Reinier de Graaf	12 (18)	15 (22)	
Erasmus MC	28 (41)	28 (39)	
Haga Hospital	3 (4)	3 (4)	
Ijsselland Hospital	6 (9)	6 (9)	
Maasstad Hospital	6 (9)	6 (9)	
Sint Franciscus Hospital	7 (10)	7 (10)	
Target lesion location, n (%)			
Head	39 (57)	34 (48)	0.003
Uncinate process	5 (7)	6 (9)	
Neck	9 (13)	4 (6)	
Corpus	9 (13)	14 (20)	
Tail	0 (0)	13 (18)	
Missing	6 (8)	0 (0)	
Target lesion size (mm), mean ± SD	28.7 ± 9.63	31.0 ± 1.37	n.s.
FNA needle size, n (%)			
19-gauge	3 (6)	1 (1)	0.016
22-gauge	31 (57)	27 (38)	
25-gauge	20 (37)	43 (61)	
Number of passes, median (IQR)	3.00 (2.00-3.00)	3.00 (2.00-3.00)	n.s.

N: number, mm: millimeter, SD: standard deviation, FNA: fine needle aspiration, n.s: non significant.

Table 4. Diagnostic outcome of SMEAR samples from cases versus controls.

Variables, n (%)	Cases (n=71)	Controls (n=68)	p-value**
Presence of artifacts	54 (76)	56 (82)	0.363
Type of artifacts*			
Poor fixation	3 (6)	3 (5)	1
Thick smear/clots	45 (83)	42 (75)	0.351
Cytolysis	25 (46)	30 (54)	0.567
Cellularity			
<50%	36 (51)	38 (56)	0.480
≥50%	35 (49)	30 (44)	
Sample diagnosis			
Impossible to determine	21 (30)	21 (31)	0.998
Benign	1 (1)	1 (1)	
Atypical/suspect for malignancy	13 (18)	12 (18)	
Malignant	36 (51)	34 (50)	
Gold standard diagnosis			
Benign	4 (6)	2 (3)	0.556
Atypical (NET, pancreatitis)	3 (4)	5 (7)	
Malignant	64 (90)	61 (90)	
Diagnostic accuracy for diagnostic classification Bethesda	51 (36)	47 (32)	0.667
Diagnostic accuracy for malignancy % (n/n)	66 (47/71)	66 (45/68)	0.998

*NET: neuroendocrine tumor, n: number, *more than one option possible, **generalized linear mixed model.*

middle-aged women, and exposure to EUS-FNA varied greatly, which corresponds well with exposure in the academic and non-academic centers.

Target lesion characteristics

71 cases and 68 controls were assessed (Table 3), with a mean lesion size of 31mm (SD± 1.37mm). Pancreatic corpus and tail lesions were somewhat over-represented in the control group (p=0.003, table 3). Most case lesions were sampled with a 25G needle (61%), while controls were mostly targeted with a 22G needle.

Smear quality

The presence of artifacts was comparable for smears prepared before and after the training session (76% versus 82%, p=0.363, table 4), as were individual types of artifacts. Also, for smear cellularity, there was no difference between cases and controls (p=0.480).

Smear diagnosis and accuracy

After a median follow-up time of 24 months (range 21-32), 70 (50%) of the smears were scored as malignant, 25 (18%) as atypical or suspect for malignancy, and 2 (1%) as benign. Smears were considered non-diagnostic in 42 lesions (30%). Gold standard diagnosis revealed 125 (90%) malignant lesions, 8 (6%) atypical lesions or suspect for malignancy (one IgG-mediated pancreatitis, two pancreatitis, five neuroendocrine tumors), and 6 (4%) benign lesions (three chronic pancreatitis, one fibrotic lesion, two non-specified benign lesions). Similar to FNA sample quality, tissue-preparation-training did not result in a significant increase in the diagnostic accuracy for malignancy (p=0.998) or the Bethesda classification (p=0.667, table 4).

DISCUSSION

With this pilot study, we aimed to evaluate the efficacy of an EUS-FNA-smear-preparation-training for endoscopy staff in centers lacking ROSE. Unfortunately, our training did not improve the smear quality or diagnostic accuracy in our regional EUS-working group. For this, several reasons may be found.

First of all, our training program may have been inadequate to achieve a significant improvement in the performance of the trainees. As official EUS-sample preparation-courses do not exist, we had to design our own program. We chose a comprehensive training, combining theoretical and practical hands-on elements. However, this program may have fallen short. It is, for example, well known that practical skills are better achieved after extensive training, and tend to grow with exposure. Therefore, it may have be more effective to intensify or repeat the training by one or more refresh sessions. In addition to this, the specimen collection period

may have been too short to allow trainees to gain sufficient experience, thereby improving their skills.

Secondly, it has been demonstrated that self-assessment and standardized feedback improves the learning curve for colonoscopy of Gastroenterologists in training [28]. Therefore, implementing standardized self-assessment forms could have increased the training effect. In addition, we could have implemented frequent multidisciplinary meetings of the trained endosonographers with the (cyto)pathologists. Such off-site feedback moments may further improve the learning curve for smear preparation.

Thirdly, our results might be inherent to the nature of the smear technique itself, since it is a manual method that is sensitive to artifacts and is prone to heterogeneous preparations. In contrast, cytological examination using a liquid-based medium (LBC), such as ThinPrep or cell block, has several advantages including less contamination by red blood cells, less drying artifacts [8].

A limitation of our study is that our power calculation was based on the training effect on our regional EUS-working group. Therefore, we could not assess the impact of the training on an individual basis. This prevents us from identifying trainees who did benefit from the training. It is known, that a learning curve can vary greatly between trainees. This has been shown for endoscopy and endoscopic retrograde cholangiopancreatography (ERCP) learning [29], and seems to have led to a more competence-based training schedule rather than a threshold number-based training for Gastroenterology residents [30, 31]. As our group comprised of endoscopy staff (both physicians and nurses) from high, medium and low volume centers with different levels of experience, differences in learning curves seem inevitable. Previous studies found that endosonographers performed equally well as compared to cytopathologists, but endoscopy nurses did not [22-25]. We did not power our study to compare the smear quality and accuracy between doctors and nurses.

Another limitation, one that hampers most EUS-FNA studies, is the inter-center variability in practice protocols. As we report in table 1, our centers use a variety of sampling and tissue preparation techniques. Although this may introduce a bias, today, this is inevitable in multicenter studies, as no consensus exists on the optimal sampling and tissue handling technique [15, 30, 32].

Furthermore, the endpoints that we used to measure EUS-FNA quality are not globally harmonized. The most important problem is that there are no uniform guidelines that advise on how to mark FNA sample diagnosis [33], and there is no consensus on how to describe sample quality. Therefore, quality definitions used in the current study were jointly created by the study group.

Taken all together, this pilot EUS-FNA smear-preparation-training for endoscopy personnel did not improve EUS-FNA smear quality or accuracy. Nevertheless, it stands to reason that endoscopy staff could benefit from some form of specimens-preparation-training, and perhaps an adjusted, more elaborate program will be more effective. However, optimization of smear

quality is only one link in the chain towards a higher diagnostic accuracy for EUS-FNA. Therefore, we also need to explore other strategies to achieve this.

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Chapter 8

Diagnostic yield and agreement on fine needle specimens from solid pancreatic lesions: comparing the conventional smear technique to liquid-based cytology

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Submitted

ABSTRACT

Introduction

The traditional 'smear technique' for the processing and assessment of endoscopic ultrasound guided fine-needle aspiration (EUS-FNA) is sensitive to artifacts. Processing and evaluation of specimens collected in a liquid medium, liquid-based cytology (LBC), may be a solution. We compared the diagnostic value of EUS-FNA smears to LBC in pancreatic solid lesions in the absence of rapid on-site evaluation (ROSE).

Methods

Consecutive patients, who required EUS-FNA of a solid pancreatic lesion were included in seven hospitals in the Netherlands, and followed for at least 12 months. Specimens of the first pass were split into two smears and a vial for LBC (using ThinPrep and/or cell block). Smear and LBC were compared in terms of diagnostic accuracy for malignancy, sample quality, and diagnostic agreement between three (cyto)pathologists.

Results

Diagnostic accuracy for malignancy was higher for LBC (82% (58/71)) than smear (66% (47/71), p=0.04), but did not differ when smears were compared to ThinPrep (71% (30/42), p=0.56) or cell block (62% (39/63), p=0.61) individually. Artifacts were less often present in ThinPrep (57% (24/42), p=0.02) or cell block samples (40% (25/63), p<0.001) than smears (76% (54/71)). Agreement on malignancy was equally good for smears and LBC (κ=0.71 versus κ=0.70, p=0.98), but lower for ThinPrep (κ=0.26, p=0.01) than smears.

Conclusion

LBC provides a higher diagnostic accuracy than the conventional smear technique for EUS-FNA of solid pancreatic lesions in the absence of ROSE. Therefore, LBC, especially in EUS-centers lacking ROSE, is a better alternative for handling FNA samples than the smear technique.

INTRODUCTION

Pancreatic cancer is one of the most lethal solid tumors [1, 2], but individualized therapies have improved progression free survival [3, 4]. As these therapies depend on pre- therapeutic tissue analysis [5], endoscopic ultrasound (EUS) guided tissue collection is increasingly being used for this purpose.

Although EUS-guided tissue sampling can reach diagnostic accuracy rates over 90%, its outcome strongly depends on performer skills, sampling tools and techniques, and tissue processing [6]. Traditionally, fine-needle aspiration (FNA) needles have been used to collect cytological samples, which were smeared onto glass slides, the so-called smear technique. This technique is cheap, easy to use and available to the majority of the EUS-centers [7]. The downside of smears is that they are very sensitive to preparation artifacts [8, 9]. A dedicated on-site pathologist (ROSE) can improve smear quality and hence diagnostic accuracy. However, in many EUS-centers ROSE is not readily available due to costs and logistic issues [7]. As a result, FNA samples are often handled by the endoscopy staff, with varying diagnostic outcomes [10-13].

An alternative for ROSE is to collect FNA samples in a liquid-based medium, the so-called liquid-based cytology (LBC) technique. This technique makes samples less vulnerable to contamination or artifacts, as debris, blood and exudates can easily be removed [14]. There are different LBC techniques, i.e. ThinPrep, Surepath, Cellprep plus, and cell block. LBC slides mimic the in situ 3-dimensional tissue architecture and provide a homogeneous cell dispersion. They also allow pathologists to perform ancillary tissue tests that could previously only be performed on histological samples.

Although, LBC is more accurate than the conventional smears for the cytological diagnosis of cervical, bile duct and gall bladder cancers [15, 16], its superiority for pancreatic cancer has not been proven. The outcome of studies that compared smear to LBC for pancreatic lesions vary greatly, and are difficult to compare due to heterogeneity in the used LBC techniques (i.e. ThinPrep, Surepath, Cellprep plus, and cell block) [9, 17-26]. As the ThinPrep and cell block technique are two commonly used LBC techniques, we compared the diagnostic performance of these techniques to the conventional smear technique for the processing of FNA specimens from solid pancreatic lesions, in the absence of an on-site pathologist.

METHODS

Study design and patient selection

This prospective multicenter study compared EUS- sample processing using the smear and LBC technique in terms of diagnostic accuracy, sample quality, and agreement on these parameters. Consecutive patients, scheduled for EUS-FNA of a suspected solid pancreatic malignancy were included in a tertiary referral center and six regional community hospitals in the Netherlands,

between April 2016 and September 2017. Patients were followed for at least 12 months, until September 2018. Prior to the study, the endoscopy personnel underwent a one-day EUS-FNA-tissue-preparation-training, to optimize their knowledge and skills. All harvested and prepared FNA samples were collected and reviewed by an expert cytopathologist and two experienced cytotechnicians of the pathology department at the Erasmus MC University Medical Center in Rotterdam, the Netherlands. The Medical Ethics Committee reviewed the study and granted a waiver of consent as the protocol did not interfere with local EUS-FNA sampling protocols (MEC-2016-022).

EUS-guided tissue sampling

All EUS-FNA procedures were performed according to a standard protocol, using a convex array echoendoscope (Pentax EG-3870 UTK, Pentax EG-3270 UK; Pentax, Tokyo, Japan, Olympus UTC 140/180, Olympus linear GF-UCT180; Olympus, Tokyo, Japan, table 1). Tissue sampling was performed by endosonographers who were formally trained for at least 1 year at a tertiary referral center, have had 1-20 years of EUS experience, and perform at least 25 EUS-guided tissue sampling procedures annually. Patients were sampled using a 19, 22- or 25-gauge FNA needle (EchoTip; Cook Medical or Expect; Boston Scientific). The number of passes, sampling technique, and use of additional techniques (e.g. applying negative suction with a syringe) were at the discretion of the performer.

Specimen handling

EUS-FNA specimens of the first pass were split into two smears (glass slides) and a vial that was processed as LBC. Smears were performed using the 'sandwich method' [27]. LBC was processed using thin layer preparation (ThinPrep®, (Hologic) and/or the cell block technique (Cellient™ automated cell block system (Hologic), the Agar technique, or Aalfix cell block, depending on local tissue handling protocols (Table 1). Subsequent passes were handled according to local standards and not included in the study. Smears and LBC were prepared on-site, by the endoscopy personnel (endoscopy nurse or endosonographer). On-site pathological assistance was only allowed after the first pass, once study material was collected.

Sample reviewing

All study samples were anonymized and sent to the Erasmus MC University Medical Center in Rotterdam for review by an expert cytopathologist and two cytotechnicians who were specialized in pancreaticobiliary diseases. Reviewers were blinded for the final clinical and pathological outcome. Sample assessment and scoring were done individually by the reviewers. Case discussion was not allowed. Smears, thin layer samples and cell blocks were analyzed consecutively.

Table 1. EUS-guided FNA and tissue processing specifics per center

Center	EUS-scope type	Annual EUS-FNA per endosonographer	ROSE available	Additional techniques	SMEAR preparation	Liquid cytology medium	Thin-layer cytology technique	cell block technique
Albert Schweitzer Hospital, Dordrecht	Olympus linear GF-UCT180	25	Yes	Slow pull or Suction	Air dry, Hemocolor	Cytolyt	ThinPrep	Cellient Hologic
Reinier de Graaf Hosptial, Delft	Olympus linear GF-UCT180	30	No	Slow pull	Air dry No stain	Cytolyt, or Polytransportbuffer*	ThinPrep	Agar
Erasmus MC University Medical Center Rotterdam	Pentax EG-3870 UTK Olympus UTC 140/180	50	Yes	Slow pull or Suction	Air dry Diff quick	Cytolyt	ThinPrep	Cellient Hologic
Haga Hospital, The Hague	Olympus linear GF-UCT180	25	Yes	Slow pull	Air dry Diff quick	Formalin	None	Paraffin cell block
Ijsselland Hospital, Rotterdam	Olympus linear GF-UCT180	25	Yes	Slow pull	Air dry Diff quick Giemsa	CytoRichRed	None	Agar
Maasstad Hospital, Rotterdam	Pentax EG-3270 UK Olympus linear GF-UCT180	30	No	Slow pull	Air dry Diff quick	CytoRichRed	None	Aalfix cell block*
Sint Franciscus Hospital, Rotterdam	Pentax EUS-scope	20	No	Slow pull or Suction	Air dry No stain	CytoRichRed	None	Agar

*medium/technique developed locally.

Endpoints, scoring variables and definitions

The primary endpoint was the comparison of the diagnostic accuracy of the conventional smear method to the LBC technique of FNA-specimens from solid pancreatic lesions. Sample diagnosis was based on the Bethesda classification, and scored as non-diagnostic, benign, atypical, or malignant [28]. The reviewing expert cytopathologist determined the final sample diagnosis. Gold standard diagnosis was based on the surgical resection specimens in operated patients, or on a compatible clinical disease course during a 12-month follow-up period. Solid-pseudopapillary neoplasms (SPN) and NET grade 2 and 3 were classified as malignant [29, 30].

Secondly, we compared sample quality, defined as sample cellularity (< or >50% target cells) and presence of preparation artifacts, such as poor fixation, thick smear/clots, obscuring blood or inflammation, or cytolysis (no/yes). In addition, we compared the interobserver agreement on sample diagnosis and quality amongst the three reviewers between the two techniques.

Other parameters that were scored included needle size, target lesion characteristics (location, size), the number of needle passes performed, type of LBC medium used, and procedure related complications (pancreatitis, infection, bleeding, other).

Statistics

Diagnostic accuracy and sample quality were compared between the smear and LBC technique, and were analysed using logistic mixed effects models [31] with subject and study center specific (random) intercepts. This method allows to take into account the clustering structure of this multicenter trial, i.e., that observations from the same study center may be correlated. Separate models were fitted for the comparison of SMEAR vs LBC and SMEAR vs ThinPrep vs cell Block. Statistical significance was established as $p<0.05$ (two-tailed).

Inter-observer agreement amongst reviewers was calculated using kappa statistics [Fleiss' κ-statistic and 95% confidence intervals (CIs)]. κ- statistics were interpreted according to convention of Landis and Koch; <0, no agreement; 0-0.20 slight agreement; 0.21-0.40, fair agreement; 0.41-0.60, moderate agreement; 0.61-0.80, substantial agreement; and 0.81-1.0; almost perfect agreement. Because not all samples were evaluated for both LBC methods, ThinPrep and cell block, some of the ratings were missing. To compare agreement coefficients, the coefficient was then calculated based on the samples for which all ratings of the methods in the current comparison were available. In settings where the agreement coefficients of three methods were compared, three pairwise tests were used and p-values were corrected for multiple testing using Holm's procedure [32]. For this, the p-values presented in this manuscript have been multiplied by the number of comparisons. Analyses were carried out using R version 3.5.1 [33], and SPSS version 23, Statistical Package for the Social Sciences, SPSS Inc., Chicago, Illinois.

Power calculation

To determine the power needed for this study, we first performed a pilot study to assess the diagnostic accuracy for malignancy for pancreatic EUS-FNA specimens prepared using the

smears and LBC method in het Erasmus MC University Medical Center. A difference in diagnostic accuracy of 20% between smear and LBC was found, and considered clinically relevant. We estimated that to find such a difference, a sample size of 59-72 pairs will have 80% power to detect a difference in proportions of 0.250 when the proportion of discordant pairs is expected to be between 0.500-0.600 and the method of analysis is a McNemar's test of equality of paired proportions with a 0.050 two-sided significance level.

RESULTS

Case characteristics

A total of 71 cases were included, of which lesion and sampling characteristics are listed in table 2. No procedure related complications were recorded. Final diagnosis comprised 64 (90%) malignancies, 3 (4%) atypical cases, including 2 NETs and 1 case of pancreatitis, and 4 (6%) benign cases. This diagnosis was based on resection specimens in 19 (29%), additional tissue biopsy (i.e. peritoneal, brain, lymph node biopsy) in 13 (20%), and follow-up in 33 (51%) cases.

Table 2. Case characteristics.

Variables	Cases (n=71)
Target lesion location, n (%)	
Head	34 (48)
Uncinate process	6 (9)
Neck	4 (6)
Corpus	14 (20)
Tail	13 (18)
Target lesion size (mm), mean ± SD	31.0 ± 1.37
FNA needle size, n (%)	
19-gauge	1 (1)
22-gauge	27 (38)
25-gauge	43 (61)
Number of passes, median (IQR)	3 (2 - 3)
Gold standard diagnosis	
Benign	4 (6)
Atypical (NET, pancreatitis)	3 (4)
Malignant	64 (90)

n: number, mm: millimeter, SD: standard deviation, IQR: interquartile range, NET: neuroendocrine tumor.

Diagnostic accuracy and sample quality for smear versus LBC

Overall, diagnostic accuracy for malignancy of the first pass was 86% (61/71). Accuracy was higher for samples processed using LBC than with the conventional smear technique (82% versus 66%, OR 2.62 95% CI 1.13-6.79, p=0.03). Overall diagnostic accuracy based on Bethesda was 80% (57/71). For this classification, smears and LBC performed equally well (51% versus

59%, OR 1.44 95% CI 0.73-2.92, p=0.30). Comparing the diagnostic accuracy for malignancy and the Bethesda classification of smears to both LBC techniques individually did not result in a significant difference in diagnostic accuracy (Table 3). Cell block had lower sample cellularity than smear (OR 0.39 95% CI 0.18-0.82, p=0.01, table 4), but there was no clear evidence of a difference between ThinPrep and smear (OR 0.51 95% CI 0.21-1.16, p=0.11). Sample quality, in terms of artifacts, was better for both LBC techniques as compared to the smears (Table 4).

Table 3. Overall diagnostic accuracy, and per tissue processing technique compared to smear.

Tissue processing technique	Accuracy for malignancy n (%)	OR (95% CI)	p-value	Accuracy for Bethesda n (%)	OR (95% CI)	p-value
Overall (n=71)	61 (86)			57 (80)		
Smear (n=71)	47 (66)	1.92 (0.75-4.83)	*	36 (51)	1.03 (0.62-1.71)	*
LBC (n=71)	58 (82)	2.62 (1.13-6.79)	0.03	42 (59)	1.44 (0.73-2.92)	0.30
ThinPrep (n=42)	30 (71)	1.29 (0.52-3.26)	0.59	26 (62)	1.61 (0.74-3.76)	0.24
cell block (n=63)	39 (62)	0.78 (0.78-1.69)	0.53	22 (35)	0.51 (0.24-1.03)	0.07

*reference category

Table 4. Sample quality per tissue processing technique, compared to smear.

Tissue processing technique	Artifacts n (%)	OR (95% CI)	p-value	Cellularity n (%)	OR (95% CI)	p-value
Smear (n=71)	54 (76)	4.09 (1.54-15.16)	*	35 (49)	0.97 (0.43-2.04)	*
LBC (n=71)						
ThinPrep (n=42)	24 (57)	0.32 (0.12-0.82)	0.02	14 (33)	0.51 (0.21-1.16)	0.11
cell block (n=63)	25 (40)	0.15 (0.05-0.35)	<0.001	18 (29)	0.39 (0.18-0.82)	0.01

*reference category

Diagnostic agreement for smear vs LBC

The diagnostic agreement amongst the cytopathologist and the two cytotechnicians was equally good for identifying malignancy in smears (κ=0.71, 95% CI 0.57-0.84) and LBC samples (κ=0.70, 95% CI 0.55-0.86, p=0.98). The same was true for their agreement on the Bethesda classification (κ=0.70, 95% CI 0.57-0.83 vs κ=0.64, 95% CI 0.50-0.78, p=0.55). When ThinPrep (κ=0.26, 95% CI 0.04-0.48) and cell block (κ=0.79, 95% CI 0.66-0.92) were assessed separately, agreement on the presence of malignancy was comparable for cell block and smears (κ=0.79 vs. κ=0.73, adjusted p=0.53, figure 1), but lower for ThinPrep than smears (κ =0.261 vs κ =0.640, adjusted p=0.04). Similar results were found for the Bethesda classification (Figure 1). Agreement on the presence of artifacts was low for all processing techniques, and did not differ significantly between the processing techniques (Figure 2). Agreement on cellularity was highest for cell block (κ=0.64, 95% CI 0.48-0.81) and smears (κ=0.60, 95% CI 0.46-0.75), and lowest for ThinPrep (κ=0.35, 95% CI 0.14-0.56).

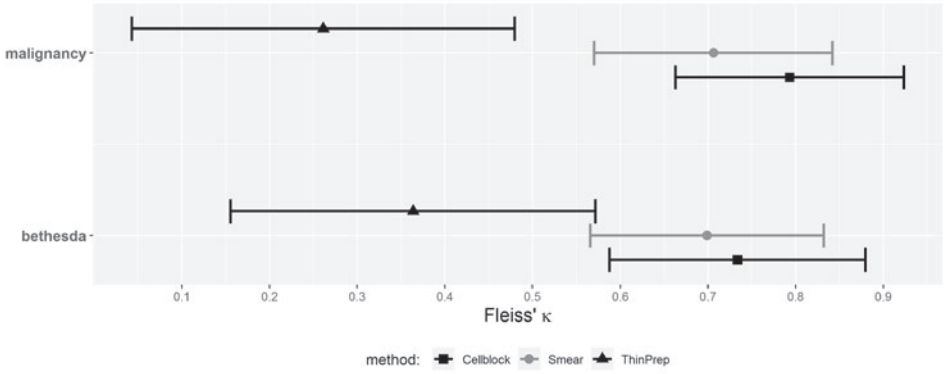

Figure 1. Agreement on diagnostic accuracy of malignancy and the Bethesda classification for the smear, ThinPrep and cell block technique.

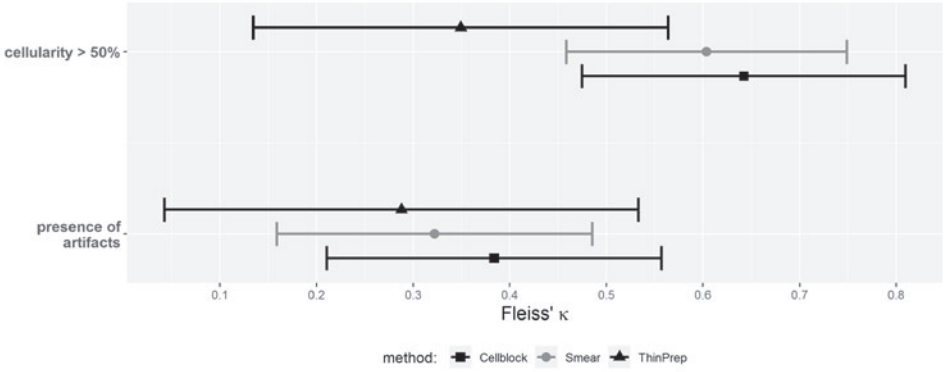

Figure 2. Agreement on sample cellularity and presence of artifacts for the smear, ThinPrep and cell block technique.

DISCUSSION

Liquid-based cytology using ThinPrep and cell block provides a higher diagnostic accuracy than and a comparable agreement compared to the conventional smear technique for FNA specimens from solid pancreatic lesions in the absence of an on-site pathologist. LBC is therefore a good alternative for the smear technique in the absence of ROSE. The higher diagnostic agreement for cell block than ThinPrep advocates for the implementation of the cell block technique for LBC.

The first explanation for the higher diagnostic accuracy of LBC than smear seems to be its lower artifact rate. It is generally accepted that smears are vulnerable to preparation artifacts, which induces interpretation errors, and may result in a lower diagnostic accuracy [25]. Despite the fact that the endoscopy staff in the current study participated in a smear-preparation-

training to optimize their performance before initiation of the study, 76% of the smears still contained artifacts. This was much higher than artifact rate for cell block (40%) and ThinPrep samples (57%).

Besides a low artifact rate, the histology-like look of cell block samples likely contributes to an easier interpretation and matching interobserver agreement. It has previously been reported that pathologists prefer histology or cell block over conventional cytology preparation, as its appearance is much closer to the in situ tissue architecture [8]. Furthermore, LBC allows for additional testing, such as immunohistochemistry, which may be deciding in challenging diagnostic cases such as auto-immune pancreatitis, or the differentiation between metastatic or primary disease. Although agreement was higher for cell block than ThinPrep, it should also be taken into account that special training of cytotechnicians and pathologists is prerequisite for accurate interpretation of these different LBC techniques [8]. Therefore, choosing the optimal LBC technique will depend upon the preference and experience of the local pathologists.

The finding that sample cellularity was lower for LBC than smears does not seem to match with its high diagnostic accuracy and agreement. It may be explained by the more homogeneous cell dispersion of LBC samples. This allows for better assessment of cell morphology, but may give the impression of a less 'cellular sample'. On the other hand, highly cellular smears may be scored as containing more than enough target cells, but if cells are packed in thick layers, this only hampers the interpretation. Despite the lack of a clear definition of 'FNA sample cellularity', a higher cellularity has been associated with a higher DNA yield for molecular testing [14]. Therefore, it is crucial to determine the specific purpose of EUS-guided tissue collection in advance, and discuss this with the involved pathologist.

It is challenging to compare our findings to previous reports, since EUS-FNA protocols and tissue handling and processing techniques vary greatly. So far, 11 studies have compared the smear to the LBC technique for solid pancreatic lesions [9, 17-26]. Six of them reported a higher diagnostic accuracy for smears than LBC [9, 18, 21-23, 26]. Half of these studies used ROSE [18, 21, 22]. Overall, only three of the eleven studies that compared smear to LBC were performed without ROSE [9, 20, 26]. Of these studies, two found a benefit of smear over LBC [9, 26], and one found a benefit of LBC, using another ThinPrep-like solution (Surepath)[20]. Each study used different ThinPrep solutions, limiting a direct comparison with our results. Of the studies that reported a diagnostic benefit for LBC, two out of three used the cell block rather than the ThinPrep technique, which seems to correspond with our findings [17, 24, 25]. Lastly, none of the above-mentioned studies assessed diagnostic agreement on the different techniques.

Compared to other studies, our overall diagnostic accuracy rate of 86% is rather high, considering the fact that material was collected from the first needle pass only. Previous studies mostly based their results on several passes. Moreover, we split the material from this first pass for smear and LBC. As a result, our samples likely contained less material as compared to other study settings. Therefore, our diagnostic accuracy rates underestimate the true diagnostic accuracy rates in our practices. Furthermore, the diagnostic accuracy of each preparation

technique alone was somewhat, lower than LBC overall. The most likely explanation for this is that ThinPrep and cell block are complementary techniques, that provide samples with a different phenotype and diagnostic possibilities.

Our study has some limitations. An important limitation of studies on EUS-guided tissue sampling is the lack of uniform guidelines on the optimal sampling and tissue handling techniques. Therefore, the resulting inter-center variation should always be considered, and may hamper general extrapolation of our findings. Secondly, we did not power our study to perform additional, subgroup analysis. Furthermore, although the participating endosonographers who performed the smears participated in a hands-on-FNA-tissue-preparation training, their experience cannot be explained to that of on-site pathologists. Therefore, this may have limited the diagnostic accuracy of the smears. Another limitation is that the reviewing pathology staff could not be blinded for the processing technique, as their appearance differs accordingly. Furthermore, we did not perform a cost-effectiveness analyses, due to the differences in local EUS-protocols between the participating centers.

In conclusion, in the absence of an on-site pathologist, diagnostic accuracy of EUS-FNA of solid pancreatic lesions can be increased with the LBC technique as compared to the conventional smear technique. As LBC provided for a higher diagnostic accuracy and a comparable interobserver agreement than smears, it should be routinely implemented in EUS-centers lacking ROSE. The higher agreement for cell block advocates for the implementation of cell block rather than ThinPrep. However, providing the optimal EUS-tissue sampling depends on many factors, including experience and skills of the involved endoscopy and pathology team, and starts with the determination of the diagnostic or therapeutic purpose of tissue acquisition.

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Part IV

Summary, general discussion and appendices

Chapter 9

General summary and discussion

GENERAL SUMMARY AND DISCUSSION

This thesis explores how to improve the diagnostic outcome of Endoscopic ultrasound (EUS)-guided tissue sampling. First, it gains insight in the current international practice patterns of endosonographers. Then, it focusses on the performance of different EUS-sampling devices. We compared the diagnostic performance of a fine needle aspiration (FNA) and fine needle biopsy (FNB) device, their diagnostic reproducibility, and the value of their combined use, in a prospective randomized manner. In addition, a meta-analysis was performed, mutually comparing FNA and the novel-generation FNB devices, comprising the ProCore (Cook Medical), SharkCore (Medtronic) and Acquire (Boston Scientific) needles. In the third part of the thesis, we explore if EUS-FNA tissue preparation techniques can be optimized by training endoscopy personnel and by comparing specimen processing with liquid based cytology (LBC) to the conventional glass-smear technique.

Current practice in EUS-guided tissue sampling

EUS-guided tissue sampling is increasingly being used, due to its high diagnostic accuracy, minimal invasive character, and good tissue yield, which is of major importance in the current era of patient tailored medicine [1]. Because of its increased use, the technique is continuously evolving. This leaves the optimal sampling strategy a subject of debate, and clinicians with limited evidence for their daily practice [2-6].

In **chapter 2,** an online survey aimed to map the practice patterns for EUS-guided tissue sampling. We found considerable intercontinental differences and deviations from the guidelines. According to the questioned American endosonographers, their all-round use of propofol rather than conscious sedation may be explained by marketing strategies of anaesthesiologists [7-9]. If this practice pattern will spread to Europe, procedure costs are expected to rise [7, 10, 11]. Another difference was the use of periprocedural acetylsalicylic acid, which was generally continued in the United States (US), according to the guidelines, in contrast to Europe and Asia. In Asia, this may be explained by the believe that Asians are more susceptible to bleeding complications [12]. As for the use of rapid on-site pathological evaluation and preparation of the collected tissue (ROSE), our survey reported that it was used by virtually all respondents from the US, while in Europe and Asia, it was only available in half of the centers, due to cost issues and disbelief in its added value. Indeed, a recent meta-analysis could not proof its benefit and the European Society of Gastroenterology (ESGE) guidelines do not recommend its use [13].

Histology was uniformly stored in formalin. The preferred preservation fluid for cytological specimens, however, differed considerably. Asians generally use alcohol or saline, while European and US practitioners use Cytolyt. This reflects the lack of recommendations on preservation and specimen handling by the current guidelines. A practice pattern that did not differ between continents was the preferred needle size and number of passes. The survey identified the 22-gauge (G) needle as the preferred size for both FNA and FNB [14-17], and participants

reported to perform less passes with FNB than FNA. Although at the time of the survey, this was not yet recommended, the updated ESGE guidelines indeed advise to perform less passes with FNB [2-3] than FNA [3-4], in the absence of ROSE [13].

The optimal EUS-sampling device

As we discussed in the introduction of this thesis, EUS-needles are, and always have been a major target of innovation in EUS-guided tissue sampling. FNB devices were introduced to overcome the limitations of FNA, mainly by providing histological specimens rather than loose target cells. However, their diagnostic performance was not convincingly better than the FNA needles [18-22]. Some reported that FNB not so much harvested histological tissue fragments, but just improved the yield of loose target cells [18, 23-29]. Others claimed that histology can also be obtained with the conventional FNA needles [18, 30, 31]. Lastly, new tissue preparation techniques, such as cell block, allow for a 'histology like' analysis of cytological material.

The first FNB devices, TruCut^{tm} (Travenol Laboratories) and Quick-Core$^{®}$ (Cook Medical), were hampered by a rigid design and difficult deployment. In response, several novel FNB needles have been introduced in the last years, including the ProCore reversed and forward facing bevel, SharkCore, and Acquire needles. As the design of these needles significantly differs from the first FNB devices and from each other, there is a growing interest in their diagnostic performance.

The 20G ProCore forward facing bevel needle was introduced first, in 2015, and its diagnostic performance has been extensively assessed. The largest study available is the international multicenter trial that is presented in **chapter 3**. In this trial, the ASPRO study (ASpiration versus PROcore), we compared the ProCore forward facing FNB needle to a commonly used conventional FNA needle, the 25G EchoTip Ultra Needle (Cook Medical), which was chosen because of its optimal flexibility and yield [2, 13].

We demonstrated that this 20G FNB needle achieves a higher diagnostic accuracy and tissue core yield within less passes than FNA. These findings equally applied for pancreatic and non-pancreatic lesions, and were irrespective of target lesion size, number of needle passes, and presence of an on-site pathologist. Moreover, despite of inter-center differences, the benefit of the new FNB needle was consistent amongst all 13 participating centers, supporting the general applicability of these findings.

The better performance of the new 20G FNB needle seems related to its design adaptations, which include a large diameter, a flexible needle sheet, a forward facing rather than a reversed bevel, and a Menghini rather than a lancet tip, which decreases resistance during tissue traversing. The fact that a large-bore FNB needle outperformed a thinner FNA needle is an important observation, because it counters the conception that the performance of larger-bore needles is hampered by their suboptimal performance in an angulated scope-position.

The better overall performance of the FNB needle was shown to be center independent, but we were well aware that all participating centers were high volume centers. To assess the

reproducibility in non-expert hands, we performed a second study. As described in **chapter 4**, we compared the diagnostic agreement between academic and non-academic pathologists. The first 125 pancreatic and lymph node cases, enrolled in the original ASPRO trial, were re-assessed by 5 academic and 5 non-academic pathologists from different countries. The diagnostic agreement on the final diagnosis was higher for FNB samples than for FNA, amongst expert academic as well as non-academic pathologists. Logistic regression analysis further showed that the pathologist's provenance (academic or non-academic) did not influence diagnostic accuracy. This endorses the use of the novel 20G FNB needle in academic as well as non-academic centers.

The most likely explanation for the better agreement on the FNB samples is their higher tissue core rate, which was positively associated with a better diagnostic agreement. Furthermore, the cytological yield of FNB was also higher than for FNA, but only the availability of tissue cores, and not cytology, contributed to a better diagnostic accuracy. Another quality parameter that seemed to contribute to the high diagnostic accuracy and agreement on samples obtained with FNB is the low sample artifact rate. Not only did the present study show a decrease in accuracy when artifacts were present, previous studies have also reported that artifacts may hamper ancillary testing [32]. Interestingly, agreement on the presence of artifacts was low for both needles (although slightly better for FNB than FNA). This is in line with the fact that agreement on all sample quality parameters was low in the current study, similar to reports from others [33, 34]. This likely results from the lack of uniform outcome definitions regarding the performance of EUS-guided tissue sampling.

To further explore the optimal sampling approach, in **chapter 5,** the role of combined FNA and FNB use is assessed. The benefits of an FNA needle; optimal flexibility to reach a target lesion and the possibility to perform on-site cytological evaluation, may complement the benefits of FNB; collection of histological tissue cores to optimize the diagnostic yield and harvest enough tissue for ancillary testing. To explore the incremental diagnostic yield of dual sampling, all ASPRO cases that were sampled with both needles during the same procedure were included. This resulted in 24 patients who were first sampled with the 25G FNA needle, and 49 cases who were first sampled with the 20G FNB device. Interestingly, dual sampling only improved diagnostic accuracy for malignancy if FNA was followed by sampling with FNB and not vice versa. The previously reported diagnostic benefit of the FNB over the FNA needle may very well explain why FNA followed by FNB resulted in a higher diagnostic accuracy than FNA alone, and also explains the limited value of the reversed approach. However, FNB may have caused blood contamination of subsequent specimens, or "tracking" within the target lesion, impeding the FNA needle from finding its own diagnostic route. Last, secondary FNA was mostly used to allow for ROSE. However, since the incremental yield of ROSE is questionable in academic high volume centers, the benefit of FNA to allow for ROSE is expected to be limited [35-37].

In **chapter 6** we zoom out from the diagnostic performance of the two specific needles and present an updated meta-analysis on the performance of all currently available EUS-FNA

and FNB needles. EMBASE, MEDLINE/PubMed, Web of Science, the Cochrane Library, and Google Scholar were systematically searched for studies comparing FNA to FNB. We included randomized controlled trials of at least 50 patients, and extracted data on diagnostic accuracy, adequacy, number of passes, presence of tissue cores, and adverse events. Study quality was assessed based on the QUADAS-2 tool.

The recent wave of publications enabled us to include a significant number of new trials that compare FNA to FNB, and limit our evaluation to studies of high quality, as compared to previous meta-analyses [18-22]. Our analysis is the first to demonstrate a diagnostic benefit of FNB over FNA for pancreatic as well as non-pancreatic lesions, in terms of diagnostic accuracy, the number of needle passes performed, and tissue core yield. The adverse event rate was equally low for FNA and FNB. Other than diagnostic accuracy, sample adequacy did not differ between FNA and FNB. Sample adequacy or cellularity may be of less importance for a correct diagnosis than the presence histological tissue cores. Despite the use of new cytological preparation techniques like ThinPrep and cell block and the abundant use of ROSE, diagnostic accuracy was still better for FNB than FNA.

With regard to the general applicability of our results, it should be noted that there was a significant heterogeneity amongst the included studies. This is mainly due to the diversity in EUS-sampling protocols, and the inconsistent use of outcome definitions. Consequently, the outcomes of EUS-device studies remain difficult to interpret and should be extrapolated with caution.

Although the current analysis showed that FNB convincingly outperforms FNA, all included trials used the ProCore needle for FNB. As the use of the other new FNB needles, such as the Acquire and SharkCore needle, is increasing, we eagerly await head to head comparison studies between different FNB devices. Preferably, these should be (international) multicenter randomized controlled studies. According to ClinicalTrials.gov, a significant number of interesting studies investigating the performance of the novel generation of FNB devices is currently running.

Improving EUS-specimen preparation and handling

As mentioned in the previous section of this discussion, new FNA-tissue preparation techniques like ThinPrep and cell block increasingly enable pathologists to perform ancillary testing on cytological specimens. These techniques have been introduced to improve FNA-sample quality and accuracy, since the traditional, so called, smear-technique, is highly sensitive to preparation and contamination artifacts. Another way to solve this issue is to invest in a dedicated, on-site pathologist to handle the collected tissue. However, most centers omit this type of service. Therefore, EUS-FNA specimens are often prepared by the endoscopy staff, generally without a specialized training.

Chapter 7 explores if a one-day-hands-on tissue preparation training for endoscopy staff could improve sample quality and thus diagnostic accuracy of EUS-FNA in centers lacking ROSE. We performed a prospective pilot study, for which we invited 10 endosonographers and 12

endoscopy nurses from 7 regional EUS-centers in the Netherlands. Participants were educated on pancreas pathology, common diagnostic pitfalls, and the cause and prevention of smear-preparation-artifacts. Subsequently, they practiced smear preparation under the supervision of a team of academic (cyto)pathologists. After the training, 71 FNA-smears were prospectively collected from solid pancreatic lesions and compared to an equal number of pre-training 'control' slides.

Unfortunately, smear quality and diagnostic accuracy did improve after the training. The sample size did not allow us to assess individual results, to identify any trainees that did benefit from the training. There may be several reasons for the limited effect of our pilot-training. First, the training program may have been too short. As practical skills are better achieved after extensive training and tend to grow with exposure, it might have been more effective to intensify or repeat the training with one or more refresh sessions. In addition, the study period may have been too short to allow trainees to gain sufficient experience. Last, the lack of quality improvement may also be inherent to the nature of the smear technique itself, since it is a manual method that is sensitive to artifacts and prone to heterogeneous preparations. In contrast, cytological examination using LBC, i.e. ThinPrep and cell block, are associated with less contamination and drying artifacts [38].

Chapter 8 compares the diagnostic benefit of LBC to the conventional smear technique for the diagnosis of solid pancreatic lesions. The participating EUS personnel had been trained in the course of the previously described study. Two smears and a vial for ThinPrep and/or cell block were prepared from the first FNA-pass, without use of ROSE, and compared in terms of diagnostic accuracy for malignancy, sample quality, and diagnostic agreement between three (cyto)pathologists. The diagnostic accuracy was higher with the LBC technique as compared to the smear technique. However, this was only true when the yield of ThinPrep and cell block were collated. Artifacts were less present in both LBC techniques. The diagnostic agreement was comparable for LBC and smears, with the highest agreement on cell block samples. Given its higher accuracy and comparable agreement, LBC could be an alternative for centers lacking ROSE, especially cell block.

Future perspectives and recommendations

The historical line between FNA and FNB is fading with the current pool of EUS-devices containing FNA and FNB needles of comparable sizes and flexibility. To complicate things even further, needle designs differ significantly within the FNA and FNB groups. Therefore, we propose to discard the distinctive nomenclature of FNA and FNB, and instead, focus on the performance of individual EUS-sampling devices.

The current thesis provides significant new information that may be used to improve EUS-guided tissue sampling. However, there is room for further development, as the updated meta-analysis in this thesis found that the diagnostic accuracy for traditional FNA ranged between 67% and 100% and for FNB between 69% and 100%. This thesis states that an FNB needle

outperforms FNA, and should be the first-choice device for EUS-guided tissue acquisition, independent of the type of solid target lesion. However, before any specific needle FNB type or design can be recommended, newer generation FNB needles need to be assessed.

Ideally, one would want to compare each available EUS-sampling device head to head, but this is challenging. First of all, due to the many available FNA and FNB sizes and designs this will be time consuming but not impossible. Other complicating issues that make it difficult to compare the result are the heterogeneity in EUS-sampling protocols and lack of uniform outcome definitions. As long as there is no evidence which sampling strategy is optimal, it will be difficult to standardize practice patterns. To overcome some of the issues, uniform outcome definitions need to be created. For this there may be a role for the European and American Societies for Gastroenterology, as was the case for the diagnostic classification of pathology samples by the Papanicolaou Society.

In addition to the optimal EUS-needle design, this thesis also focused on the optimal tissue preparation of FNA specimens. One may question if future research should continue to focus on this, since our study proved FNB to be superior to FNA. However, we did not directly compare FNA specimens in liquid-based cytology to FNB cores in formalin. It would be interesting to compare this in an international multicenter setting. Furthermore, it should be noted that tissue collection for liquid preparation techniques is easy for the endosonographer, but requires a well-equipped pathology laboratory and trained personnel. Therefore, introducing and implementing novel techniques and innovations for EUS-guided tissue sampling should always be done in close cooperation with the pathology department.

Lastly, in light of ever-increasing burden of health-care costs, it is crucial that, before making any recommendation regarding the most optimal EUS-guided sampling technique and tool, cost-effective analyses should be performed.

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Chapter 10

Nederlandse samenvatting en discussie

NEDERLANDSE SAMENVATTING EN DISCUSSIE

Dit proefschrift onderzoekt hoe de diagnostische opbrengst van endo-echografisch (EUS)-geleide weefselafname kan worden verbeterd. EUS is een techniek waarbij men een inwendige echo maakt, middels een flexibele endoscoop met aan het uiteinde een echoapparaat. Via een speciaal kanaal kunnen instrumenten, zoals naalden of biopteurs, worden ingebracht. Dit maakt het mogelijk om – echogeleid – weefsel af te nemen van verdachte gebieden in organen rondom het maagdarmstelsel.

Het eerste deel van dit proefschrift geeft inzicht in de huidige toepassing van EUS-geleide weefselafname en welke hulpmiddelen en technieken hierbij worden gebruikt. Het tweede deel richt zich op de verschillende naalden voor weefsel afname. We vergeleken de diagnostische prestaties van een dunne 'aspiratienaald' (fine needle aspiration, afgekort FNA) en een dikkere 'biopsienaald' (fine needle biopsy, afgekort FNB) in een prospectief gerandomiseerde studie. Ook bekeken we de reproduceerbaarheid van deze uitkomsten en de waarde van het gecombineerd gebruik van beide naalden gedurende één procedure. In een meta-analyse vergeleken we vervolgens de prestaties van FNA met de nieuwe generatie EUS-FNB-naalden, waaronder de ProCore (Cook Medical), SharkCore (Medtronic) en Acquire naalden (Boston Scientific). In het derde deel van dit proefschrift onderzochten we of de kwaliteit van EUS-FNA-preparaten kan worden verbeterd door endoscopiepersoneel te trainen in het verwerken hiervan, en door het verkregen weefsel te verzamelen in een vloeibaar medium (liquid based cytology, afgekort LBC), in plaats van het uit te smeren op een dekglaasje (de conventionele 'smear-techniek').

EUS-geleide weefselafname in de huidige praktijk

EUS-geleide weefselafname wordt steeds vaker gebruikt vanwege de diagnostische nauwkeurigheid, het minimaal invasieve karakter en de hoge weefselopbrengst. Dit laatste is extra belangrijk in het tijdperk van gepersonaliseerde geneeskunde [1]. Vanwege het groeiende gebruik evolueert de techniek voortdurend en staat de optimale strategie nog ter discussie. Door het gebrek aan wetenschappelijk bewijs moeten endo-echoscopisten terugvallen op hun eigen ervaring en aanbevelingen van experts uit veelal verouderde richtlijnen [2-6].

Hoofdstuk 2 beschrijft de uitkomsten van een onlinevragenlijst die wij naar een internationale groep endo-echoscopisten uitstuurden, om de gebruikte technieken en materialen bij EUS-geleide weefselafname in kaart te brengen. We vonden aanzienlijke intercontinentale verschillen tussen en deviaties van de geldende richtlijnen. Volgens de Amerikaanse endo-echoscopisten wordt sedatie met Propofol daar veel vaker gebruikt dan het zogenaamde roesje. Wanneer dit gebruik zich naar Europa uitbreidt, zullen de kosten van EUS-geleide weefselafname ook hier toenemen [7, 10, 11].

Een ander verschil bleek het gebruik van acetylsalicylzuur rondom de procedure. Conform de geldende richtlijnen werd dit in de Verenigde Staten (VS) veelal gecontinueerd, terwijl het in Europa en Azië vaker gestaakt werd. In Azië kan dit worden verklaard door de overtuiging dat Aziaten vatbaarder zijn voor bloedingscomplicaties [12]. De reden dat Europese endo-echoscopisten hier afwijken van de richtlijnen blijft onduidelijk. Wat betreft het inzetten van een patholoog voor 'on-siteweefselbeoordeling' en verwerking (ROSE), rapporteerden alle respondenten uit de VS hier gebruik van te kunnen maken, terwijl dit in Europa en Azië slechts in de helft van alle centra het geval was. Redenen hiervoor waren volgens de Europese en Aziatische respondenten hoge kosten en een beperkt geloof in de toegevoegde waarde. Deze bevindingen sluiten aan bij de resultaten van een recente meta-analyse die inderdaad geen toegevoegde waarde van ROSE liet zien. Het komt daarnaast ook overeen met de huidige Europese richtlijn, waarin het gebruik van ROSE niet wordt aanbevolen [13].

Histologie (intacte weefselfragmenten) werd in alle continenten verzameld en bewaard in formaline. Het weefselmedium voor cytologie (losse cellen) verschilde aanzienlijk. De meeste Aziaten verzamelden cytologie in alcohol of zoutoplossing. Europese en Amerikaanse artsen gebruikten hiervoor Cytolyt. Deze verschillen kunnen goed worden verklaard door het gebrek aan richtlijnen voor het bewaren en bewerken van EUS-weefsel. Een item dat niet verschilde tussen de continenten was de keuze van de naalddikte (gauge) en het aantal puncties dat werd verricht. De 22-gauge (G) werd het meest gebruikt, zowel voor FNA als FNB [14-17]. Deelnemers verrichten minder puncties van een verdachte laesie met een FNB-naald in vergelijking met een FNA-naald. Hoewel er op het moment van het uitsturen van de vragenlijst nog geen aanbevelingen werden gedaan, adviseren de huidige richtlijnen van de European Society of Gastroenterology (ESGE) inmiddels om inderdaad minder puncties te verrichten met FNB (2 tot 3) dan FNA (3 tot 4), mits er geen patholoog aanwezig is tijdens de procedure [13]. In dat geval kan deze beoordelen of voldoende weefsel is afgenomen voor verdere diagnostiek.

De optimale EUS-naald

Zoals in de inleiding van dit proefschrift al genoemd, was en is het verbeteren van de EUS-naalden een belangrijk doelwit van innovatie. FNB-naalden werden geïntroduceerd om de beperkingen van FNA te overkomen, voornamelijk door de collectie van histologie in plaats van cytologie. De diagnostische prestaties van de eerste FNB-naalden waren echter niet overtuigend beter dan die van de conventionele FNA-naalden [18-22]. Sommigen meldden dat FNB niet de histologische, maar slechts de cytologieopbrengst verbeterde [18, 23-29]. Anderen beweerden dat histologie ook verkregen kon worden met FNA-naalden [18, 30, 31]. Ten slotte maken nieuwe weefsel-collectie-media en bewerkingstechnieken, zoals de cell block techniek, een 'histologieachtige' analyse van cytologisch materiaal mogelijk.

De eerste FNB-naalden, waaronder de TruCut^{tm} (Travenol Laboratories) en Quick-Core$^{®}$ (Cook Medical) naalden, waren relatief stug en lastig te hanteren, hetgeen weefsel afname vanuit een sterk gebogen endoscoop positie, zoals het duodenum, bemoeilijkte. Als reactie

hierop zijn de afgelopen jaren verschillende nieuwe FNB-naalden geïntroduceerd, waaronder twee typen ProCore naalden (reversed en forward facing bevel) en de SharkCore en Acquire naalden. Aangezien deze naalden qua ontwerp niet alleen aanzienlijk verschillen van de eerste generatie FNB-naalden, maar ook van elkaar, is er veel interesse in hun diagnostische prestaties.

De 20G ProCore forward facing bevel naald werd als eerste geïntroduceerd, in 2015, en de diagnostische prestaties zijn inmiddels uitgebreid onderzocht. De grootste studie is de internationale multicenterstudie (de ASPRO-studie; ASpiration versus PROcore), die in **hoofdstuk 3** wordt gepresenteerd. In deze studie wordt deze ProCore naald vergeleken met een veelgebruikte FNA-naald, de 25G EchoTip Ultra Needle (Cook Medical), gekozen vanwege zijn grote flexibiliteit en opbrengst [2, 13].

Onze studie demonstreerde een hogere weefselopbrengst en diagnostische nauwkeurigheid voor de 20G FNB-naald. Met FNB waren bovendien minder puncties nodig dan met FNA. Deze bevindingen golden zowel voor pancreas- als niet-pancreaslaesies en waren onafhankelijk van de afmetingen van de afwijking, het aantal verrichte puncties en de aanwezigheid van een patholoog. Bovendien presteerde de nieuwe FNB-naald consequent beter dan de FNA-naald in alle 13 deelnemende centra. Dit pleit voor de algemene toepasbaarheid van onze bevindingen, ook buiten de studiepopulatie.

De betere prestaties van de nieuwe 20G FNB-naald lijken te berusten op ontwerpaanpassingen, waaronder een grotere naalddiameter, een nieuwe coating van de huls van de naald voor meer flexibiliteit, een naar voren in plaats van naar achteren gerichte inkeping voor weefselcollectie aan de zijkant van de naald en gebruik van een Menghini in plaats van een lancetpunt. Dit laatste om de weerstand tijdens de punctie te verminderen. Het feit dat een FNB-naald met een grote diameter beter presteert dan een dunnere FNA-naald is een belangrijke observatie, omdat dit ingaat tegen het bestaande idee dat de opbrengst van naalden met een grotere diameter wordt beperkt door hun suboptimale prestatie vanuit een gebogen endoscoopopositie.

Hoewel de betere prestaties van de nieuwe 20G FNB-naald algemeen toepasbaar leken, namen alleen hoog volume of 'expertcentra' deel aan de studie. Om de reproduceerbaarheid van de bevindingen in minder ervaren handen te onderzoeken, werd een tweede studie uitgevoerd. Zoals beschreven **in hoofdstuk 4**, vergeleken we de diagnostische overeenkomst tussen het oordeel van academische en niet-academische pathologen. De eerste 125 pancreas- en lymfeklierpuncties, vervaardigd tijdens de oorspronkelijke studie, werden herbeoordeeld door 5 academische en 5 niet-academische pathologen uit verschillende landen. Monsters afgenomen met FNB gaven een hogere mate van overeenstemming voor het stellen van de diagnose dan de FNA-monsters. Dit gold zowel voor de academische als niet-academische pathologen. Logistische regressie toonde bovendien aan dat de diagnostische nauwkeurigheid van FNB niet werd beïnvloed door de achtergrond van de patholoog (academisch of niet-academisch). Dit ondersteunt het gebruik van de nieuwe 20G FNB-naald in zowel academische als niet-academische centra.

De meest waarschijnlijke verklaring voor de hogere mate van overeenstemming voor FNB-monsters is dat deze vaker intacte weefselfragmenten bevatten. De kans dat pathologen het eens waren over de diagnose was namelijk groter voor histologische monsters. Hoewel ook de cytologische opbrengst voor FNB hoger was dan voor FNA, droeg alleen histologie bij aan een hogere diagnostische nauwkeurigheid. Een andere kwaliteitsparameter die de hogere diagnostische nauwkeurigheid en overeenstemming van FNB-samples kan verklaren, is het minimaal aantal artefacten in de FNB-samples. Onze bevinding dat weefselartefacten de diagnostische nauwkeurigheid van EUS-monsters verlaagd, wordt ondersteund door eerder onderzoek, dat aantoonde dat artefacten aanvullende tests lastiger maken [32]. De overeenstemming over de aanwezigheid van artefacten was vrij laag voor FNA en FNB, hoewel iets beter voor FNB. Dit is in overeenstemming met het feit dat de pathologen het überhaupt weinig eens waren over weefsel-kwaliteitsparameters. Dit werd ook door anderen vastgesteld [33, 34] en lijkt het gevolg van het gebrek aan uniforme kwaliteitsparameters voor EUS-geleide weefselafname.

Om de optimale techniek voor EUS-geleide weefselafname vast te stellen onderzoeken we in **hoofdstuk 5** de rol van het gecombineerde gebruik FNA- en FNB-naalden. De voordelen van een FNA-naald: optimale flexibiliteit om een target laesie te bereiken en de mogelijkheid van cytologische evaluatie door een patholoog ter plaatse, zouden de voordelen van FNB kunnen aanvullen: het verkrijgen van histologisch weefsel om de diagnostische opbrengst te optimaliseren en genoeg weefsel over te houden voor aanvullende diagnostiek. Om de waarde van gecombineerd FNA- en FNB-gebruik, of 'dual-sampling', nader te onderzoeken, includeerden wij alle 73 ASPRO-casussen waarbij tijdens dezelfde procedure met beide naalden was gepuncteerd. Bij 24 patiënten werd eerst de 25G FNA-naald gebruikt en in 49 eerst de FNB-naald. Interessant is dat de diagnostische nauwkeurigheid voor het vaststellen van een maligniteit alleen toenam als een punctie met FNA werd gevolgd door FNB en niet andersom. Het eerder bewezen diagnostische voordeel van de 20G FNB- ten opzichte van de FNA-naald kan dit uiteraard goed verklaren. Een andere verklaring is dat eerst prikken met FNB mogelijk meer bloedcontaminatie veroorzaakt voor de daaropvolgende FNA-punctie, of dat de er door de grotere diameter van de FNB-naald "tracking" ontstaat, waardoor de diagnostische route van de FNA-naald negatief wordt beïnvloed. Tot slot werd een punctie met FNA na FNB meestal gebruikt voor ROSE. Aangezien de toegevoegde waarde van ROSE twijfelachtig is voor expert-centra, was de meerwaarde van ROSE in de deelnemende centra waarschijnlijk beperkt [35-37].

In **hoofdstuk 6** verlaten we de twee eerdergenoemde EUS-naalden en geven we in een meta-analyse een overzicht van de prestaties van alle gangbare EUS-naalden. Hiervoor voerden we een systematische zoekopdracht uit in EMBASE, MEDLINE / PubMed, Web of Science, de Cochrane Library en Google Scholar naar alle studies die FNA met de eerder genoemde nieuwe generatie FNB naalden vergeleken. We beperkten de selectie tot gerandomiseerde studies van ten minste 50 deelnemers en extraheerden hieruit gegevens over de diagnostische nauwkeurigheid, de weefselkwaliteit, de aanwezigheid van histologie in de samples, het aantal

verrichtte puncties en de kans op complicaties. Hiernaast werd de studiekwaliteit beoordeeld met behulp van de QUADAS-2-tool.

In vergelijking met eerdere meta-analyses maakte de recente golf aan publicaties het mogelijk een aanzienlijk aantal nieuwe studies te includeren en ons te beperken tot studies van hoge kwaliteit [18-22]. Voor het eerst werd een diagnostisch voordeel van FNB ten opzichte van FNA aangetoond, zowel voor laesies in als buiten de pancreas. FNB leverde een hogere diagnostische nauwkeurigheid en monsters bevatten vaker intacte histologische weefselfragmenten. Bovendien waren hier minder puncties voor nodig dan met FNA. De kans op complicaties was laag voor zowel FNA als FNB en verschilde niet significant. Hoewel FNB vaker tot een correcte diagnose leidde, werden monsters niet vaker als voldoende geschikt bevonden voor diagnostiek. Dit kan worden verklaard door het feit dat de mate van geschiktheid voor diagnostiek vaak wordt beoordeeld op basis van het aantal losse cellen, de cytologie. Mogelijk is dit minder belangrijk voor het stellen van een correcte diagnose dan de aanwezigheid van histologisch intacte weefselfragmenten. Zelfs ondanks het gebruik van nieuwe verwerkingstechnieken voor cytologie, zoals ThinPrep en cell block, en het gebruik van ROSE, was de diagnostische nauwkeurigheid beter voor FNB.

Bij het extrapoleren van onze bevindingen naar andere centra dient men rekening te houden met de significante heterogeniteit tussen de studies in de meta-analyse. Dit komt vooral door de diversiteit in EUS-weefselafnameprotocollen en het inconsistente gebruik van uitkomstdefinities. Dit maakt de resultaten van EUS-studies moeilijk te interpreteren. Hoewel de huidige meta-analyse aantoonde dat FNB overtuigend beter presteerde dan FNA, betrof dit alleen de ProCore FNB-naald, aangezien alle studies in de meta-analyse met deze FNB-naald waren uitgevoerd. Er zijn op dit moment nog geen studies van voldoende kwaliteit beschikbaar waarin één van de andere nieuwe generatie FNB-naalden, zoals de Acquire en SharkCore naald, is getest, alhoewel volgens ClinicalTrials.gov er enkelen onderzoeken lopen.

Verbeteren van EUS-weefselpreparatie en -verwerking

Zoals eerder vermeld, stellen nieuwe FNA-weefselpreparatietechnieken zoals ThinPrep en cell block pathologen in toenemende mate in staat om aanvullende testen uit te voeren op cytologische samples. Deze technieken zijn geïntroduceerd om de kwaliteit en nauwkeurigheid van FNA-monsters te verbeteren, aangezien de traditionele smear-techniek gevoelig is voor artefacten. Een andere manier om dit probleem op te lossen is investeren in de aanwezigheid van een patholoog tijdens de procedure om het verzamelde weefsel direct te beoordelen en te verwerken. In de meeste centra ontbreekt echter deze mogelijkheid. Daarom wordt de eerste bewerking van EUS-FNA-materiaal vaak door endoscopiemedewerkers gedaan, meestal zonder formele training in weefselcollectie en -preparatie.

Hoofdstuk 7 onderzoekt of een eendaagse 'hands-on' EUS-weefselpreparatietraining voor endoscopiepersoneel de kwaliteit en dus de diagnostische nauwkeurigheid van FNA-monsters kan verbeteren in centra zonder ROSE. In een prospectieve pilotstudie volgden 10 endo-

echoscopisten en 12 endoscopieverpleegkundigen uit 7 regionale EUS-centra in Nederland een training. Zij werden onderwezen in pancreaspathologie, algemene diagnostische valkuilen en de oorzaak en preventie van artefacten van weefseluitstrijkpreparaten. Vervolgens oefenden zij het maken van uitstrijkpreparaten onder supervisie van een team van academische (cyto) pathologen. Na de training verzamelden we prospectief 71 FNA-weefseluitstrijken van solide pancreaslaesies en vergeleken we deze met een gelijk aantal 'controleuitstrijkpreparaten', welke waren vervaardigd voor de training.

Helaas verbeterde onze training de kwaliteit en nauwkeurigheid van de EUS-FNA-weefseluitstrijken niet significant. Door de beperkte steekproefomvang konden we de individuele resultaten niet analyseren en dus niet beoordelen of er individuen wel baat had van de training. Voor het beperkte effect van onze pilot-training zijn meerdere verklaringen denkbaar. Ten eerste was de training zelf misschien te kort of was één training te weinig. Aangezien praktische vaardigheden over het algemeen beter worden na een uitgebreide training en verder verbeteren na frequente en repeterende oefening, was het wellicht effectiever geweest om de training te intensiveren of te herhalen. De studieperiode was daarbij mogelijk te kort om de deelnemers voldoende ervaring op te laten doen. Ten slotte kan het gebrek aan kwaliteitsverbetering ook inherent zijn aan de aard van de uitstrijktechniek zelf, omdat het een handmatige methode is die gevoelig is voor artefacten en heterogene preparaten. De eerdergenoemde alternatieve technieken: ThinPrep en cell block zijn hiervoor minder gevoelig [38].

Hoofdstuk 8 vergelijkt de diagnostische prestaties van deze alternatieve cytologieverwerkingstechnieken, LBC, met de conventionele weefseluitstrijktechniek voor EUS-FNA van solide pancreaslaesies. Het EUS-personeel van de eerdergenoemde 7 regionale EUS-centra was voor het starten van deze studie reeds getraind in het uitstrijken van FNA-weefsel, volgens de training beschreven in hoofdstuk 7. Voor deze studie vervaardigden de deelnemers van elke eerste FNA-punctie monsters twee weefseluitstrijkjes en een flesje met weefsel in een vloeibaar medium, ThinPrep en/of cell block. Er mocht hierbij uiteraard geen patholoog op de kamer aanwezig zijn. We vergeleken de diagnostische waarde van beide technieken op basis van de weefselkwaliteit, de diagnostische nauwkeurigheid voor maligniteit en de overeenstemming over de weefseldiagnose onder de drie (cyto)pathologen.

De studie liet zien dat de diagnostische nauwkeurigheid hoger was voor LBC dan voor de uitstrijktechniek. Dit was echter alleen waar indien de opbrengst van ThinPrep en cell block werden samengenomen. Artefacten waren minder aanwezig in beide LBC-technieken. De pathologen bereikten even vaak overeenstemming over de diagnose voor LBC en weefseluitstrijkjes en waren het vaker eens over de cell block samples. Gezien de hogere nauwkeurigheid en vergelijkbare diagnostische overeenstemming onder de (cyto)pathologen, zou LBC een alternatief kunnen zijn voor centra zonder ROSE. Gezien de hoge mate van overeenstemming tussen de pathologen zou de cell block techniek de voorkeur hebben boven ThinPrep.

Toekomstperspectieven en aanbevelingen voor de toekomst

De historische grens tussen FNA en FNB lijkt te vervagen doordat de naalden vrijwel niet meer op basis van hun design te onderscheiden zijn. Om de zaken nog verder te compliceren, zijn er beduidende ontwerpverschillen binnen de FNA- en FNB-groepen. Hieruit voortvloeiend zouden wij willen pleiten voor het verlaten van de huidige nomenclatuur van FNA en FNB, en in plaats hiervan de nadruk willen leggen op de prestaties van individuele EUS-naalden.

Dit proefschrift biedt belangrijke nieuwe informatie die kan worden gebruikt om EUS-geleide weefselafname te verbeteren. Er is echter ruimte voor verdere ontwikkeling, aangezien onder meer de meta-analyse in dit proefschrift aantoont dat de diagnostische nauwkeurigheid voor FNA nog steeds varieert tussen 67% en 100% en voor FNB tussen 69% en 100%. Dit proefschrift stelt dat een FNB-naald beter presteert dan FNA, en dus de eerste keuze zou moeten zijn voor door EUS-geleide weefselafname, onafhankelijk van de soort afwijking (gelegen binnen de pancreas of daarbuiten). Voordat men echter één specifieke EUS-naald kan aanbevelen moeten de diagnostische prestaties van alle nieuwe generatie FNB-naalden in kaart worden gebracht en vergeleken.

Hoewel men idealiter elk type EUS-naald 'head to head' zou willen vergelijken, zal dit een uitdaging zijn. Allereerst is het een tijdrovende, maar niet onmogelijke klus vanwege de vele beschikbare FNA- en FNB-formaten en -designs. Andere complicerende factoren zijn de heterogeniteit in EUS-weefselafnameprotocollen en het ontbreken van uniforme uitkomstdefinities. Zolang er geen bewijs is voor de optimale EUS-geleide weefselafnamestrategie zal het moeilijk zijn om uniforme EUS-strategieën te implementeren. Makkelijker te bewerkstellen is het creëren van uniforme uitkomstdefinities. Hierin zouden de Europese en Amerikaanse verenigingen voor gastro-enterologie een belangrijke rol kunnen spelen, zoals ook de Papanicolaou Society (de internationale pathologievereniging) deed voor de diagnostische classificatie van EUS-weefsel.

Naast de optimale EUS-naald, focust dit proefschrift zich op de preparatie en verwerking van EUS-FNA-monsters. Men kan zich afvragen of toekomstig onderzoek zich hierop moet blijven richten, aangezien wij aantoonden dat FNB beter is dan FNA. We hebben echter niet direct onderzocht of FNB-weefsel nog steeds beter presteert indien FNA-weefsel is verwerkt middels LBC. Het zou interessant zijn dit te onderzoeken in een internationale multicenterstudie. In dit kader is het belangrijk te benoemen dat weefselbewerking middels LBC gemakkelijk is voor de endo-echoscopisten, maar niet voor het pathologiepersoneel, aangezien men hiervoor een goed uitgerust pathologielaboratorium en getraind personeel nodig heeft. Om die reden zal het implementeren van nieuwe technieken en innovaties voor EUS-geleide weefselafname idealiter altijd moeten worden afgestemd met de pathologieafdeling. Ten slotte is het cruciaal dat, gezien de toenemende kosten van de gezondheidszorg, kosteneffectiviteitsanalyses plaatsvinden voordat innovaties in de praktijk geïmplementeerd worden.

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Appendices

LIST OF PUBLICATIONS

This thesis:

1. **Van Riet PA**, Poley JW, Cahen DL, Bruno MJ. Mapping international practice patterns in EUS-guided tissue sampling: outcome of a global survey. *Endosc Int Open* 2016; 04(03): E360-E370.

2. **Van Riet PA**, Larghi A, Attili F, Rindi G, Nguyen NQ, Ruszkiewicz A, Kitano M, Chikugo T, Aslanian H, Farrell J, Robert M, Adeniran A, Van Der Merwe S, Roskams T, Chang K, Lin F, Lee JG, Arcidiacono PG, Petrone M, Doglioni C, Iglesias-Garcia J, Abdulkader I, Giovannini M, Bories E, Poizat F, Santo E, Scapa E, Marmor S, Bucobo JC, Buscaglia JM, Heimann A, Wu M, Baldaque-Silva F, Moro CF, Erler NS, Biermann K, Poley JW, Cahen DL, Bruno MJ. A multicenter randomized trial comparing a 25-gauge EUS fine-needle aspiration device with a 20-gauge EUS fine-needle biopsy device. *Gastrointest Endosc*. 2019 Feb;89(2):329-339.

3. **Van Riet PA**, Cahen DL, Biermann K, Hansen B, Larghi A, Rindi G, Fellegara G, Arcidiacono PG, Doglioni C, Decarli NL, Iglesias-Garcia J, Abdulkader I, Iglesias HL, Kitano M, Chikugo T, Yasukawa S, Van Der Valk H, Nguyen NQ, Ruszkiewicz A, Giovannini M, Poizat F, Van Der Merwe S, Roskams T, Santo S, Marmor S, Chang K, Lin F, Farrell J, Robert M, Bucobo JC, Heimann A, Baldaque-Silva F, Moro CF, Bruno MJ. Agreement on endoscopic ultrasonography-guided tissue specimens: Comparing a 20-G fine-needle biopsy to a 25-G fine-needle aspiration needle among academic and non-academic pathologists. *Dig Endosc.* 2019 Jul 10. doi: 10.1111/den.13424. *[Epub ahead of print]*

4. **Van Riet PA,** Arcidiacono PG, Petrone M, Nguyen NQ, Kitano M, Chang K, Larghi A, Iglesias-Garcia J, Giovannini M, Van Der Merwe S, Santo S, Baldaque-Silva F, Bucobo JC, Bruno MJ, Aslanian HR, Cahen DL*, Farrell J*. Combined versus single use of the 20G fine-needle biopsy and the 25G fine-needle aspiration needle for ultrasound-guided tissue sampling of solid gastrointestinal lesions. *Endoscopy. 2019 Jul 22. doi: 10.1055/a-0966-8755. [Epub ahead of print]*

5. **Van Riet PA**, Erler NS, Bruno MJ, Cahen DL. The optimal EUS sampling-strategy: a meta-analysis of FNA and new generation FNB devices. *Submitted*

6. **Van Riet PA**, Quispel R, Cahen DL, Snijders-Kruisbergen MC, Van Loenen P, Erler NS, Poley JW, van Driel LMJW, Mulder SA, Veldt BJ, Leeuwenburgh I, Anten MPGF, Honkoop P, Thijssen AY, Hol L, Hadithi M, Fitzpatrick CE, Schot I, Bergmann JF, Bhalla A, Bruno MJ*, Biermann K*. Diagnostic yield and agreement on fine needle specimens from solid pancreatic lesions: comparing the conventional smear technique to liquid-based cytology. *Submitted*

7. **Van Riet PA**, Quispel R, Cahen DL, Erler NS, Snijders-Kruisbergen MC, Van Loenen P, Poley JW, van Driel LMJW, Mulder SA, Veldt BJ, Leeuwenburgh I, Anten MPGF, Honkoop P, Thijssen AY, Hol L, Hadithi M, Fitzpatrick CE, Schot I, Bergmann JF, Bhalla A, Bruno MJ, Biermann K. Optimizing tissue handling of EUS-FNA of solid pancreatic lesions: a pilot study to the effect of a smear preparation training for endoscopy personnel on sample quality and diagnostic accuracy. *Submitted*

Other publications (not covered in this thesis):

8. **Van Riet PA**, Poley JW, Cahen DL, Bruno MJ. EUS guided FNA of cystic lesions of the pancreas: indications, technique, and complications. Book chapter in Testoni et al (red). Endoscopic management of pancreatico-biliary cancer and precancerous conditions. *Minerva Medica*. 2015, pp 35 – 43.

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Courses and workshops

	Year	Workload
Photoshop and Illustrator CS6, Molecular medicine postgraduate school, Rotterdam	2016	8 hours
Indesign CS6, Molecular medicine postgraduate school, Rotterdam	2016	4 hours
Introduction course on statistics & survival analysis, Molecular medicine postgraduate school, Rotterdam	2016	8 hours
Biomedical English Writing and Communication, Erasmus MC Rotterdam	2015	40 hours
Research Integrity course, Erasmus MC Rotterdam	2014	16 hours
Endnote workshop, Erasmus MC Rotterdam	2014	6 hours
Pubmed workshop, Erasmus MC Rotterdam	2014	6 hours
BROK-cursus, Consultatiecentrum Patiëntgebonden onderzoek (CPO), Erasmus MC Rotterdam. Certificate Good Clinical Practice	2013	24 hours

Invited lectures

	Year	Workload
Results of the ASRPO study and future research. MEDSURG, Cook Medical, Lisbon, Portugal	2019	24 hours
Outcome of the ASPRO study. EUS-ENDO, Marseille, France	2018	12 hours
Mapping international practice patterns in EUS-guided tissue sampling: outcome of a global survey. Endo Live Roma, Italy	2015	24 hours

Oral presentations

	Year	Workload
Follow-up of neoplastic pancreatic cysts (PACYFIC-study). Pancreasdag, Utrecht, the Netherlands	2016	12 hours
Follow-up of neoplastic pancreatic cysts (PACYFIC-study). Diner Pensant Pancreas, Rotterdam, the Netherlands	2016	12 hours
Interobserver agreement on the diagnostic outcome of the new 20-gauge ProCore needle amongst a group of international pathologists. Fit for the Future in Gastroenterology, Berlin, Germany	2016	24 hours
Mapping international practice patterns of EUS-guided tissue sampling; outcome of a global survey. Fit for the Future in Gastroenterology, Berlin, Germany	2015	24 hours

Poster presentations

	Year	Workload
Agreement on EUS-guided tissue specimens: comparing a 20G FNB to a 25G FNA needle amongst academic and non-academic pathologists. United European Gastroenterology Week, Vienna, Austria	2018	12 hours
The value of combined versus single use of the 20G FNB needle and the 25G FNA needle for EUS-guided tissue sampling of solid Gastrointestinal lesions. United European Gastroenterology Week, Vienna	2018	12 hours
A multicenter randomized trial comparing a 25-gauge EUS fine-needle aspiration device with a 20-gauge EUS fine-needle biopsy device. Digestive Disease Week, Chicago, USA	2018	12 hours
The value of combined versus single use of the 20G FNB needle and the 25G FNA needle for EUS-guided tissue sampling of solid Gastrointestinal lesions. Digestive Disease Week, San Diego, USA	2018	12 hours
Mapping international practice patterns of EUS-guided tissue sampling: outcome of a global survey. United European Gastroenterology Week, Barcelona, Spain	2017	12 hours
Mapping international practice patterns of EUS-guided tissue sampling: outcome of a global survey. Digestive Disease Week, Washington DC, USA	2015	12 hours

Attended (inter)national conferences

	Year	Workload
EUS-ENDO, Marseille, France	2018	16 hours
United European Gastroenterology Week, Vienna, Austria	2018	28 hours
Digestive Disease Week, Washington, USA	2018	28 hours
European Pancreas Club, Liverpool, UK	2016	16 hours
Digestive Disease Week, Chicago, USA	2017	28 hours
United European Gastroenterology Week, Vienna, Austria	2016	28 hours
Digestive Disease Week, San Diego, USA	2016	28 hours
Najaarscongres, Nederlandse vereniging voor Gastro-enterologie, Veldhoven, the Netherlands	2016	12 hours
EURO EUS, Marseille, France	2015	16 hours
United European Gastroenterology Week, Barcelona, Spain	2015	28 hours
Digestive Disease Week, Washington DC, USA	2015	28 hours
Najaarscongres, Nederlandse vereniging voor Gastro-enterologie, Veldhoven, the Netherlands	2015	12 hours
Voorjaarscongres, Nederlandse vereniging voor Gastro-enterologie, Veldhoven, the Netherlands	2015	12 hours
United European Gastroenterology Week Vienna, Austria	2014	28 hours
Digestive Disease Week, Chicago, USA	2014	28 hours
European Pancreas Club, Southampton, UK	2014	16 hours
Najaarscongres, Nederlandse vereniging voor Gastro-enterologie, Veldhoven, the Netherlands	2014	12 hours
Voorjaarscongres, Nederlandse vereniging voor Gastro-enterologie, Veldhoven, the Netherlands	2014	12 hours

Attended seminars

	Year	Workload
Postgraduate course, United European Gastroenterology Week, Vienna, Austria	2018	16 hours
Erasmus Liver day, Rotterdam, the Netherlands	2018	6 hours
Postgraduate course, United European Gastroenterology Week, Vienna, Austria	2016	16 hours
Pancreasdag, Utrecht, the Netherlands	2016	6 hours
Erasmus Liver day, Rotterdam, the Netherlands	2016	6 hours
10e Jaarsymposium Gastro-enterologie	2015	6 hours
6e Lagerhuisdebat Hepatitis B en C, Utrecht, the Netherlands	2014	2 hours
Pancreasdag, Utrecht, the Netherlands	2014	6 hours

Awards

	Year
United European gastroenterology week young investigator bursary	2018
United European gastroenterology week young investigator bursary	2015
Winner of Poster pitch session, poster of excellence, United European gastroenterology week, Barcelona, Spain	2015

Educational activities

	Year	Workload
Tutor medical students Erasmus MC Rotterdam	2015	24 hours

Memberschips

	Year
De Jonge Specialist en LAD, artsen in dienstverband	2018-present
Netherlands Society of Gastroenterology (NVMDL)	2017-present
Netherlands Association of Gastroenterology (NVGE)	2013-present
Dutch Pancreatic Cancer Group (DPCG)	2013-presrnt
Dutch Pancreatitis Group (PWN)	2013-present

Reviewing activities

	Year	Workload
Endoscopy	2019	8 hours

ABOUT THE AUTHOR

Priscilla van Riet was born on November 25th in Amsterdam, the Netherlands and grew up in Rotterdam and Leiden. In 2006 she finished her pre-university education at the Rijnlands Lyceum in Oegstgeest. From 2006 to 2007 she studied Biomedical Sciences at the University of Amsterdam, for which she collected her first-year-diploma. Subsequently, she started medical school at the Erasmus MC University Medical Center in Rotterdam. In her second year of medical school, she applied for an extracurricular research project on penetrating neck and thoracic injuries at the department of trauma and emergency surgery of the Erasmus MC Rotterdam. For this, she also did a scientific and clinical internship at the department of surgery at Sint Maarten, Netherlands Antilles. In the last two years of medical school, during the clinical rotations, her interest switched from surgery to gastroenterology after an inspiring clinical internship at the department of gastroenterology in the Maasstad hospital in Rotterdam. In her final year of medical school, she did a two-month clinical internship at the department of gastroenterology, at the Groote Schuur hospital, in Cape town, South-Africa. Subsequently, her final clinical internship was done at the department of gastroenterology and hepatology in the Erasmus MC. After obtaining her medical degree in November 2013, she started her PhD at the Erasmus MC at department of gastroenterology and hepatology, under the supervision of prof. Dr. M.J. Bruno and Dr. D.L. Cahen. As of June 2017, she started with her two-year internal medicine residency at the Ikazia hospital in Rotterdam as part of the formal postgraduate training in gastroenterology and hepatology (cluster Erasmus MC University Medical Center, Rotterdam, program director prof. Dr. C.J. Van Der Woude). In September 2019 she continued her training in gastroenterology and hepatology in the Erasmus MC.

DANKWOORD

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