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CLINICAL—PANCREAS

Endoscopic Ultrasound–guided Fine-needle Biopsy With or Without Rapid On-site Evaluation for Diagnosis of Solid Pancreatic Lesions: A Randomized Controlled Non-Inferiority Trial

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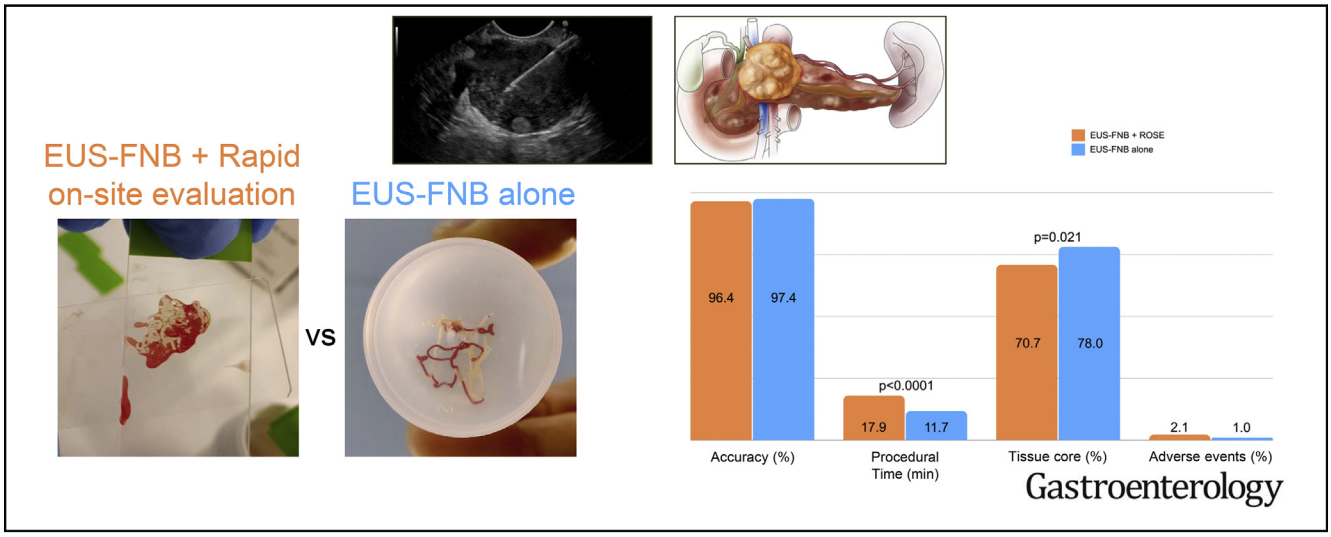

BACKGROUND AND AIMS: The benefit of rapid on-site evaluation (ROSE) on the diagnostic accuracy of endoscopic ultrasound–guided fine-needle biopsy (EUS-FNB) has never been evaluated in a randomized study. This trial aimed to test the hypothesis that in solid pancreatic lesions (SPLs), diagnostic accuracy of EUS-FNB without ROSE was not inferior to that of EUS-FNB with ROSE. **METHODS:** A noninferiority study (noninferiority margin, 5%) was conducted at 14 centers in 8 countries. Patients with SPLs requiring tissue sampling were randomly assigned (1:1) to undergo EUS-FNB with or without ROSE using new-generation FNB needles. The touch-imprint cytology technique was used to perform ROSE. The primary endpoint was diagnostic accuracy, and secondary endpoints were safety, tissue core procurement, specimen quality, and sampling procedural time. **RESULTS:** Eight hundred patients were randomized over an 18-month period, and 771 were analyzed (385 with ROSE and 386 without). Comparable diagnostic accuracies were obtained in both arms (96.4% with ROSE and 97.4% without ROSE, $P = .396$). Noninferiority of EUS-FNB without ROSE was confirmed with an absolute risk difference of 1.0% (1-sided 90% confidence interval, −1.1% to 3.1%; noninferiority $P < .001$). Safety and sample quality of histologic specimens were similar in both groups. A significantly higher tissue core rate was obtained by EUS-FNB without ROSE (70.7% vs. 78.0%, $P = .021$), with a significantly shorter mean sampling procedural time (17.9 ± 8.8 vs 11.7 ± 6.0 minutes, $P < .0001$). **CONCLUSIONS:** EUS-FNB demonstrated high diagnostic accuracy in evaluating SPLs independently on execution of ROSE. When new-generation FNB needles are used, ROSE should not be routinely recommended. (ClinicalTrial.gov number NCT03322592.)

Keywords: Pancreatic Cancer; Preoperative Sampling; Endoscopic Ultrasound Tissue Acquisition; Diagnostic Accuracy.

Endoscopic ultrasound (EUS)-guided fine-needle aspiration (EUS-FNA) with cytologic rapid on-site evaluation (ROSE) represents an efficient diagnostic modality for evaluation of solid pancreatic lesions (SPLs).[1–3] Despite controversial results on various meta-analyses,[4–7] ROSE has convincing advantages of providing timely feedback on sample adequacy and optimizing the number of needle passes performed. However, the complexity of pancreatic cytopathologic expertise development and related costs has restricted the availability of ROSE in tertiary care centers and, even more importantly, in community hospitals.[8] Consequently, limited accuracy of EUS-FNA in the absence of ROSE might have constrained widespread utilization of EUS-guided sampling globally.[9] Additionally, performance of immunohistochemical staining, required to diagnose certain diseases, may be limited on cytologic samples.[10–14]

To overcome the limitations of ROSE, efforts have been made to succeed in acquiring samples for histologic evaluation.[15] Indeed, architecturally intact histologic tissue specimens are more easily assessable by most pathologists, and immunohistochemical stains are easier to perform on tissue than on cytologic samples. Moreover, EUS formalin-fixed paraffin-embedded histologic samples are suitable to perform molecular diagnostics.[16]

The newest generation of histology needles have recently become available, divided into those with a modified tip with cutting edges and those with a forward-facing bevel on a side fenestration. All needles demonstrated higher histologic and diagnostic yield compared with standard FNA needles,[17–20] and a recent randomized trial[21] and a meta-analysis[22] demonstrated that EUS-guided fine-

Abbreviations used in this article: **AE, adverse event; CI, confidence interval; EUS, endoscopic ultrasound; EUS-FNA, endoscopic ultrasound–guided fine-needle aspiration; EUS-FNB, endoscopic ultrasound–guided fine-needle biopsy; ROSE, rapid on-site evaluation; SPL, solid pancreatic lesion; TIC, touch-imprint cytology.**

Most current article

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WHAT YOU NEED TO KNOW

BACKGROUND

EUS-FNB showed equal diagnostic accuracy of EUS-FNA performed with ROSE. Whether ROSE can improve EUS-FNB performance for diagnosis of solid pancreatic lesions is still unknown.

FINDINGS

The accuracy of EUS-FNB without ROSE demonstrated noninferiority to EUS-FNB with ROSE (97.4% vs 96.4%; noninferiority margin, 5%) with an absolute risk difference of 1.0% (2-sided 90% CI, −1.1 to 3.1, noninferiority $P < .001$).

IMPLICATIONS FOR PATIENT CARE

EUS-FNB could be done without ROSE, with important implications for costs, hospital resources allocation, and global widespread EUS-sampling utilization.

needle biopsy (EUS-FNB) has equal diagnostic yield compared with EUS-FNA+ROSE.

Importantly, valuable cytologic specimens can also be obtained from samples acquired with FNB needles and performing ROSE using the touch-imprint cytology (TIC) technique.[23,24] TIC showed an even higher accuracy than conventional ROSE.[24] Theoretically, the availability of both cytologic and histologic specimens could represent a diagnostic advantage over EUS-FNB alone, as suggested by recent large retrospective studies.[25,26] However, EUS-FNB alone performed with new FNB needles demonstrated diagnostic accuracy of greater than 90% in prospective studies,[27,28] and no randomized trial has investigated the benefit of ROSE with TIC on FNB specimens. Therefore, we hypothesized that EUS-FNB alone was noninferior to EUS-FNB+ROSE, and we aimed to compare the diagnostic accuracy of EUS-FNB alone versus EUS-FNB+ROSE with TIC in patients with newly diagnosed SPLs.

Methods

Study Design

This international, multicenter, randomized, noninferiority study was approved by the ethics committee of the provinces of Verona and Rovigo on October 17, 2017 (protocol no. 50348) and subsequently by ethics committees and institutional review boards of all participating centers. The protocol was registered at ClinicalTrial.gov (NCT03322592). All patients signed informed consent before the procedure. All authors had access to the study data and reviewed and approved the final manuscript.

Patient Population

Consecutive patients with SPLs referred for EUS evaluation were assessed for study eligibility. Patients were included if they were ≥18 years, with a lesion likely to be visualized and sampled by EUS, and able to provide informed consent. Patients with bleeding disorders (uncorrectable with co-factors or fresh frozen plasma), concomitant anticoagulant use (not to be discontinued), international normalized ratio > 1.5, platelet count < 50,000, lesions with cystic component, included in another study, or pregnant were excluded.

Included patients were randomly assigned to undergo EUS-FNB or EUS-FNB+ROSE. Randomization (1:1) was automatically generated by a web-based randomization module at the time of the EUS procedure through a designated study website using random 10-patient block sizes for allocation concealment between groups. Data were collected using online case record forms.

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. Selection of needle type (22G SharkCore [Medtronic, Minneapolis, MN], 22G Acquire [Boston Scientific, Marlborough, MA], or 20G ProCore [Cook Medical, Bloomington, IN]) and sampling technique (eg, suction, slow-pull, or wet suction) were left at the discretion of the endosonographer, who used the fanning technique whenever possible.[29]

In the EUS-FNB+ROSE arm, collected specimens were first processed for ROSE using the TIC technique. Acquired material was placed on a smear by gently pushing and rubbing down with other slide(s), allowing transfer of superficial cells. Any remaining preserved worm-like tissue was removed from the slide and transferred in formalin for histologic evaluation.[23,30] The procedure was repeated until adequacy was reached according to the cytopathologist up to a maximum of 3 passes.[31,32] In case of adequate ROSE, after the first or second pass additional passes up to 3 were performed for histologic evaluation.

In the EUS-FNB alone arm, 3 needle passes were performed following the suggestion of the European Society of Gastrointestinal Endoscopy[32] guidelines unless adverse events (AEs) occurred, with all specimens placed into a single vial with formalin for subsequent histopathologic evaluation. Patients were excluded if the lesion was impossible to puncture.

Definition of Study Endpoints

Primary endpoint. The primary endpoint was diagnostic accuracy defined as the percentage of sampled lesions that corresponded to the final diagnosis.[31] Definitive diagnosis was defined by histopathologic evaluation of the surgical specimen or in nonresected patients by the evolution of the disease assessed for at least 6 months by a combination of clinical course, imaging studies, and/or additional tissue sampling.[31] Follow-up was performed by the study investigators at each participating center by outpatient visits, electronic chart review, and telephone contacts and was terminated in case of surgical resection or death.

Histologic and ROSE evaluations followed the Papanicolaou classification.[33] Specimens defined as malignant and suspicious for malignancy (category 5) were categorized as positive. Those specimens reporting a specific tumor (low-grade or benign, eg, neuroendocrine tumor, solid pseudopapillary tumor, gastrointestinal stromal tumor, paraganglioma, solid serous cystadenoma) or disease/condition (eg, autoimmune pancreatitis, intrapancreatic spleen) were counted as positive and deemed accurate if the specific tissue diagnosis matched the final diagnosis. Nonspecific benign conditions (eg, chronic pancreatitis)

and atypical samples (category 3) were considered negative. Specimens that contained inadequate material were not excluded from the primary analysis and were considered false negatives. Additional analyses using more restrictive criteria (samples reported as suspicious for malignancy [category 5] categorized as negative) were also performed.

Secondary endpoints. Safety was defined by the rate of AEs classified according to the international American Society for Gastrointestinal Endoscopy lexicon.[34] AEs were assessed by telephone contact or by outpatient visit 3 and 15 days after the procedure.[35]

Positive core procurement yield was defined by the procurement of an intact piece of tissue of at least 550 μm.[35,36] Time of the sampling procedure was calculated from needle insertion in the echoendoscope working channel for the first pass until its removal after the third pass. Sample quality was evaluated by applying a previously used score for tissue quantity and blood contamination (Supplementary Table 1).[35,37] Diagnostic accuracy, AE rates, core procurement, and sample blood contamination were compared between different needles used.

Sample Size

Sample size was calculated based on evaluation of the primary outcome of diagnostic accuracy for evaluation of SPLs. A sample size of 365 patients per arm was required, based on the expected diagnostic accuracy of 92% for both arms,[3,38] a noninferiority margin of 5%, power of 80%, and an alpha level of 5% (1-sided). The sample size was increased to 400 patients per arm to account for 9.5% loss to follow-up and drop-out. Noninferiority was met for the primary endpoint if the lower bound of the 2-sided 90% confidence interval (CI) (equivalent to the lower bound of a 1-sided 97.5% CI) for the difference excluded a 5% or greater difference in favor of the control arm. Quality indicators for EUS sampling[39] recommended a performance target higher than 85%, thus a modest noninferiority margin of 5% was chosen.

Statistical Analysis

A strict intention-to-treat analysis was not possible because of withdrawals of consent and loss to follow-up. Therefore, a modified intention-to-treat analysis, including all patients with confirmed SPLs who underwent randomization and technical success was achieved and for whom data regarding the primary outcome was available, was used to establish if the lower confidence limit for the difference in effect between arms was above −5%. No major protocol deviations occurred; thus, the per-protocol population analysis completely reflected the modified intention-to-treat analysis.

Continuous variables were expressed as mean ± SD or as median with interquartile range, and categorical variables were expressed as frequencies with percentages. Comparisons of secondary outcomes were performed using the Student's t test for normally distributed continuous variables, Wilcoxon rank-sum test for non-normally distributed continuous variables, and the χ^2 or Fisher's exact test for categorical variables. A comparison of operating characteristics (sensitivity, specificity, and accuracy) of ROSE and EUS-FNB+ROSE for the patients randomized to the EUS-FNB+ROSE arm was performed using McNemar's test (paired, binary data).

Factors influencing the diagnostic accuracy of procedures were assessed by using a logistic regression model. The coefficients obtained from the logistic regression analysis were expressed in terms of odds ratio. Adjustments were made for multiple comparisons by using the Tukey test. A $P < .05$ was considered statistically significant. Data were analyzed using R software (version 4.0.3).

Results

Among 845 patients evaluated, 800 patients were considered eligible for the study and randomized between April 2018 and September 2019. Twenty-nine patients were subsequently excluded (Figure 1); thus, the study cohort was made of 771 randomized patients (men, 56.4%; mean age, 67.5 ± 11.5), of whom 385 were allocated to the EUS-FNB+ROSE arm and 386 to the EUS-FNB alone arm. No differences in patient demographics and clinical characteristics were observed (Table 1). Final diagnosis for the analyzed patients according to the study groups is listed in Table 1. Pathologic results of EUS samples according to study arm and reference standard are shown in Supplementary Table 2.

Primary Outcome

Performance characteristics of EUS-FNB+ROSE and EUS-FNB alone are summarized in Table 2. In the EUS-FNB+ROSE arm, the cumulative rate of adequate samples on ROSE was 59.2% (228/385) on first biopsy pass, 76.4% (294/385) on the second pass, and 90.4% (348/385) on the third pass, with an overall diagnostic accuracy of 82.9% (319/385). After evaluation of histologic specimens collected during or after ROSE, adequacy was raised to 98.4% (379/385) with a correct diagnosis obtained in 371 of 385 patients, corresponding to a diagnostic accuracy of 96.4% (95% CI, 94.0%–98.0%), a sensitivity of 96.0% (95% CI, 93.5%–97.7%), a specificity of 100% (95% CI, 73.5%–100%), a positive predictive value of 100% (95% CI, 99.0%–100%), and a negative predictive value of 46.2% (95% CI, 26.6%–66.6%). Detailed comparative analyses of the results of the ROSE evaluation alone vs ROSE plus additional histologic specimens acquired by EUS-FNB in patients in the EUS-FNB+ROSE arm are reported in the Supplementary Methods.

In the EUS-FNB alone arm, adequacy rate was 98.7% (381/386), with a correct diagnosis obtained in 376 of 386 patients corresponding to a diagnostic accuracy of 97.4% (95% CI, 95.3%–98.8%), a sensitivity of 97.3% (95% CI, 95.1%–98.7%), a specificity of 100% (95% CI, 78.2%–100%), a positive predictive value of 100% (95% CI, 99.0%–100%), and a negative predictive value of 60.0% (95% CI, 38.7%–78.9%). The difference in accuracy between study arms was 1.0% (2-sided 90% CI, −1.1% to 3.1%). Because the lower limit of CI for this difference exceeded the noninferiority margin of −5% (Figure 2), noninferiority was shown for diagnostic accuracy ($P <$ .001). Results were similar when samples interpreted as suspicious for malignancy were considered as negative findings (Supplementary Table 3). Comparison of other

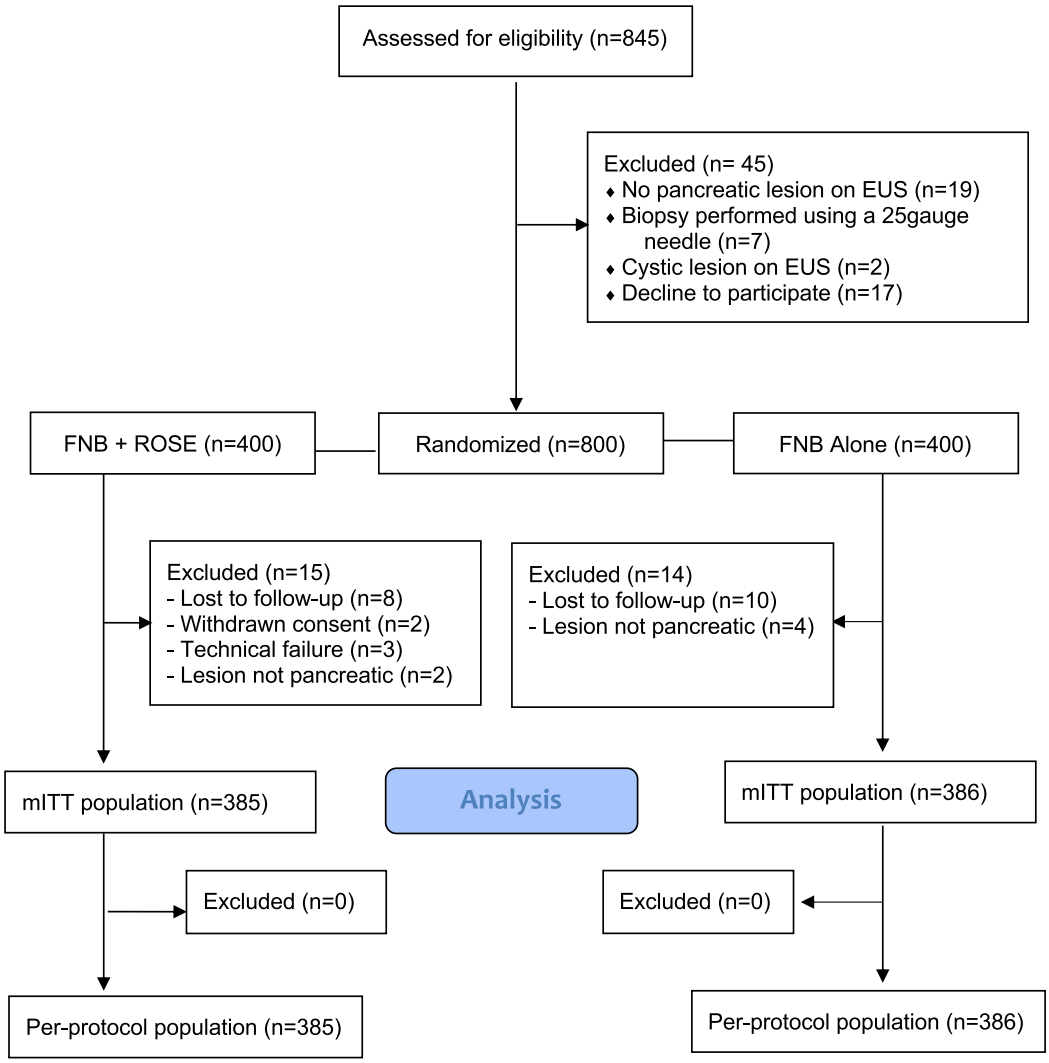

Figure 1. CONSORT flowchart of the study. CONSORT, Consolidated Standards of Reporting Trials; mITT, modified intention-to-treat.

diagnostic measures confirmed the robustness of the study hypothesis showing noninferiority (equivalence) of EUS-FNB alone vs EUS-FNB+ROSE (Table 2). On multivariable analysis, only needle type was associated with diagnostic accuracy, in favor of end-cutting needles (Supplementary Table 4). However, additional analyses of the population stratified for each needle type used showed a similar diagnostic yield of EUS-FNB with or without ROSE (Supplementary Table 5). Diagnostic accuracy, sensitivity, and negative predictive value of each sampling technique (ie, suction, slow-pull, or wet suction) were also comparable in the 2 study arms (Supplementary Table 6).

Secondary Outcomes

Safety, core procurement yield, procedural time, and tissue quality. The results of secondary outcomes are shown in Table 3. Overall, 12 postprocedural AEs (1.6%) were observed with similar rates in the 2 arms ($P = .262$). In the EUS-FNB+ROSE arm there were 6 episodes of acute pancreatitis managed conservatively, 1 pancreatic leak with pseudocyst formation treated endoscopically, and 1 episode of stroke. In the EUS-FNB alone arm 1 patietn had acute pancreatitis that was managed conservatively, 1 case of bleeding with a >2-g/dL drop in hemoglobin not requiring transfusion, 1 case of syncope, and 1 death after a single combined session of EUS sampling followed by ERCP from unexplained causes (autopsy was not performed).

A significantly higher rate of tissue core was obtained in the EUS-FNB alone arm compared with EUS-FNB+ROSE (78.0% vs 70.7%, $P = .021$), with a significant shortened procedural time (mean time, 11.7 ± 6.0 vs 17.9 ± 8.8 minutes; $P < .0001$). Tissue integrity and blood contamination scores were comparable between the 2 arms ($P = .154$ and $P = .338$, respectively).

Diagnostic measures according to needle type. In a nonrandomized comparison, lower accuracy and core procurement rates and higher blood contamination of samples were observed using the 20G side-fenestrated needle compared with 22G end-cutting needles. The safety profile was similar between all needles (Table 4).

Table 1. Demographic and Clinical Characteristics of Patients with SPLs Who Underwent EUS-FNB With or Without ROSE

	EUS-FNB+ROSE (n = 385)	EUS-FNB Alone (n = 386)	Overall (n = 771)
Gender			
Male	225 (58.4)	210 (54.4)	435 (56.4)
Female	160 (41.6)	176 (45.6)	336 (43.6)
Age, y, mean ± SD	67.5 ± 11.6	67.3 ± 11.4	67.4 ± 11.5
Needle used			
Side-fenestrated 20G	77 (20.0)	80 (20.7)	157 (20.4)
Fork-tip 22G	77 (20.0)	86 (22.3)	163 (21.1)
Franseen-tip 22G	231 (60.0)	220 (57.0)	451 (58.5)
Lesion site			
Uncinate	42 (10.9)	46 (11.9)	88 (11.4)
Head	183 (47.5)	193 (50.0)	376 (48.8)
Neck	25 (6.5)	24 (6.2)	49 (6.4)
Body	96 (24.9)	83 (21.5)	179 (23.2)
Tail	39 (10.1)	40 (10.4)	79 (10.2)
Lesion size, mm			
Mean ± SD	31.6 ± 12.6	31 ± 12.8	31.3 ± 12.7
Median (IQR)	30 (24–39)	30 (22–37)	30 (23–38)
Biopsy route			
Stomach	163 (42.3)	153 (39.6)	316 (41.0)
Duodenal bulb	114 (29.6)	131 (33.9)	245 (31.8)
Second duodenum	108 (28.1)	102 (26.4)	210 (27.2)
Sampling technique			
Suction	125 (32.5)	120 (31.1)	245 (31.8)
Slow-pull	237 (61.6)	237 (61.4)	474 (61.5)
Wet suction	21 (5.4)	29 (7.5)	50 (6.5)
No suction	2 (0.5)	0	2 (0.3)
Fanning	358 (93)	358 (92.8)	716 (92.9)
No. of passes, mean ± SD	2.88 ± 0.39	2.95 ± 0.25	2.91 ± 0.37
Final diagnosis			
Pancreatic ductal adenocarcinoma	311 (80.8)	304 (78.8)	615 (79.8)
Malignant other[a]	18 (4.7)	29 (7.5)	47 (6.1)
Pancreatic neuroendocrine tumor	26 (6.8)	27 (7.0)	53 (6.9)
Neoplastic other[b]	8 (2.1)	2 (0.5)	10 (1.3)
Inflammatory/benign[c]	22 (5.7)	24 (6.2)	46 (6.0)
Follow-up			
Surgical resections	101 (26.2)	91 (23.6)	192 (24.9)
Evaluation of clinical course	284 (73.8)	295 (76.4)	579 (75.1)
Median follow-up time in nonresected patients, days (IQR)	274 (175–212)	270 (153–403)	273 (169–367)
Repeated sampling	5 (1.3)	9 (2.3)	14 (1.8)
Centers			
Verona	46 (11.9)	48 (12.4)	94 (12.2)
Palermo (ARNAS)	44 (11.4)	42 (10.9)	86 (11.2)
Adelaide	39 (10.1)	40 (10.4)	79 (10.2)
Palermo (ISMETT)	34 (8.8)	34 (8.8)	68 (8.8)
Milan (ASST Rhodense)	30 (7.8)	34 (8.8)	64 (8.3)
Milan (Humanitas)	25 (6.5)	25 (6.5)	50 (6.5)
Wakayama	23 (6.0)	25 (6.5)	48 (6.2)
Brussels	25 (6.5)	21 (5.4)	46 (6.0)

Discussion

In this multicenter, international, randomized controlled trial using new-generation biopsy needles for evaluation of SPLs, we found that EUS-FNB alone was not inferior to EUS-FNB+ROSE. Until now, EUS-FNA+ROSE has been the preferred technique for EUS-guided tissue acquisition, with ROSE as the most important factor affecting EUS-FNA

Table 1. Continued

	EUS-FNB+ROSE (n = 385)	EUS-FNB Alone (n = 386)	Overall (n = 771)
Barcelona	22 (5.7)	22 (5.7)	44 (5.7)
Rome	20 (5.2)	20 (5.2)	40 (5.2)
Charlottesville	20 (5.2)	20 (5.2)	40 (5.2)
Rotterdam	21 (5.4)	17 (4.4)	38 (4.9)
Stockholm	18 (4.7)	20 (5.2)	38 (4.9)
Tokyo	18 (4.7)	18 (4.7)	36 (4.7)

Values are n (%) unless otherwise defined.
[a]Malignant other includes metastases (n = 23), neuroendocrine carcinoma (n = 8), undifferentiated carcinoma (n = 6), lymphoma (n = 4), acinar carcinoma (n = 2), mixed ductal/acinar neuroendocrine carcinoma (n = 2), hepatoid carcinoma (n = 1), and cholangiocarcinoma (n = 1).
[b]Neoplastic other includes solid pseudopapillary tumor (n = 7), gastrointestinal stromal tumor (n = 1), paraganglioma (n = 1), and myeloma (n = 1).
[c]Inflammatory/benign includes chronic pancreatitis (n = 26), autoimmune pancreatitis (n = 7), intrapancreatic spleen (n = 5), pseudosolid serous cystadenoma (n = 3), steatonecrosis (n = 3), and lymphoid tissue (n = 2).

diagnostic yield, as confirmed in a large randomized controlled trial on 351 patients.[40] On the other hand, newly introduced needles specifically designed for EUS-FNB have recently achieved a significantly better diagnostic accuracy over standard FNA needles in various randomized studies[17,18,41] and in a large retrospective study on 2127 SPLs.[24] Based on these results and a subsequent meta-analysis,[19] some authors claimed that EUS-FNB could replace EUS-FNA for SPL sampling. Nevertheless, 2 recent retrospective studies found that cytologic samples obtained with the TIC technique and standard ROSE with FNB and FNA needles, respectively, were comparable.[23,25] One of these studies revealed that EUS-FNB alone had a significantly lower accuracy than EUS-FNB+ROSE (80.7% vs 93.1%, $P = .001$), thus suggesting a potential benefit of ROSE even during EUS-FNB.[25]

To better clarify this issue, we performed a study based on the hypothesis that EUS-FNB alone would not be inferior to EUS-FNB+ROSE. Our results showing comparable diagnostic accuracies of EUS-FNB alone and EUS-FNB+ROSE (97.4% vs 96.4%) confirmed our assumption to be correct. Importantly, the 96.4% accuracy of EUS-FNB+ROSE was reached only by adding histologic samples collected during or after TIC, which raised accuracy by 13.5% from 82.9%. De Moura et al[25] reported a similar but less relevant increase in diagnostic accuracy with the adjunct of histologic samples to EUS-FNA+ROSE. Our results coupled with the above-mentioned findings strongly support that EUS-FNB could definitively replace EUS-FNA+ROSE.[17–21]

Overall, diagnostic accuracy exceeded 95% in both arms, a finding that has relevant implications on EUS service organization and resource allocation. Centers with an established ROSE service can re-evaluate the need for it, whereas institutions lacking a ROSE service can reach an outstanding diagnostic accuracy using EUS-FNB alone. In our view, EUS-FNB availability will facilitate widespread use of EUS-guided sampling, especially in the community, which has been limited by the need for ROSE.[9]

The significantly higher rate of tissue core procurement observed in the EUS-FNB alone arm compared with the EUS-FNB+ROSE arm is easily explained by reduction of the overall histologic material in the ROSE arm because of its partial use of TIC. Conversely, both tissue quantity and blood contamination were comparable in both arms. Interestingly, needle design had an impact on diagnostic accuracy in the multivariable analysis in favor of end-cutting needles,

Table 2. Diagnostic Measures of EUS-FNB+ROSE and EUS-FNB Alone for Evaluation of SPLs

	EUS-FNB+ROSE (n = 385)		EUS-FNB Alone (n = 386)		
Diagnostic Measures	% (n/N)	95% CI	% (n/N)	95% CI	P[a]
Sensitivity[b]	96.0 (359/373)	93.5–97.7	97.3 (361/371)	95.1–98.7	.324
Specificity	100 (12/12)	73.5–100	100 (15/15)	78.2–100	NS
Positive predictive value	100 (359/359)	99.0–100	100 (361/361)	99.0–100	NS
Negative predictive value	46.2 (12/26)	26.6–66.6	60.0 (15/25)	38.7–78.9	.738
Accuracy[b]	96.4 (371/385)	94.0–98.0	97.4 (376/386)	95.3–98.8	.396

CI, confidence interval; NS, not significant.
[a]*P*-value for the null hypothesis of inferiority (ie, EUS-FNB alone inferior to EUS-FNB+ROSE).
[b]Inadequate samples were included in the calculation of sensitivity and accuracy as false-negative pathologist's interpretation.

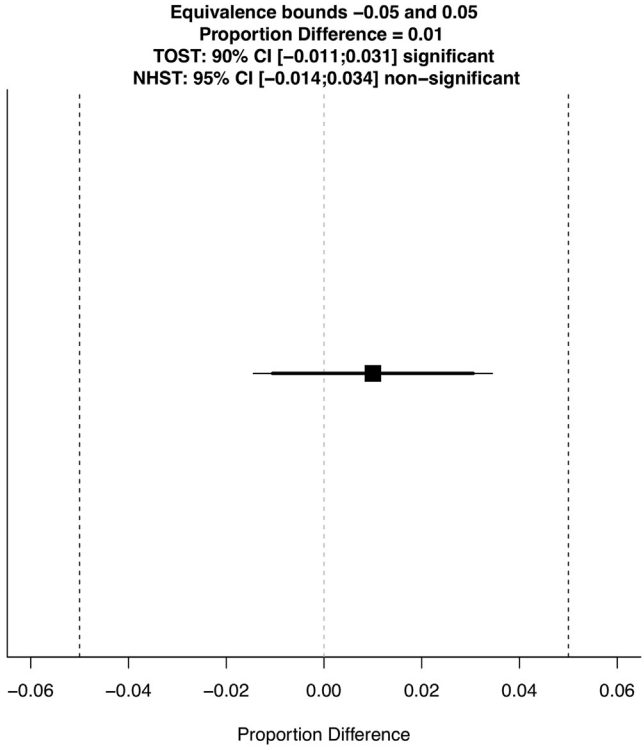

Figure 2. Results of noninferiority for diagnostic accuracy. The noninferiority margin was –0.05. The null hypothesis test was not significant ($P = .396$). The equivalence test based on Fisher's exact z-test was significant ($P < .001$). CI, confidence interval; NHTS, confidence interval for the null hypothesis test; TOST (2 one-sided tests), confidence interval for the equivalence (noninferiority) hypothesis.

Table 3. Secondary Outcomes

Outcome	EUS-FNB +ROSE (n = 385)	EUS-FNB Alone (n = 386)	*P*
Adverse events	8 (2.1)	4 (1.0)	.268
Core[a] procurement	272 (70.7)	301 (78.0)	.021
Time of the sampling, min			<.0001
Mean ± SD	17.9 ± 8.8	11.7 ± 6.0	
Median (interquartile range)	15 (11–21)	10 (7–15)	
Tissue quantity			.154
No tissue	6 (1.6)	1 (0.3)	
Limited cytologic interpretation	10 (2.6)	8 (2.1)	
Adequate cytologic samples	21 (5.4)	14 (3.6)	
Microfragments	76 (19.7)	62 (16.0)	
1–5 cores[a]	140 (36.4)	144 (37.3)	
6–10 cores[a]	76 (19.7)	83 (21.5)	
>10 cores[a]	56 (14.6)	74 (19.2)	
Blood contamination			.338
Only blood	0	1 (0.2)	
>50% of the slide	70 (18.2)	77 (20.0)	
25%–50% of the slide	162 (42.1)	142 (36.8)	
<25% of the slide	153 (39.7)	166 (43.0)	

Values are n (%) unless otherwise defined.
[a]Cores were 550 μm.

which also provided a better sample quality compared with the side-fenestrated needle, in agreement with results of 2 recent randomized controlled trials comparing these needles for SPL sampling.[42,43]

Obtaining tissue core biopsy samples for histologic examination represents a clear major breakthrough in SPL evaluation. Indeed, in patients with unresectable pancreatic ductal adenocarcinomas, which account for 80%–85% of all SPLs and all pancreatic ductal adenocarcinoma cases, tissue samples represent the only available histologic material on which molecular characterization and therapeutic stratification markers can be performed. Molecular profiling from both formalin-fixed paraffin-embedded and frozen specimens acquired by EUS-FNB has been reported to be highly feasible.[16] Moreover, repeated EUS-FNB after neoadjuvant chemotherapy might soon become standard of care to detect therapy-induced molecular changes, reinforcing the importance of pretreatment histologic tissue acquisition.[44,45] Finally, assessment of the Ki-67 index for risk stratification of pancreatic neuroendocrine tumors is more reliable on EUS-FNB than cytologic specimens.[46] High-quality histologic samples acquired with EUS-FNB, in our view, will pave the road for personalized treatment development in patients with both pancreatic ductal adenocarcinomas and neuroendocrine tumors.

Our study represents the largest prospective study assessing AE rates of EUS-FNB performed with new-generation needles. Twelve postprocedural AEs (1.6%) were observed, without statistically significant differences between the 2 study arms and needle type used. Our study, however, was not powered to detect difference in AE rates, and further studies to specifically assess this issue are warranted. The rate we observed is comparable with the 2.4% reported in a systematic review on EUS-FNA for SPLs,[47] with mild acute pancreatitis as the most commonly observed AE. One fatal event occurred in a patient who underwent a single-session EUS/ERCP. The patient did not experience any specific symptoms, and necropsy was not performed; thus, we were unable to identify the cause of death.

The sampling procedure was significantly shorter in the absence of ROSE, with a mean difference of about 6 minutes in favor of EUS-FNB alone. A recent randomized controlled trial demonstrated a shorter pathology viewing time for histologic compared with cytologic samples,[18] suggesting that EUS-FNB with off-site histologic evaluation can be timesaving and cost-effective, especially in high-volume centers. On the other hand, ROSE can provide immediate sampling adequacy in a relevant number of cases, reducing the overall turnaround time. These outcomes, however, were not evaluated in our study, precluding us from drawing any definitive conclusion.

This study has some limitations. First, some pathologists despite being fully trained in standard ROSE had limited experience with TIC, leading to a possible bias in favor of EUS-FNB alone. To overcome this drawback, all centers performed at least 10 cases to become confident with the TIC technique in SPLs before starting enrollment.[35] Second, all involved centers were highly experienced, and results

Table 4. Comparison of Operating Characteristics by Needle Type

Needle	Accuracy % (95% CI)	Core Procurement[a] % (95% CI)	Blood Contamination[b] % (95% CI)	Adverse Events n (%)
ProCore 20G (157)	93.6 (88.6–96.9)	68.2 (60.3–75.4)	Little, 32.5 (25.3–40.4) Medium, 44.0 (36.2–51.7) High, 23.5 (16.9–30.2)	2 (1.3)
SharkCore 22G (163)	98.8 (95.7–99.8)	73.0 (65.5–79.7)	Little, 52.1 (44.5–60.0) Medium, 25.8 (19.1–32.5) High, 22.1 (15.7–28.4)	5 (3.2)
Acquire 22G (451)	97.3 (95.4–98.6)	76.9 (72.8–80.8)	Little, 40.6 (36.0–45.3) Medium, 42.8 (38.2–47.4) High, 16.6 (13.2–20.0)	5 (1.1)

Comparisons	P^c	P^c	P^c	P^c
ProCore 20G vs SharkCore 22G	.077	.557	.001	.449
ProCore 20G vs Acquire 22G	.091	.070	.168	NS
Acquire 22G vs SharkCore 22G	.572	.557	.032	.140

Results are averaged over the levels of study arms. CI, confidence interval; NS; not significant.
[a]Presence of at least 1 core of tissue of 550 μm.
[b]Little blood contamination (surface area $<$ 25% of slide) vs medium (25%–50% of the slide), or high blood contamination ($>$50% of the slide or only blood).
[c]Two-sided *P*; *P* adjustment: Tukey method for comparing a family of 3 estimates.

might not be reproducible in other settings. Third, this study was performed using 3 different histologic needles without randomization. However, the concept behind the study was to assess performance of EUS-FNB with and without ROSE, independently of which FNB needle type was used, which was left to the discretion of each endosonographer. Moreover, subgroup analyses showed similar results among the 3 needle types used. Fourth, in the EUS-FNB alone arm histologic samples were collected in a single formalin vial; thus, a minimum number of passes to reach noninferiority could not be assessed. Finally, sample size was calculated for the primary outcome but not for secondary outcomes, which should be considered as exploratory with the need for specifically designed trials to confirm our findings.

In conclusion, in patients with SPLs our study demonstrated that diagnostic accuracy of EUS-FNB alone with 3 needle passes reached 97% and was noninferior compared with EUS-FNB+ROSE, rendering ROSE not routinely recommended. Based on these results, we strongly believe that EUS-FNB should become standard of care for SPL evaluation.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org and at https://doi.org/10.1053/j.gastro.2021.06.005.

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Conflicts of interest
These authors disclose the following: Dr Crinò is a consultant for Boston Scientific. Dr Deprez is a consultant for Boston Scientific. Dr Carrara received a research grant from Boston Scientific. Dr Shami is a consultant for Interpace Diagnostics and Olympus America. Dr Poley is a consultant for and receives travel compensation from Cook Medical, Boston Scientific, and Pentax. Dr Ginès is a consultant for Cook Endoscopy. Dr van Malenstein is a consultant for Boston Scientific. Dr Larghi is a consultant for Pentax and Boston Scientific and received a teaching fee from Medtronic and Boston Scientific. The remaining authors disclose no conflicts.

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Supplementary Methods

Comparative Analysis of ROSE vs EUS-FNB+ROSE for Patients Randomized to EUS-FNB+ROSE Arm

In the EUS-FNB+ROSE arm, the cumulative rate of adequate samples on ROSE was 59.2% (228/385) on the first biopsy pass, 76.4% (294/385) on the second pass, and 90.4% (348/385) on the third pass. Of the 37 ROSE-inadequate patients, 31 (83.8%) became adequate on the final interpretation (ie, including histologic specimens collected during/after TIC), whereas 6 cases (16.2%) remained inadequate, indicating an incremental value of adequacy of EUS-FNB over ROSE of 8.0% (ROSE-adequate rate, 90.4% vs EUS-FNB+ROSE, 98.4%; $P < .001$). Among ROSE-adequate cases (348 cases), the overall proportion of agreement between ROSE and EUS-FNB+ROSE interpretation was 93.1% (95% CI, 87.8%–94.0%), with substantial agreement (Cohen's $\kappa = 0.70$; 95% CI, 0.60–0.80) (Supplementary Table 7).

Changes in interpretations were found. Of the 41 initially reported benign or atypical findings on ROSE, 25 of 41 (61%) were upgraded to be malignant or suspicious for malignancy, whereas 16 of 41 (39%) persisted as benign or atypical (Supplementary Table 7). Interesting, of the 281 initially reported malignant or suspicious for malignancy on ROSE, only 1 (0.3%) was downgraded to neoplastic low grade (acinar cell carcinoma on ROSE vs neuroendocrine tumor on EUS-FNB). In another case (0.3%), malignancy was found on ROSE, but only atypical cells were detected on FNB. Compared with the true final status, diagnostic accuracy for EUS-FNB+ROSE was significantly higher than for ROSE (96.4% vs 82.9%; incremental diagnostic accuracy over ROSE, 13.5%; $P < .001$) (Supplementary Table 8).

Supplementary Table 1. Scores Used to Evaluate the Quality of the Tissue Retrieved

Tissue integrity score	
Score	Explanation
0	Insufficient material for interpretation
Cytology	
1	Sufficient material for limited cytologic interpretation; probably not representative
2	Sufficient material for adequate cytologic interpretation
Histology (2–5)	
3	Sufficient material for low-quality histologic interpretation (microfragments < 550 μm in greatest axis)
4	Sufficient material for good-quality histologic interpretation (1–5 cores > 550 μm in greatest axis)
5	Sufficient material for high-quality histologic interpretation (6–10 cores > 550 μm in greatest axis)
6	Sufficient material for excellent-quality histologic interpretation (>10 cores > 550 μm in greatest axis or total tissue length > 5.500 μm)
Blood contamination score	
Score	Explanation
0	Only blood
1	High blood contamination, surface area $> 50\%$ of the slide
2	Medium blood contamination, surface area 25%–50% of the slide
3	Little blood contamination, surface area $< 25\%$ of slide

Supplementary Table 2. Pathologic Findings of Samples Collected in Patients With SPLs Who Underwent EUS-FNB With or Without ROSE Stratified According To The Papanicolaou Classification and Presented With Results Of The Reference Standard

	Reference Standard (n = 385)			
EUS-FNB+ROSE Arm	Positive Findings (n = 373)		Negative Findings (n = 12)	
EUS-FNB+ROSE diagnosis	True Positive[a] (n = 359)	False Negative (n = 14)	True Negative (n = 12)	False Positive (n = 0)
Malignant	292 (78.3)	0 (0)	0 (0)	0 (0)
Suspicious for malignancy	28 (7.5)	0 (0)	0 (0)	0 (0)
Neoplastic benign/other	32 (8.6)	0 (0)	0 (0)	0 (0)
Atypical NOS	0 (0)	6 (1.6)	1 (8.3)	0 (0)
Benign (negative for malignancy)	7 (1.9)	2 (0.5)	11 (91.7)	0 (0)
Inadequate	0 (0)	6 (1.6)	0 (0)	0 (0)
All diagnoses	359 (93.2)	14 (6.8)	12 (100)	0 (0)

	Reference Standard (n = 386)			
EUS-FNB Alone Arm	Positive Findings (n = 371)		Negative Findings (n = 15)	
EUS-FNB alone diagnoses	True Positive[a] (n = 361)	False Negative (n = 10)	True Negative (n = 15)	False Positive (n = 0)
Malignant	297 (80.1)	0 (0)	0 (0)	0 (0)
Suspicious for malignancy	29 (7.8)	0 (0)	0 (0)	0 (0)
Neoplastic benign/other	31 (8.4)	0 (0)	0 (0)	0 (0)
Atypical NOS	0 (0)	1 (0.3)	0 (0)	0 (0)
Benign (negative for malignancy)	4 (1.1)	4 (1.1)	15 (100)	0 (0)
Inadequate	0 (0)	5 (1.3)	0 (0)	0 (0)
All diagnoses	361 (97.3)	10 (2.7)	15 (100)	0 (0)

Values are n (%). NOS, not otherwise specified.
[a]For specific benign conditions/neoplasms (eg, autoimmune pancreatitis, solid serous cystadenoma, intrapancreatic spleen), outcome was determined with respect to the exact diagnosis (ie, considered true positive or false negative when the specific diagnosis was achieved or not, respectively).

Supplementary Table 3. Diagnostic Measures in the 2 Arms Using Strict Criteria

	EUS-FNB+ROSE (n = 385)		EUS-FNB Alone (n = 386)	EUS-FNB+ROSE vs EUS-FNB Alone P^a
	ROSE	EUS-FNB+ROSE		
True positive	252 (64.7)	331 (86.0)	332 (86)	—
True negative	11 (2.3)	12 (3.1)	15 (3.9)	—
False positive	0	0	0	—
False negative	122 (32.2)	42 (10.9)	39 (10.1)	—
Sensitivity[b]	66.8 (61.7–71.5)	88.7 (85.1–91.8)	89.5 (85.9–92.4)	.814
Specificity	75.0 (42.8–94.5)	100 (73.5–100)	100 (78.2–100)	1.00
Negative predictive value	6.8 (4.8–9.4)	22.2 (17.7–27.5)	27.8 (22.2–34.1)	.657
Positive predictive value	98.8 (96.9–99.6)	100 (99.0–100)	100 (99.0–100)	1.00
Accuracy	67.0 (62.1–71.7)	89.1 (85.5–92.0)	89.9 (86.5–92.7)	.726

Values are n (%) or % (95% confidence interval). The difference in accuracy of EUS-FNB alone vs EUS-FNB+ROSE was 0.8% (2-sided 90% confidence interval, –2.8% to 4.4%; noninferiority $P = .004$).
[a]*P*-value for the null hypothesis of inferiority (ie, EUS-FNB alone inferior to EUS-FNB+ROSE).
[b]Inadequate samples were included in the calculation of sensitivity and accuracy as false-negative pathologist's interpretation.

Supplementary Table 4. Multivariable Analysis of Diagnostic Accuracy

Variable	Odds Ratio (95% Confidence Interval)	*P*
Study arm		
EUS-FNB alone	1	
EUS-FNB+ROSE	0.68 (0.29–1.61)	.378
Patient gender		
Male	1	
Female	1.20 (0.48–2.98)	.235
Patient age (as a continuous variable mean centered)	0.98 (0.94–1.03)	.744
Lesion size (as a continuous variable mean centered)	1.02 (0.99–1.06)	.518
Lesion location		
Body/tail	1	
Head/uncinate/neck	0.48 (0.17–1.36)	.167
Needle type[a]		
ProCore 20G (n = 157)	1	
SharkCore 22G (n = 163)	5.23 (1.01–28.80)	.049
Acquire 22G (n = 457)	2.40 (0.77–7.15)	.155
Needle fanning		
No	1	
Yes	0.77 (0.16–3.80)	.701
Blood contamination		
Medium or high concentration	1	
Little concentration	2.06 (0.79–5.31)	.430

Results from a logistic regression model.
[a]Odds ratio and (2-sided) *P*-value after adjustment for multiple comparisons.

Supplementary Table 5. Diagnostic Performance in the 2 Arms Stratified by Needle Design

Needle	Diagnostic Performance % (95% Confidence Interval)	EUS-FNB+ROSE (n = 77)	EUS-FNB alone (n = 80)	*P*
Procore 20G (n = 157)	Sensitivity	93.3 (85.1–97.8)	93.4 (85.3–97.8)	1.00
	Negative predictive value	28.6 (14.6–48.3)	44.4 (25.5–65.1)	.633
	Accuracy	93.5 (85.5–97.9)	93.7 (86.0–97.9)	1.00
		(n = 77)	(n = 86)	
SharkCore 22G (n = 163)	Sensitivity	98.7 (92.7–99.9)	97.6 (91.6–99.7)	1.00
	Negative predictive value	75.0 (30.0–95.5)	60.0 (27.6–85.5)	1.00
	Accuracy	98.7 (93.0–99.9)	98.8 (93.7–99.9)	1.00
		(n = 231)	(n = 220)	
Acquire 22G (n = 451)	Sensitivity	96.4 (93.1–98.5)	98.1 (95.3–99.5)	.534
	Negative predictive value	46.7 (30.7–63.3)	63.6 (39.9–82.2)	.404
	Accuracy	96.5 (93.3–98.5)	98.2 (95.4–99.5)	.416

Supplementary Table 6. Diagnostic Performance in the 2 Arms Stratified by Sampling Technique

	Diagnostic Performance % (95% Confidence Interval)	EUS-FNB+ROSE (n = 125)	EUS-FNB alone (n = 120)	*P*
Suction (n = 245)	Sensitivity	98.3 (94.1–99.8)	96.5 (91.3–99.0)	.641
	Negative predictive value	71.4 (29.0–96.0)	50.0 (15.7–84.3)	.751
	Accuracy	98.4 (94.3–99.8)	95.8 (90.5–98.6)	.411
		(n = 237)	(n = 237)	
Slow-pull (n = 474)	Sensitivity	94.8 (91.1–97.3)	97.4 (94.3–99.0)	.240
	Negative predictive value	33.3 (13.0–59.0)	60.0 (32.1–83.7)	.238
	Accuracy	94.9 (91.3–97.4)	97.5 (94.5–99.1)	.230
		(n = 21)	(n = 29)	
Wet suction (n = 50)	Sensitivity	95.0 (75.1–99.9)	100.0 (87.6–100.0)	.864
	Negative predictive value	50.0 (1.3–98.7)	100.0 (2.5–100.0)	1.000
	Accuracy	95.2 (76.2–99.9)	100.0 (88.0–100.0)	.870

Supplementary Table 7. Agreement Between ROSE and EUS-FNB+ROSE in Classification of SPLs

	ROSE-Adequate				
EUS-FNB+ROSE	Malignant or Suspicious for Malignancy	Neoplastic Lesions	Benign or Atypical NOS	Inadequate	Total
Malignant or suspicious for malignancy	279	1	25	15	320
Neoplastic lesions	1	24	0	7	32
Benign or atypical NOS	1	1	16	9	27
Inadequate	0	0	0	6	6
Total	281	26	41	37	385

NOS, not otherwise specified.

Supplementary Table 8. Comparison of Operating Characteristics on ROSE and Pathologic EUS-FNB+ROSE Results (Paired Data)

	ROSE		
EUS-FNB+ROSE	True Positive	False Negative	Total
Sensitivity[a]			
True positive	310	49	359
False negative	0	14	14
Total	310	63	373

	True Negative	False Positive	Total
Specificity[b]			
True negative	9	3	12
False positive	0	0	0
Total	9	3	12

	Correct Diagnosis	Incorrect Diagnosis	Total
Accuracy[c]			
Correct diagnosis	319	52	371
Incorrect diagnosis	0	14	14
Total	319	66	385

Any inadequate sample was included as false negative in the analysis of accuracy.
[a]Difference in sensitivity, 359/373 (96.3%) for EUS-FNB+ROSE vs 310/373 (83.1%) for ROSE, $P < .001$.
[b]Difference in specificity, 12/12 (100%) for EUS-FNB+ROSE vs 9/12 (75.0%) for ROSE, $P = .25$.
[c]Difference in accuracy, 371/385 (96.4%) for EUS-FNB+ROSE vs 319/385 (82.9) for ROSE, $P < .001$.