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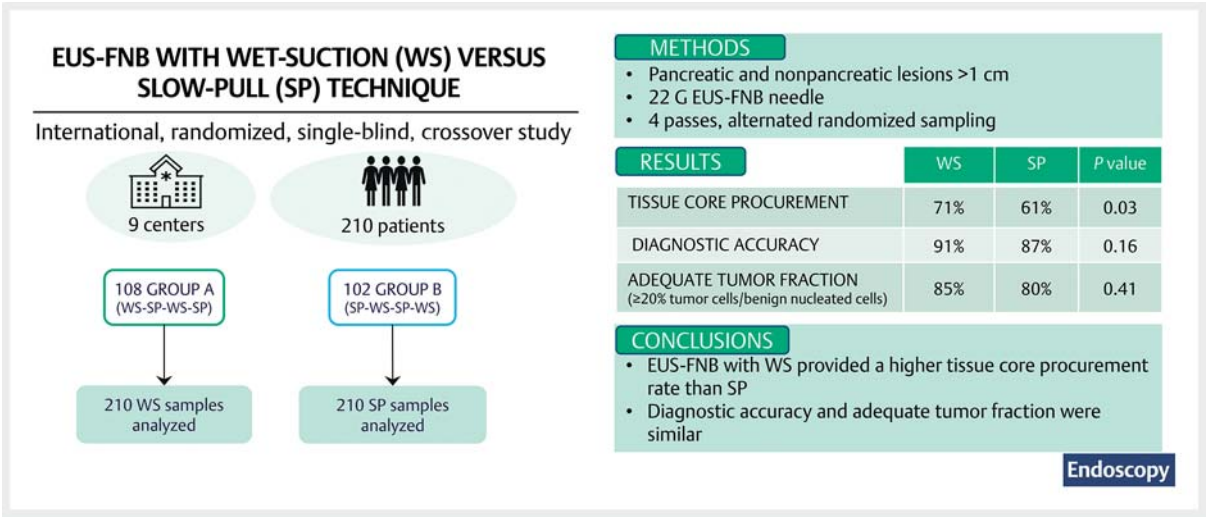
Wet-suction versus slow-pull technique for endoscopic ultrasound-guided fine-needle biopsy: a multicenter, randomized, crossover trial

GRAPHICAL ABSTRACT



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ABSTRACT

Background It is unknown whether there is an advantage to using the wet-suction or slow-pull technique during endoscopic ultrasound-guided fine-needle biopsy (EUS-FNB) with new-generation needles. We aimed to compare the performance of each technique in EUS-FNB.

Methods This was a multicenter, randomized, single-blind, crossover trial including patients with solid lesions of ≥ 1 cm. Four needle passes with 22 G fork-tip or Franseen-type needles were performed, alternating the wet-suction and slow-pull techniques in a randomized order. The primary outcome was the histological yield (samples containing an intact piece of tissue of at least 550 µm). Secondary end points were sample quality (tissue integrity and blood contamination), diagnostic accuracy, and adequate tumor fraction.

Results Overall, 210 patients with 146 pancreatic and 64 nonpancreatic lesions were analyzed. A tissue core was retrieved in 150 (71.4 %) and 129 (61.4 %) cases using the wet-suction and the slow-pull techniques, respectively (P = 0.03). The mean tissue integrity score was higher using wet suction (P = 0.02), as was the blood contamination of samples (P < 0.001). In the two subgroups of pancreatic and nonpancreatic lesions, tissue core rate and tissue integrity score were not statistically different using the two techniques, but blood contamination was higher with wet suction. Diagnostic accuracy and tumor fraction did not differ between the two techniques.

Conclusion Overall, the wet-suction technique in EUS-FNB resulted in a higher tissue core procurement rate compared with the slow-pull method. Diagnostic accuracy and the rate of samples with adequate tumor fraction were similar between the two techniques.

Introduction

Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) has become an essential tool for the diagnosis of solid pancreatic and nonpancreatic lesions, with 85 % sensitivity and 98 % specificity [1]. Several factors have been previously evaluated to optimize outcomes of EUS-FNA [1], such as use of rapid on-site evaluation (ROSE) for immediate cytopathological assessment [2, 3], use of needles of different calibers and types [4], number of needle passes [5], and different sampling techniques [6].

In the past decade, new EUS needles for the acquisition of histological specimens (fine-needle biopsy [EUS-FNB]) have been developed to overcome the limitations of cytology, facilitating the differential diagnosis of rare conditions through performance of specific immunohistochemical staining [7], and obviating the need for ROSE [8]. Two end-cutting needles (i. e. fork-tip SharkCore needle, Covidien/Medtronic, Boston, Massachusetts, USA; and the Franseen-type needle, Acquire, Boston Scientific, Marlborough, Massachusetts, USA) have shown excellent histological yields [9, 10], with comparable diagnostic performance in two randomized controlled trials (RCTs) and two metanalyses [11–14]. Importantly, EUS-FNB samples have been demonstrated to be suitable for next-generation sequencing [15] when containing a tumor fraction of ≥ 20 %, either for pancreatic ductal adenocarcinoma (PDAC) [16] or pancreatic neuroendocrine tumors (pNETs) [17]. Consequently, current practice has almost completely shifted from EUS-FNA to EUS-FNB [18].

Different sampling techniques have been introduced and compared with the standard suction technique, including the slow-pull and wet-suction techniques. With standard suction, the stylet is removed and an air-filled pre-vacuum 10 mL or 20 mL syringe is attached to the proximal end of the needle and opened once inside in the lesion to apply negative pressure suction [19]. In the slow-pull technique, negative pressure is created by slowly withdrawing the stylet from the needle [20]. In the wet-suction technique, the needle is flushed with saline to replace the column of air, and a pre-vacuum 10 mL or 20 mL syringe is utilized to apply suction [21]. The standard suction and slow-pull techniques have been widely studied for EUS-FNA. A recent meta-analysis, including seven RCTs comparing them for sampling of solid pancreatic lesions, demonstrated similar adequacy and accuracy, with less blood contamination for the slow-pull method [22]. The wet-suction technique has been introduced more recently and two RCTs comparing standard and wet suction for EUS-FNA reported significant higher specimen

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cellularity, adequacy, and accuracy with wet suction, both for solid pancreatic and nonpancreatic lesions [21, 23].

To date, a single-center pilot RCT has evaluated different sampling techniques on EUS-FNB of solid pancreatic lesions, comparing standard, slow-pull, and wet-suction methods, using both the fork-tip and Franseen-type needles. No difference in cellularity scores or blood contamination were found, regardless of the technique or needle type used [24]. However, the small sample size, inclusion of only solid pancreatic lesions, and the single-center design did not allow definitive conclusions to be drawn, leaving these issues still open for further evaluation.

We performed a multicenter RCT with the primary aim of comparing the histological yield of EUS-FNB using the slow-pull and wet-suction techniques in patients with pancreatic and nonpancreatic solid lesions. Secondary aims included evaluation of sample quality, tumor fraction, and diagnostic accuracy.

Methods

Study design

This study was an international, single-blinded, crossover, randomized study involving nine centers. It was first approved by the ethics committee of the provinces of Verona and Rovigo on 29 March 2021 (protocol number 18440), and subsequently by ethics committees and institutional review boards of all participating centers. All patients signed informed consent before inclusion into the study.

Patient population

Consecutive adult patients with a solid lesion of ≥ 1 cm who were referred for EUS evaluation and were able to provide informed consent were assessed for study eligibility. Patients with bleeding disorders (uncorrectable with coagulation factors or fresh frozen plasma), concomitant anticoagulants use (not to be discontinued), an international normalized ratio of > 1.5, platelet count of < 50 000, pregnant or breastfeeding, lesions with cystic component (> 50 % of the volume), or included in another study, were excluded.

EUS procedure and specimen processing

EUS procedures were performed by expert endosonographers without involvement of trainees. Once the target lesion was visualized on EUS, interposed vessels were excluded using color Doppler. A 22 G end-cutting needle, fork-tip or Franseen-type, was used in all cases, with the choice of needle type left to the endosonographer's discretion or according to availability at each center. Four passes were performed using the same needle, alternating the sampling techniques according to the randomization list (see below). During each pass, regardless of the sampling procedure, approximately 10 to-and-fro movements of the needle were performed inside the lesion, utilizing the fanning technique whenever possible [25]. For wet suction, the stylet was removed and the needle pre-flushed with 1–2 mL of saline. The lesion was then punctured, and suction applied using a 10 mL pre-vacuum syringe [23]. For the slow-pull method, after puncturing the lesion, the stylet was slowly and gradually withdrawn for at least 40 cm. The samples collected were finally pushed into two formalin vials. The samples retrieved with the same sampling technique (i. e. 1st/3 rd passes and 2nd/4th passes) were placed in the same container. Specimens were processed as standard biopsies (e. g. after being embedded in paraffin, sections of 5 µm thickness were cut from paraffin blocks and stained with hematoxylin and eosin). ROSE was not used in any case. Patients were excluded if the lesion was impossible to puncture and dropped out if at least one needle pass with each technique was not performed.

Randomization and blinding

Once the eligibility for inclusion into the trial was verified, patients were randomized in a 1:1 ratio in a crossover design into Group A and Group B, based on a computer-generated randomized block sequence (block size of 10). For Group A, the pass sequence was wet suction, slow pull, wet suction, slow pull. For Group B, the pass sequence was slow pull, wet suction, slow pull, wet suction. The randomization list was stratified by the type of lesion (pancreatic vs. nonpancreatic).

A data manager not involved in the data analysis or patient enrollment generated the randomization list. At each center, sealed envelopes containing the group assignment were prepared and opened after obtaining study consent and EUS baseline assessment, just prior to EUS-FNB.

The pathologists designated for sample evaluation were blinded to patients' randomization and type of EUS-FNB technique performed during the entire study.

Definition of study end points

Primary end point

The primary end point was the tissue core procurement rate. A tissue "core" was defined as at least one intact piece of tissue of 550 µm or more in length [26, 27]. The length of histological fragments was measured using dedicated software at each participating center.

Secondary end points

Sample quality was evaluated in terms of tissue integrity and blood contamination, applying a previously utilized, simplified, score [28–31]. Briefly, a score ranging from 0 to 3 was assigned to the specimens by a blinded pathologist. For both tissue integrity and blood contamination, the higher the score the better the sample quality (▶ **Table 1**).

The rate of samples containing an adequate tumor fraction was assessed for PDAC and pNET cases. Tumor fraction was considered adequate if the ratio of tumor cells in a background of benign nucleated cells exceeded 20 % [16, 17].

Diagnostic accuracy was measured as conventional "malignancy" analysis using strict criteria (i. e. samples reported as suspicious for malignancy were categorized as negative). Definitive diagnosis was assessed on surgical specimens whenever available, while in nonresected patients it was based on the diagnostic work-up (combined outcomes of imaging studies and any additional biopsy sample result) and clinical course of the

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▶ **Table 1** Sample quality scores (tissue integrity and blood contamination).

Score	Tissue integrity
0	No cells/tissue
1	Cytological specimen (disaggregated cells representative of the target lesion not allowing for tissue architectural assessment)
2	Histological microfragments (sample adequate for histological evaluation, namely an architecturally intact piece of tissue but without a "core")
3	Histological "core" (defined as an architecturally intact piece of tissue measuring at least 550 µm)
Score	Blood contamination
0	Only blood
1	High blood contamination (> 50 % of the surface of the slide)
2	Moderate blood contamination (25 %–50 % of the surface of the slide)
3	Low blood contamination (< 25 % of the surface of the slide)

disease of at least 6 months [32]. Follow-up was performed by the study investigators at each center by outpatient visits, electronic chart review, and telephone contacts, and was terminated in case of surgical resection or death. The Papanicolaou classification [33] was used to classify both EUS-FNB samples and surgical specimens of pancreatic masses. Low grade tumors (e. g. neuroendocrine tumor, solid pseudopapillary tumor) were considered malignant. Lymph nodes were simply classified as benign or malignant. Specimens that contained inadequate material were considered as negative for malignancy.

Sample size

The sample size was calculated in the context of the primary binary outcome and considering the crossover study design with each lesion sampled using the two techniques. Assuming an expected pooled histological yield of 95 % with wet suction [23] and 85 % with slow pull [34, 35], with $\alpha = 0.05$, power = 0.9, and calculating the proportion of discordant pairs (equal to 0.18) with the approximation of Machin D et al. [36] due to the lack of data in the current literature, the total required sample size was established to be of 185 patients. Assuming an approximate 8 % drop-out rate, a sample size of 200 patients was initially calculated. After the study was initiated, some concerns about a potential higher drop-out rate were raised. Therefore, an amendment to the protocol was made and the drop-out rate was increased to 20 %, resulting in a final sample size of 220 patients.

Statistical analysis

The characteristics of the samples were summarized by descriptive statistics (mean with SD for continuous variables and frequency distributions for categorical variables). The follow-up time in nonresected patients was reported as median with 95 %CI.

Rate of tissue cores, samples containing an adequate tumor fraction, and diagnostic accuracy (defined as true positive + true negative divided by total number of patients) were compared using McNemar test, whereas tissue quality scores were compared by means of Wilcoxon signed-rank test. All the analyses were performed in the overall cohort and in the subgroups of pancreatic and nonpancreatic lesions. A Bonferroni correction for multiple comparisons was applied in the subgroup analyses. A further sub-analysis concerning the primary outcome was performed according to the different needles utilized. Both intention-to-treat (i. e. including all patients who underwent at least one needle pass with each technique) and per-protocol (i. e. two passes with each technique were performed) analyses were scheduled.

In order to account for eventual center effects in the analysis, a random-effects analysis fitting a logistic regression model was performed according to the formula

$$\text{log it}\ (\pi_{ij}) = \alpha + \beta_{treat} X_{ij} + u_j,$$

where π_{ij} is the probability of an event for the ith patient in the jth center, β_{treat} indicates the log odds ratio for treatment, X_{ij} indicates whether the patient received the treatment or control, and u_j is the effect of the jth center [37].

All analyses were performed using R Statistical Software 3.0.2 (Foundation for Statistical Computing, Vienna, Austria) with a statistical level of significance of 5 % and respective 95 % CIs. Sample size calculation was performed with R Statistical Software 3.0.2 (*pwr* package). A two-tailed distribution was used and statistical significance was considered for $P < 0.05$.

Results

Between April 2021 and October 2021, 244 patients were assessed for eligibility. A total of 24 patients were excluded and 220 underwent EUS-FNB. Two and eight patients in groups A and B, respectively, dropped out due to the inability to perform the second needle pass as a result of the onset of anesthesiological complications in three patients (desaturation in two cases and bradycardia in one) and perilesional self-limiting hematoma in seven cases. Consequently, a strict intention-to-treat analysis was not possible due to the lack of comparative samples in these cases, and therefore only a per-protocol analysis was performed. No protocol deviation occurred in the analyzed patients.

A total of 210 patients (mean age 65.9 [SD 11.3] years; 55.5 % male) were analyzed: 108 in Group A and 102 in Group B. The flow chart of the study is presented in ▶ **Fig. 1**. No differences in patient demographics and lesion characteristics were observed (▶ **Table 2**). Overall, there were 146 pancreatic and 64 nonpancreatic lesions, with a mean size of 35.1 (SD 17.5) mm.

Primary outcome

Results of the primary outcome are shown in ▶ **Table 3**. Overall, a tissue core was obtained in 71.4 % and 61.4 % of patients using the wet-suction and slow-pull techniques, respectively ($P = 0.03$). According to the random-effects model adjusted for cen-

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▶ **Table 2** Patient demographic details, lesion characteristics, procedures performed, and outcomes in 210 patients who underwent endoscopic ultrasound-guided fine-needle biopsy performed using the wet-suction and the slow-pull sampling techniques.

Variable	Overall (n = 210)	Group A (wet suction first) (n = 108)	Group B (slow pull first) (n = 102)	*P* value
Age, mean (SD), years	65.9 (11.3)	65.4 (12.0)	66.4 (10.5)	0.51
Sex, n (%)				0.70
▪ Male	114 (54.3)	60 (55.6)	54 (52.9)	
▪ Female	96 (45.7)	48 (44.4)	48 (47.1)	
Lesion site, n (%)				0.17
Submucosal	18 (8.6)	7 (6.5)	11 (10.8)	
▪ Esophagus	1	0	1	
▪ Stomach	13	6	7	
▪ Duodenum	3	0	3	
▪ Rectum	1	1	0	
Pancreatic	146 (69.5)	72 (66.7)	74 (72.5)	
▪ Head/uncinate	80	35	45	
▪ Neck/body	46	27	19	
▪ Tail	20	10	10	
Lymph node	20 (9.5)	13 (12.0)	7 (6.9)	
Other	26 (12.4)	16 (14.8)	10 (9.8)	
▪ Retroperitoneum	8	5	3	
▪ Liver	8	4	4	
▪ Adrenal	4	4	0	
▪ Rectum (perianastomotic granuloma)	1	1	0	
▪ Lung	2	1	1	
▪ Pelvic	1	0	1	
▪ Gallbladder	1	0	1	
▪ Spleen	1	1	0	
Lesion size, mean (SD), mm	35.1 (17.5)	33.5 (17.6)	36.7 (17.3)	0.18
Needle type, n (%)				0.25
▪ Fork-tip 22 G	124 (59.0)	63 (58.3)	61 (59.8)	
▪ Franseen-type 22 G	86 (41.0)	45 (41.7)	41 (40.2)	
Use of fanning technique, n (%)	183 (87.1)	93 (86.1)	90 (88.2)	0.31
Follow-up				0.20
▪ Surgical resection, n (%)	42 (20.0)	18 (16.7)	24 (23.5)	
▪ Evaluation of clinical course, n (%)	168 (80.0)	90 (83.3)	78 (76.5)	
▪ Follow-up in non-resected patients, median (95 %CI), days	187 (141–225)	191 (142–228)	183 (140–222)	
Final diagnosis, n (%)				0.77
▪ Pancreatic ductal adenocarcinoma	113 (53.8)	53 (49.1)	60 (58.8)	
▪ Pancreatic neuroendocrine tumor	19 (9.0)	10 (9.3)	9 (8.8)	
▪ Pancreatitis	9 (4.3)	6 (5.6)	3 (2.9)	

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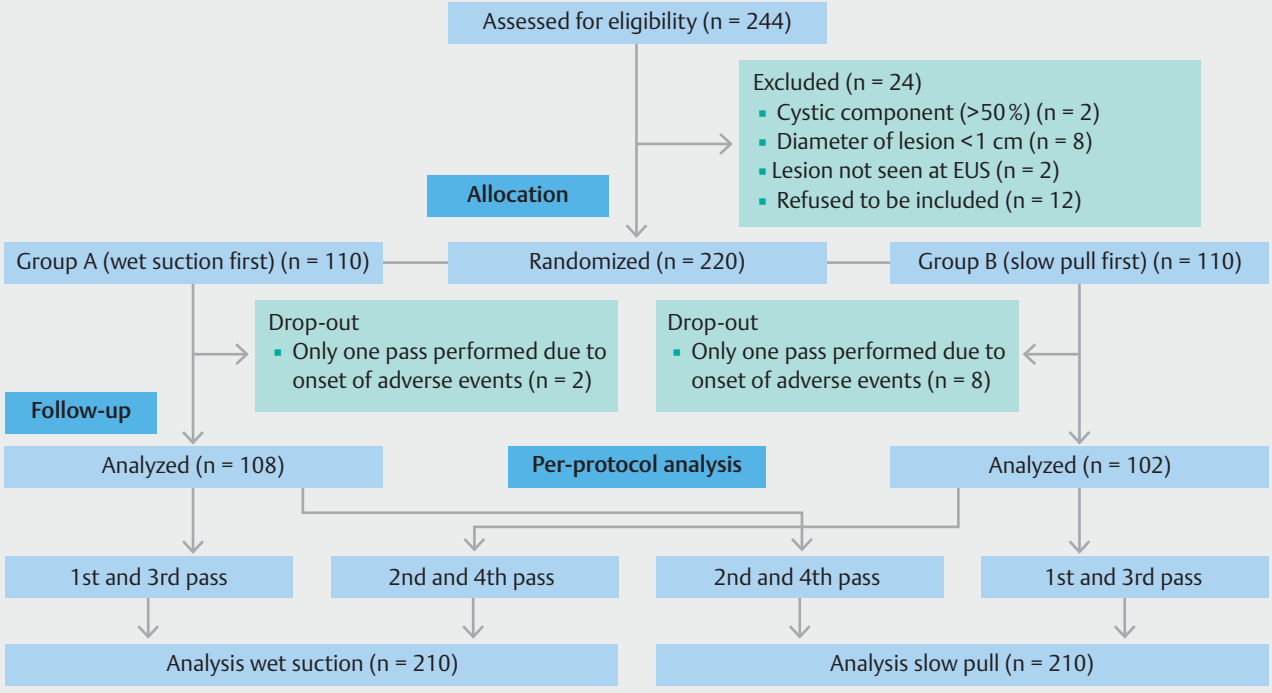
▶ **Table 2** (Continuation)

Variable	Overall (n = 210)	Group A (wet suction first) (n = 108)	Group B (slow pull first) (n = 102)	*P* value
▪ GIST	13 (6.2)	6 (5.6)	7 (6.9)	
▪ Liver metastasis	6 (2.9)	3 (2.8)	3 (2.9)	
▪ Retroperitoneal cancer	6 (2.9)	3 (2.8)	3 (2.9)	
▪ Malignant lymph node	18 (8.6)	12 (11.1)	6 (5.9)	
▪ Other*	26 (12.4)	15 (13.9)	11 (10.8)	

IQR, interquartile range; GIST, gastrointestinal stromal tumor.
* Other includes: pancreatic metastasis (3), intrahepatic cholangiocarcinoma (2), leiomyoma (2), lipoma (2), benign lymph node (2), lung cancer (2), benign adrenal nodule (2), adrenal metastasis (2), gallbladder cancer (1), spleen lymphoma (1), cervical cancer (1), retroperitoneal schwannoma (1), retroperitoneal lymphoepithelial cyst (1), pancreatic solid pseudopapillary neoplasm (1), pancreatic serous cystadenoma (1), duodenal neuroendocrine tumor (1), perianastomotic rectal granuloma (1).



▶ **Fig. 1** CONSORT flow chart of the study [38]. EUS, endoscopic ultrasound.

ter effects, the odds ratio (OR) for tissue core acquisition was 1.58 (95%CI 1.05–2.38; $P=0.03$). No difference for the primary outcome in the subgroup analysis of solid pancreatic lesions (73.3% vs. 67.1%, respectively; $P=0.25$) and in the subgroup of nonpancreatic lesions (67.2% vs. 48.4%, respectively; $P=0.03$ with a significance threshold set at 0.025 based on Bonferroni adjustment for multiple comparisons) was observed between the wet-suction and slow-pull techniques, respectively.

The study population was further stratified according to the needle type. The two needles showed similar performance in obtaining a tissue core based on sampling technique for both solid pancreatic lesions (fork-tip needle: 54/82 (65.9%) for wet suction vs. 54/82 (65.9%) for slow pull, $P>0.99$; Franseen-type needle: 53/64 (82.8%) for wet suction vs. 44/64 (68.8%) for slow pull, $P=0.09$) and nonpancreatic lesions (fork-tip needle: 26/42 (61.9%) for wet suction vs. 18/42 (42.9%) for slow pull, $P=0.12$; Franseen-type needle: 17/22 (77.3%) for wet suction vs. 13/22 (59.1%) for slow pull, $P=0.33$).

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▶ **Table 3** Results of the primary and secondary aims in the overall cohort and in the subgroups of patients with solid pancreatic and nonpancreatic lesions who underwent endoscopic ultrasound-guided fine-needle biopsy performed using the wet-suction and slow-pull sampling techniques.

	Wet suction	Slow pull	*P* value
Presence of tissue core[1], n (%)			
▪ Overall population (n = 210)	150 (71.4)	129 (61.4)	0.03
▪ Pancreatic lesions (n = 146)	107 (73.3)	98 (67.1)	0.25[2]
▪ Nonpancreatic lesions (n = 64)	43 (67.2)	31 (48.4)	0.03[2]
Tissue integrity score, mean (SD)			
▪ Overall population (n = 210)	2.63 (0.62)	2.48 (0.74)	0.02
▪ Pancreatic lesions (n = 146)	2.66 (0.61)	2.55 (0.71)	0.16[2]
▪ Nonpancreatic lesions (n = 64)	2.57 (0.66)	2.30 (0.79)	0.04[2]
Blood contamination score, mean (SD)			
▪ Overall population (n = 210)	2.09 (0.81)	2.44 (0.74)	< 0.001
▪ Pancreatic lesions (n = 146)	2.15 (0.77)	2.45 (0.74)	< 0.001[2]
▪ Nonpancreatic lesions (n = 64)	1.95 (0.88)	2.42 (0.75)	< 0.001[2]
Adequate tumor fraction rate[3], n/N (%)	112/132 (84.8)	106/132 (80.3)	0.41
Diagnostic accuracy, n (%)			
▪ Overall population (n = 210)	192 (91.4)	183 (87.1)	0.16
▪ Pancreatic lesions (n = 146)	132 (90.4)	126 (86.3)	0.28[2]
▪ Nonpancreatic lesions (n = 64)	60 (93.7)	57 (89.1)	0.64[2]

[1] Intact piece of tissue of at least 550 µm.
[2] Based on Bonferroni correction for multiple comparisons, significance threshold was set at 0.025 in subgroup analysis.
[3] Tumor fraction rate was evaluated including only cases of pancreatic ductal adenocarcinomas and neuroendocrine tumors.

Secondary outcomes

Results of secondary outcomes are summarized in ▶ **Table 3**. Overall, tissue integrity score was higher with the wet-suction compared with the slow-pull technique ($P = 0.02$). Center effects-adjusted OR for tissue integrity score was 1.66 (95 %CI 1.12–2.41; $P = 0.02$). However, in the two subgroup analyses, the tissue integrity score was not significantly different between the two techniques ($P = 0.16$ and $P = 0.04$ for solid pancreatic lesions and nonpancreatic lesions, respectively, with a significance threshold set at 0.025 based on Bonferroni adjustment for multiple comparisons). The blood contamination score was higher among slow-pull specimens, both in the overall population, and in the pancreatic and nonpancreatic subgroups, respectively ($P < 0.001$). Center effects-adjusted OR for blood contamination score was 0.56 (95 %CI 0.12–0.81; $P < 0.001$).

The rate of samples with adequate tumor fraction was evaluated among the 132 cases of PDAC and pNETs, and was similar with wet-suction and slow-pull techniques (84.8 % vs. 80.3 %, respectively; $P = 0.41$). Based on random-effects model adjusted for center effects, the OR for adequacy of tumor fraction was 1.35 (95 %CI 0.70–2.55; $P = 0.39$).

Six and eight specimens were deemed not diagnostically adequate using the wet-suction and slow-pull techniques, respectively. Overall, among specimens collected using the wet-suction technique, there were 173 true positives, 19 true negatives, 0 false positives, and 18 false negatives, corresponding to a diagnostic accuracy of 91.4 % (95 %CI 86.8–94.8). Specimens collected using the slow-pull technique were assessed as 163 true positives, 20 true negatives, 0 false positives, and 27 false negatives, corresponding to a diagnostic accuracy of 87.1 % (95 %CI 81.5–91.4), with no significant difference between the two techniques ($P = 0.16$). Based on random-effects model adjusted for center effects, the OR for diagnostic accuracy was 1.58 (95 %CI 0.81–2.93; $P = 0.16$). Similarly, diagnostic accuracy was also comparable when evaluating the subgroup of solid pancreatic lesions ($P = 0.28$) and nonpancreatic lesions ($P = 0.64$).

Discussion

Possible differences in tissue core procurement between available sampling techniques using fork-tip and Franseen-type needles have not been fully investigated. In 2021, Bang et al. published an RCT comparing standard suction vs. slow-pull technique vs. no suction with various types of needles [39]. This study showed that, in contrast to side-fenestrated reverse-bevel, and Menghini-tip needles, for Franseen-type and fork-tip needles there was no difference between the standard

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suction and slow-pull techniques, and neither technique significantly improved the rate of diagnostic adequacy and accuracy when compared with no suction [39]. However, the wet-suction method was not evaluated, and the potential advantage of this technique remains unknown. To better clarify this issue, we performed a randomized, crossover trial with the primary aim of comparing wet-suction and slow-pull techniques in their capability to acquire proper tissue "core" samples for histological evaluation.

In the overall study population evaluated in the present study, wet suction showed a higher rate of tissue core acquisition. However, this statistical difference was mainly related to the higher rate of tissue core retrieved in the subgroup of nonpancreatic lesions (67.2 % vs. 48.4 %). In this subgroup of patients, we also found that wet suction provided a higher tissue integrity score. For both outcomes, in this subgroup of patients, statistical significance was not reached but a trend toward significance was observed ($P = 0.03$ and $P = 0.04$ for tissue core rate and tissue integrity score, respectively, with a significance threshold set at 0.025 based on Bonferroni adjustment for multiple comparisons) and it is likely that, with a larger population, significance would have been achieved. In contrast, for solid pancreatic lesions, tissue core procurement rate and tissue integrity score were similar between the two techniques. The present study seems to support the findings of a previous pilot study comparing wet suction with slow pull and standard suction for sampling of solid pancreatic lesions using end-cutting needles, where all three techniques resulted in similar histological yields [24].

As previously described for EUS-FNA [22], slow-pull specimens resulted in lower blood contamination compared with wet-suction samples. This finding differs from the pilot study mentioned above, in which blood contamination was similar regardless of the sampling technique used [24]. However, the blood contamination score used was extremely simplified (score 0 = blood present and 1 = blood clots present), thus limiting the possibility of accurately differentiating and stratifying the results. On the other hand, the significance of blood contamination in the assessment of histological samples could be questioned. Indeed, no data demonstrated that blood contamination impairs diagnostic accuracy or the capability to retrieve histological tissue. Indeed, in the present study, samples collected using wet suction were highly contaminated with blood. Nevertheless, the other outcomes were similar to or even better than those obtained with the slow-pull technique.

In particular, diagnostic accuracy was slightly higher with wet suction for both solid pancreatic (90.4 % vs. 86.3 %) and nonpancreatic (93.7 % vs. 89.1 %) lesions, despite a significant difference not being observed. A similar result was observed for tumor fraction adequacy. Based on these results, in patients with solid pancreatic lesions, the choice of technique between slow pull and wet suction strongly depends on agreement with and preference of the pathologist, considering the lower blood contamination using the slow-pull method. In patients with nonpancreatic lesions, our study seems to favor the use of wet suction over slow pull due to the higher rates of tissue core acquisition, with higher tissue integrity score. However, our findings should be interpreted with caution for different reasons. First, the number of patients included may be insufficient to detect a significant difference in clinically important outcomes such as diagnostic accuracy. Second, we included all nonpancreatic lesions, and further confirmation in specifically designed and adequately powered studies focused on these types of lesions are needed. Nonetheless, to the best of our knowledge, this is the first study to compare wet-suction and slow-pull techniques for EUS-FNB of nonpancreatic lesions.

The difference we observed between solid pancreatic lesions and nonpancreatic lesions can be easily explained by the nature of the lesion biopsied. In fact, most solid pancreatic lesions were PDAC, which are characterized by a large amount of fibrous stroma increasing mass stiffness. Therefore, it is plausible that the end-cutting design of the needles used in this study allowed the coring of hard lesions such as PDACs regardless of the sampling technique. In contrast, nonpancreatic lesions are usually softer, and, therefore, the application of suction might have impacted the quantity of aspirated tissue into the needle. No differences in the primary outcome between the two needles used in this study were detected. This result is consistent with previous literature reporting the same performance of the fork-tip and Franseen-type needles [11–14].

Our study has some limitations. First, all involved centers were highly experienced, and results might not be reproducible in other settings. Second, we excluded very small lesions because performing four needle passes on lesions of < 1 cm is often difficult and in the case of solid pancreatic lesions may increase the risk of acute pancreatitis. Therefore, we cannot be sure that our results would be similar in small lesions, especially considering the reported impact of lesion size on the outcome of EUS sampling [40]. Third, we used a single needle caliber, and our findings could be different with both larger and smaller needles. Fourth, both fork-tip and Franseen-type needles were used without randomization. Further studies, adequately powered, should investigate and compare the performance of these two needle types using different sampling techniques. Fifth, we included both pancreatic and nonpancreatic lesions, and subgroup analyses were performed. Because the performance of EUS-FNB can be different in pancreatic and nonpancreatic lesions, and this trial was not powered to compare the two techniques based on the lesion type, further studies are needed to properly evaluate this matter. Sixth, despite the quite large number of patients included, our study may not have been adequately powered, as the definitions of histological yield and tissue core are not standardized, and the sample size calculation was based on previous literature that reported a very high histological yield, which was different from the tissue core rate we observed in the present study. Finally, we did not evaluate the standard suction technique. However, a recent meta-analysis of EUS-FNA RCTs comparing the slow-pull technique with standard suction demonstrated similar adequacy, but lower blood contamination and slightly higher accuracy using the slow-pull technique, making this technique preferred over standard suction in modern practice [22].

In conclusion, our study demonstrated that EUS-FNB performed with wet suction provided a higher tissue core procure-

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ment rate than a slow-pull technique. Diagnostic accuracy and rate of samples with adequate tumor fraction were slightly higher using wet suction, but no statistically significant difference between the two techniques was observed. Further large, specifically powered, multicenter studies are needed to definitively recommend one of these two techniques.

Competing Interest

S.F. Crinò has received speaker fees from Steris Endoscopy. A. Larghi is a consultant for Pentax and Boston Scientific, and has received a research grant from Medtronic and teaching fee from Taewoong. À. Ginès is a consultant for Cook Endoscopy. R. Di Mitri is a consultant for Boston Scientific. O. Sendino is a consultant for and has received speaker fees from B. Braun. M.C. Conti Bellocchi, F. Inzani, M. Rimbas, A. Lisotti, G. Manfredi, A.Y.B. Teoh, B. Mangiavillano, L. Bernardoni, E. Manfrin, D. Scimeca, E. Unti, A. Carlino, T. Voiosu, R.B. Mateescu, P. Fusaroli, S. Lega, E. Buscarini, L. Pergola, S.M. Chan, L. Lamonaca, G. Fernández-Esparrach, and A. Facciorusso declare that they have no conflict of interest.

Clinical trial

Trial Registration: ClinicalTrials.gov | Registration number (trial ID): NCT04834193 | Type of study: Prospective, randomized, multi-center, cross-over study

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