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.

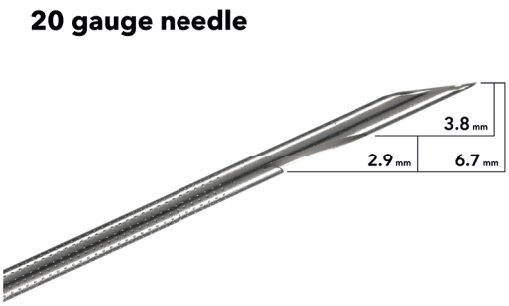

Figure 1. Needle tip design and dimensions of the 20-gauge FNB needle with a forward facing bevel and a Menghini tip-design.

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).

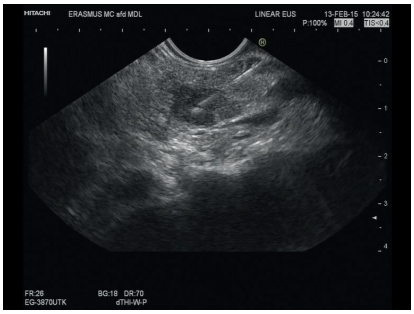

Figure 2. Endoscopic ultrasound image of a hypodense lesion of the pancreatic head, 2 cm in size, irregular borders.

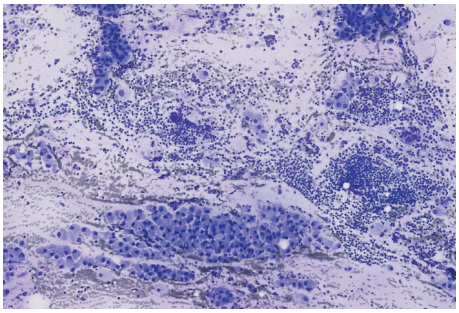

Figure 3. Cytology collected with the 25-gauge FNA needle, showing a monotonous cell population, with enlarged nucleoli, and mucus producing cells (May Grunwald Giemsa stain).

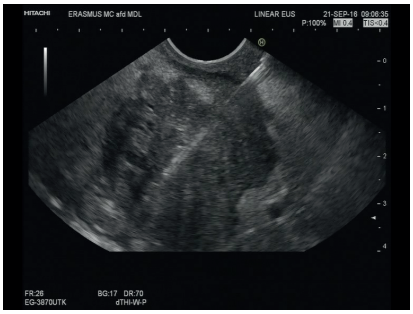

Figure 4. Endoscopic ultrasound image of a hypodense pancreatic head lesion, 4cm in size, irregular borders, and a close relation with SMA.

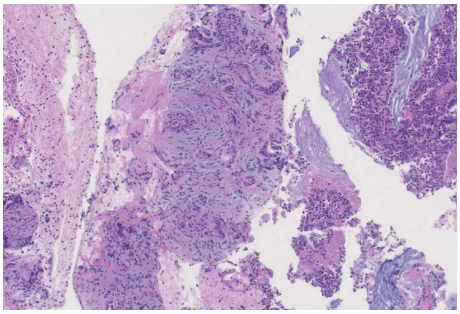

Figure 5. Histology obtained with the 20 FNB needle shows a well-differentiated adenocarcinoma with clear invasive groups of tumor cells, (hematoxylin-eosin stain).

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.

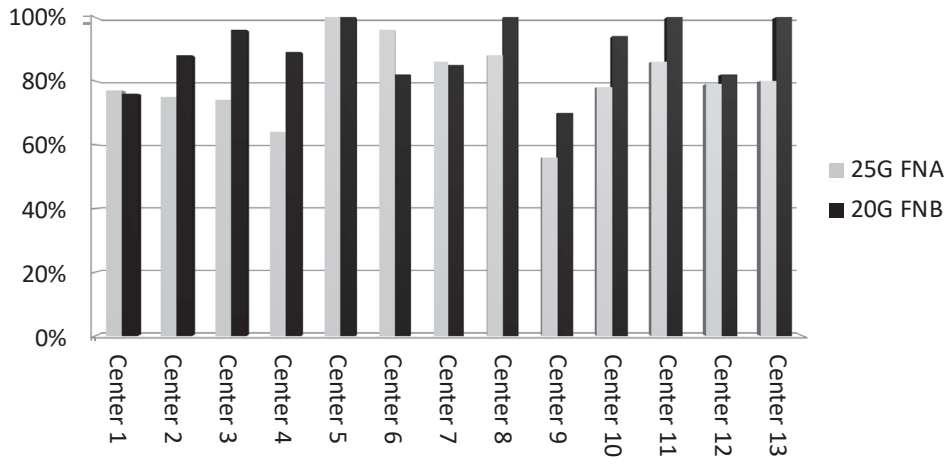

Figure 6. Diagnostic accuracy per center for the 25G FNA and 20G FNB needle.

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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