

Operator-rated puncture performance and first-pass lesion tissue area in pancreatic ductal adenocarcinoma: an exploratory single-center sub-analysis of endoscopic ultrasound-guided tissue acquisition

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Research Article

Keywords: Endoscopic ultrasound-guided tissue acquisition, fine-needle biopsy, pancreatic ductal adenocarcinoma, puncture performance, histological tissue area

Posted Date: October 22nd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11273454/v1>

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Additional Declarations: No competing interests reported.

Abstract

Background:

Adequate tissue acquisition is important for histological assessment and ancillary testing in pancreatic ductal adenocarcinoma (PDAC). We primarily explored the association between operator-rated puncture performance and first-pass lesion tissue area during endoscopic ultrasound-guided tissue acquisition (EUS-TA) in patients with PDAC. A secondary analysis examined whether this association was observed in the overall cohort, irrespective of final diagnosis.

Methods:

This exploratory single-center sub-analysis used prospective data from a multicenter randomized trial of 22-gauge fine-needle biopsy needles. Endoscopists rated overall puncture performance on a five-point scale after EUS-TA. Ratings were analyzed using two post hoc dichotomizations: very easy/easy versus indeterminate/difficult/very difficult, and very easy versus all other ratings. First-pass lesion tissue area was quantified automatically on histological slides using a digital microscope with image-analysis software. Multivariable linear regression assessed associations between ratings and tissue areas in each cohort, adjusting for needle type, puncture route, operator experience, and lesion size. Firth logistic regression assessed associations between ratings and exploratory $\geq 4\text{-mm}^2$ benchmark attainment in the PDAC cohort. Trends in tissue area across very easy, easy, and remaining ratings were assessed using the Jonckheere–Terpstra test. Comprehensive genomic profiling (CGP) suitability was summarized descriptively.

Results:

Of 62 patients, 48 had PDAC. In the PDAC cohort, the very easy/easy comparison yielded inconclusive adjusted associations with tissue area and benchmark attainment. A very easy rating was associated with greater tissue area (median, 5.45 vs. 1.90 mm²; adjusted difference, 1.93 mm²; 95% confidence interval [CI], 0.12–3.74) and benchmark attainment (57.7% vs. 22.7%; adjusted odds ratio, 4.44; 95% CI, 1.17–21.06). Tissue area decreased with less favorable ratings (p for trend = 0.016). Two of six patients assessed for CGP were considered suitable. In the overall cohort, lesion size remained associated with tissue area, whereas adjusted associations for both rating dichotomizations were inconclusive. Tissue area decreased with less favorable ratings (p for trend = 0.034).

Conclusions:

In patients with PDAC, an endoscopist's very easy rating was associated with greater first-pass lesion tissue area after adjustment for lesion size and procedural factors. Prospective pass-by-pass studies are

needed to validate this exploratory association and determine its utility in EUS-TA.

Trial registration:

Parent trial: Japan Registry of Clinical Trials (jRCT), jRCT1042220099, registered on 25 November 2022.

Background

Obtaining sufficient histological tissue is important in pancreatic ductal adenocarcinoma (PDAC) for pathological diagnosis and ancillary examinations, including immunohistochemical and molecular analyses. Endoscopic ultrasound-guided tissue acquisition (EUS-TA) with fine-needle biopsy (FNB) needles is widely used for this purpose, with current guidelines favoring 22-gauge FNB needles [1]. Histological tissue area has been used as a quantitative measure of tissue yield in EUS-TA studies [2,3], and larger areas have been associated with successful histological diagnosis [2].

Tissue acquisition may depend on lesion characteristics, puncture route, sampling technique, and operator experience [4–6]. Contemporary FNB needles incorporate modified tip designs to improve lesion penetration and tissue acquisition. In the primary report of this multicenter randomized trial [7], sharpened-tip, three-prong 22-gauge FNB needles improved initial puncture success and overall operator-rated puncture performance compared with a conventional Franseen needle but did not increase tissue area or diagnostic performance. Operator-rated puncture performance may reflect multiple aspects of the procedure; however, its association with quantitatively measured histological tissue yield remains unclear.

In this exploratory sub-analysis, we examined the association between operator-rated puncture performance and first-pass lesion tissue area in patients with PDAC. We also assessed attainment of a $\geq 4\text{-mm}^2$ tissue-area benchmark derived from comprehensive genomic profiling (CGP) criteria [8]. Additional analyses included the overall cohort irrespective of final diagnosis and explored clinical and procedural factors associated with operator-rated puncture performance.

Methods

Study design and participants

This single-center exploratory sub-analysis was conducted using prospectively collected data from patients enrolled at Nagoya University Hospital between February and October 2023 in a multicenter, open-label randomized trial comparing three 22-gauge FNB needles for EUS-TA of solid pancreatic lesions [7]. The trial was approved by the Nagoya University Clinical Research Review Board (approval number: 2022 – 0300) on November 11, 2022, and registered in the Japan Registry of Clinical Trials (jRCT1042220099) on 25 November 2022, and all participants provided written informed consent. This study was conducted in accordance with the principles of the Declaration of Helsinki. This exploratory

sub-analysis is reported in accordance with the applicable CONSORT 2010 recommendations. The sample size was determined by the number of eligible participants at this center in the parent trial.

Eligible patients were aged ≥ 20 years, had a solid pancreatic lesion ≥ 10 mm on computed tomography or magnetic resonance imaging, and required histological diagnosis by EUS-TA. Exclusion criteria included conditions precluding safe EUS-TA, inability to manage antithrombotic therapy according to the trial protocol, uncontrolled infection, serious comorbidities, severe psychiatric illness, or investigator-determined ineligibility.

EUS-TA procedure and assessments

EUS-TA was performed according to the standardized trial protocol [7]. Participants were randomly assigned to either the conventional-needle or novel-needle group at a ratio of 1:2. All needles were 22 gauge: the conventional group used a Franseen needle (Acquire; Boston Scientific, Marlborough, MA, USA), whereas the novel group used a Trident (Micro-Tech Endoscopy, High Wycombe, UK) or 3-point needle (TopGain; Medi-Globe, London, UK). After confirming a safe puncture route, the assigned needle was advanced into the target under EUS guidance. At our institution, fanning, slow-pull, and 20 actuations per pass were standardized. Specimen adequacy was assessed by rapid on-site evaluation (ROSE), which guided decisions to perform additional passes or complete EUS-TA.

Immediately after EUS-TA, the performing endoscopist rated the overall puncture performance on a five-point ordinal scale: very easy, easy, indeterminate, difficult, or very difficult. To ensure consistency in subjective assessments among the endoscopists, a kick-off meeting was held before the study to standardize the use of a predefined scale.

Specimens from each needle pass were processed separately for cytological and histological assessments. Histological slides prepared from the first-pass specimens were evaluated using a digital microscope (BZ-X800; Keyence, Osaka, Japan). Lesion tissue and blood clot areas were quantified in mm² using the microscope's image-analysis software, which performed automated color-based tissue selection and area calculation, as previously described [9]. The entire specimen was analyzed using uniform color-selection settings without manual delineation or correction. The operator performing the tissue measurements was blinded to both the puncture-performance rating and the needle type for each specimen. A representative PDAC specimen and the region included in the lesion tissue measurements are shown in Fig. 1.

Definitions

Patients with a final diagnosis of PDAC constituted the PDAC cohort, whereas the overall cohort included all eligible patients irrespective of the final diagnosis. Operators were classified as experts (≥ 10 years of EUS experience) or trainees (< 10 years). Puncture routes were classified as transgastric or

transduodenal, with the latter including puncture through the duodenal bulb or descending duodenum. Lesion size was defined as the maximum diameter of the target lesion measured during EUS-TA.

Two post hoc dichotomizations of puncture-performance ratings were examined: very easy/easy versus indeterminate/difficult/very difficult, and very easy versus all other ratings. For ordinal analyses, the ratings were grouped as very easy, easy, and indeterminate/difficult/very difficult. The first-pass lesion tissue area was defined as the measured area of tissue derived from the target lesion. Blood-clot area was measured separately, and total specimen area was defined as the sum of lesion tissue and blood-clot areas. Procedure-related adverse events (AEs) were graded according to the American Society for Gastrointestinal Endoscopy lexicon for endoscopic AEs [10].

Outcomes

The main analysis examined the association between operator-rated puncture performance and first-pass lesion tissue area in the PDAC cohort. Achievement of a first-pass lesion tissue area $\geq 4 \text{ mm}^2$ was evaluated as an exploratory tissue-quantity benchmark derived from CGP suitability criteria [8]. CGP suitability was assessed when clinically indicated and summarized descriptively alongside available clinical and specimen characteristics. Additional analyses extended the tissue-area assessment to the overall cohort and explored factors associated with operator-rated puncture performance. Correlations between lesion size and first-pass lesion tissue area, differences in total specimen and blood-clot areas between rating groups, and trends in lesion tissue area across ordered rating categories were examined in both cohorts. First-pass and overall histological diagnostic accuracy and procedure-related AEs were also evaluated.

Statistical analysis

All analyses were exploratory. Continuous variables are presented as medians with interquartile ranges (IQRs) and categorical variables as numbers with percentages. Comparisons between the groups defined by each dichotomization were performed using the Mann–Whitney U test for continuous variables and Fisher's exact test for categorical variables.

In both cohorts, correlations between lesion size and first-pass lesion tissue area were assessed using Spearman's rank correlation coefficient. Trends in lesion tissue area across the three ordered rating categories were assessed using the Jonckheere–Terpstra test. For each dichotomization, multivariable linear regression models were fitted separately in the PDAC and overall cohorts, adjusting for needle type, puncture route, operator experience, and lesion size modeled per 10-mm increase. Adjusted coefficients and 95% confidence intervals (CIs) are reported; coefficients represent absolute differences in tissue area in mm^2 . For the comparison of the most favorable category with all other ratings, an interaction between rating group and continuous lesion size was explored in each cohort by adding their product term to the corresponding model.

In the PDAC cohort, achievement of a first-pass lesion tissue area $\geq 4 \text{ mm}^2$ was examined for each dichotomization using Firth penalized-likelihood logistic regression with profile penalized-likelihood CIs. Given the limited number of events, the main models included the rating group, needle type, and lesion size per 10-mm increase. Extended models additionally included puncture route and operator experience. Adjusted odds ratios (aORs) and 95% CIs are reported. In the overall cohort, multivariable logistic regression explored associations of needle type, puncture route, operator experience, and lesion size (> 20 versus $\leq 20 \text{ mm}$) with the very easy versus all other ratings. Analyses were conducted using R software (version 4.4.2; R Foundation for Statistical Computing, Vienna, Austria). A two-sided $p < 0.05$ was considered statistically significant, without adjustment for multiple comparisons.

Results

Study population

Of the 66 patients enrolled at Nagoya University Hospital, one withdrew consent before puncture and three did not undergo EUS-TA because of difficulty visualizing the target lesion, elevated C-reactive protein level, or identification of a cystic lesion during EUS. The remaining 62 constituted the overall cohort. The conventional and novel needles were used in 21 and 41 patients, respectively. Puncture performance was rated as very easy in 33 patients (53.2%), easy in 19 (30.6%), indeterminate in seven (11.3%), and difficult in three (4.8%). One patient with an indeterminate rating experienced mild abdominal pain. Patient and lesion characteristics as well as procedural, diagnostic, and safety outcomes, are summarized in Table 1.

Analysis in the PDAC cohort

Among the 48 patients diagnosed with PDAC, 41 received very easy/easy ratings and seven received the remaining ratings. Median first-pass lesion tissue areas were 3.60 and 1.50 mm^2 , respectively ($p = 0.165$; Supplementary Table S1A). After multivariable adjustment, this comparison remained inconclusive (adjusted coefficient, 1.56 mm^2 ; 95% CI, -1.02 – 4.14 ; $p = 0.229$; Supplementary Table S1B).

For very easy versus all other ratings, 26 and 22 patients were included, respectively. The first-pass lesion tissue area was greater in the very easy group than in the other ratings group (median, 5.45 mm^2 vs 1.90 mm^2 ; $p = 0.018$; Table 2A). Lesion size was moderately correlated with the first-pass lesion tissue area (Spearman's $\rho = 0.47$, $p < 0.001$; Figure 2). After multivariable adjustment, a very easy rating (adjusted coefficient, 1.93 mm^2 ; 95% CI, 0.12–3.74) and each 10-mm increase in lesion size (adjusted coefficient, 0.60 mm^2 ; 95% CI, 0.01–1.19) were associated with a greater first-pass lesion tissue area (Table 2B). No significant interaction was observed between puncture performance rating and lesion size (p for interaction = 0.101). The first-pass lesion tissue area decreased with less favorable puncture performance ratings (p for trend = 0.016; Figure 3A).

The $\geq 4\text{-mm}^2$ benchmark was achieved in 20 of 48 patients (41.7%). Rates were 18/41 (43.9%) for very easy/easy ratings and 2/7 (28.6%) for the remaining ratings ($p = 0.683$). The association was

inconclusive in both the main and extended Firth models (aORs, 1.71 [95% CI, 0.30–12.91] and 1.61 [95% CI, 0.28–12.63], respectively; Supplementary Table S1C). For very easy versus all other ratings, achievement rates were 15/26 (57.7%) and 5/22 (22.7%), respectively ($p = 0.020$). In the main model, a very easy rating (aOR, 4.44; 95% CI, 1.17–21.06) and lesion size (aOR per 10-mm increase, 1.85; 95% CI, 1.01–4.50) were associated with benchmark attainment. In the extended model, only a very easy rating remained significantly associated (aOR, 3.82; 95% CI, 1.02–17.40; Table 2C).

CGP suitability was assessed in six patients; all received a very easy rating, and two were considered suitable. Median lesion size was 49.5 mm in suitable cases and 23.5 mm in unsuitable cases, with corresponding median first-pass lesion tissue areas of 8.9 and 2.9 mm², respectively. Individual findings are presented in Supplementary Table S2.

Analysis in the overall cohort

Patients diagnosed with PDAC had larger lesions than those with other diagnoses (median, 24.0 vs 16.5 mm; $p = 0.003$). For very easy/easy versus remaining ratings, 52 and 10 patients were included, respectively. Median first-pass lesion tissue areas were 3.05 and 1.75 mm² ($p = 0.146$; Supplementary Table S3A), and the adjusted association was inconclusive (coefficient, 1.49 mm²; 95% CI, –0.64–3.63; $p = 0.167$; Supplementary Table S3B).

For very easy versus all other ratings, first-pass lesion tissue area was greater in the very easy group (median, 3.50 vs. 2.20 mm²; $p = 0.044$), whereas total specimen and blood-clot areas did not differ significantly (Table 3A). After adjustment, the association with a very easy rating was inconclusive (coefficient, 1.34 mm²; 95% CI, –0.28–2.96; $p = 0.103$). Lesion size was moderately correlated with the first-pass lesion tissue area (Spearman's $\rho = 0.50$, $p < 0.001$; Figure 2) and remained associated after adjustment (coefficient per 10-mm increase, 0.78 mm²; 95% CI, 0.27–1.29; Table 3B). No significant interaction was detected between the very easy rating and lesion size (p for interaction = 0.427). The lesion tissue area also decreased with less favorable puncture performance ratings (p for trend = 0.034; Figure 3B).

Novel needle use was associated with a very easy rating in the multivariable analysis (aOR, 5.60; 95% CI, 1.59–19.70), whereas puncture route, operator experience, and lesion size >20 mm were not significantly associated with the rating (Table 4).

Discussion

This study yielded three main findings. First, in the PDAC cohort, a very easy rating was associated with a greater first-pass lesion tissue area and achievement of an area ≥ 4 mm². Second, in the overall cohort, lesion size remained independently associated with tissue area after adjustment, whereas the association with a very easy rating was not statistically significant. Third, novel sharpened-tip FNB needles were associated with a very easy rating but with no clear association with tissue area.

Previous studies have reported greater sample adequacy for solid pancreatic lesions measuring > 20 mm [4] and a higher sufficient-core acquisition rate for pancreatic malignancies measuring > 3 cm [11]. Consistent with these reports, lesion size was moderately correlated with first-pass lesion tissue area and remained associated with greater tissue area after multivariable adjustment. In this study, the very easy rating remained independently associated after adjustment for lesion size. ROSE helps assess specimen adequacy and guide further sampling [12], but does not necessarily reflect histological tissue area [13]. This distinction highlights the potential value of evaluating operator-rated puncture performance in relation to tissue quantity. The rating may integrate anatomical and technical conditions, ROSE feedback, and perceived sampling success. The observed decrease in tissue area with less favorable ratings supports an association between this overall assessment and tissue quantity. The broader very easy/easy comparison was not statistically significant, possibly reflecting the small PDAC reference group of seven patients and relatively similar tissue areas in the easy and indeterminate/difficult groups.

The CGP suitability of PDAC EUS-TA specimens depends on both tissue quantity and tumor cell content, which are not consistently adequate even with FNB sampling [8,14,15]. In previous studies using 22-gauge FNB needles, CGP adequacy was 53.5% in clinical practice [14], and the final quality-control success rate was 76% despite a median of three needle passes [15]. The association of a very easy rating with greater tissue area and achievement of the $\geq 4\text{-mm}^2$ benchmark suggests that it may indicate favorable conditions for obtaining sufficient tissue quantity. However, our limited CGP observations showed that an adequate tissue area did not ensure sufficient tumor-cell content. PDAC often contains dense desmoplastic stroma, and a large tissue area may still have low tumor-cell content, whereas severe fibrosis and avascular regions may further reduce sample adequacy or diagnostic sensitivity [6, 16]. Thus, a very easy rating may complement the quantitative assessment of tissue yield but cannot replace the pathological evaluation of tumor cellularity or establish CGP suitability.

In the overall cohort, the adjusted association between a very easy rating and first-pass lesion tissue area remained inconclusive. The relevance of tissue quantity varies among histological entities. Sufficient core acquisition was not associated with diagnostic yield in pancreatic neuroendocrine tumors, whereas lymphoma and AIP require adequate tissue for immunohistochemistry and preservation of the characteristic architecture [11,17,18]. Therefore, smaller non-PDAC lesions and between-diagnosis differences in sampling requirements may have increased the variability and weakened the adjusted association.

The association between novel needles and a very easy rating was consistent with the primary analysis of the trial [7]. For TopGain specifically, bench testing showed a lower maximum puncture resistance than Acquire, possibly reflecting differences in tip sharpness, slit length, and needle–sheath mechanics [19,20]. These mechanical differences suggest that the association between the needle type and rating primarily reflects the ease of lesion entry. In contrast, our study found no association between needle type and tissue area, consistent with randomized trials showing broadly similar diagnostic performance among FNB needles despite differences in tissue quantity or cellularity [21–23]. Tumor location may also

affect sampling adequacy [6], whereas needle design and echoendoscope angulation influence resistance to needle advancement [20], and sampling technique affects core procurement, tissue integrity, and blood contamination [24]. Kataoka et al. reported greater tissue area with 15 than with five actuations per pass using a 22-gauge Franseen needle [25]. Although fanning, slow-pull, and 20 actuations per pass were standardized, scope angulation and needle movement dynamics may still have varied. A randomized EUS-FNA trial found improved specimen quality with higher needle acceleration [26]. These findings suggest that scope configuration and needle manipulation are potential targets for improving puncture and sampling effectiveness. The overall rating may reflect these unmeasured conditions, supporting the interpretation that puncture performance and tissue acquisition are related but distinct aspects of EUS-TA.

This study had several limitations. First, the small sample and single-center design limit precision and generalizability. Both rating dichotomizations were post hoc, and multiple comparisons were performed without adjustment. An optimal cutoff between rating categories remains to be established. Second, puncture performance was subjective, non-validated, and recorded after the entire procedure; therefore, it may have been influenced by subsequent passes or the operator's impression of sampling success, and inter-operator reproducibility was not evaluated. Third, CGP suitability was assessed in only a small number of patients, and the actual CGP success was not evaluated. Finally, the intralesional stroke length, scope stability, viable target width, and tumor fibrosis were unavailable, and residual confounding factors and limited precision could not be excluded.

Conclusions

A very easy rating was associated with greater first-pass lesion tissue area in patients with PDAC. Prospective pass-by-pass assessment studies are needed to validate puncture-performance ratings and determine their utility in EUS-TA.

Declarations

Ethics Statement

Approval of the research protocol: The trial was approved by the Nagoya University Clinical Research Review Board (approval number: 2022 – 0300) on November 11, 2022, registered in the Japan Registry of Clinical Trials (jRCT1042220099) on November 2022, and all participants provided written informed consent.

Consent for publication

Not applicable.

Competing interests

All authors declare no conflict of interest for this article.

Funding information

None.

Author Contribution

Author contributions Conception and design: Y.T Data analysis and interpretation: Y.T Writing of the manuscript: Y.T Critical manuscript revision: T. Ishikawa, K.Y, Y.M, T.Iida, H.A, R.K, Y.K, and H.K Final approval of the article: All authors

Acknowledgements

None.

Availability of data and materials

The datasets used and/or analysed during the current study are not publicly available because they contain patient-level clinical information but are available from the corresponding author on reasonable request, subject to applicable institutional approvals.

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Tables

Data are presented as median (interquartile range) or number (percentage). The novel needles used were the Trident or TopGain, and the conventional needle used was the Acquire. Transduodenal puncture included puncture through the duodenal bulb or descending duodenum, and the trainee operators had <10 years of EUS experience. Other pancreatic carcinomas included adenosquamous carcinoma, anaplastic carcinoma, and intraductal papillary mucinous carcinoma, whereas pancreatic neuroendocrine neoplasms included pancreatic neuroendocrine tumors and neuroendocrine carcinomas. First-pass histological diagnostic accuracy was based solely on the first-pass specimen, whereas the overall accuracy was based on all available specimens. Procedure-related AEs were graded according to the ASGE lexicon [10].

AE, adverse event; ASGE, American Society for Gastrointestinal Endoscopy; EUS, endoscopic ultrasound

Data are presented as median (interquartile range). Areas were measured on histological slides prepared from first-pass specimens. Total specimen area was the sum of lesion tissue and blood-clot areas; lesion tissue area included associated stroma. All other ratings comprise easy, indeterminate, difficult, and very difficult. Comparisons used the Mann–Whitney U test.

PDAC, pancreatic ductal adenocarcinoma.

The model included all variables shown, with lesion size modeled continuously per 10-mm increase. Coefficients represent adjusted absolute differences in first-pass lesion tissue area in mm²; positive coefficients indicate greater area. Reference categories were all other ratings, conventional needle, transgastric puncture, and expert operator. All other ratings comprised easy, indeterminate, difficult, and very difficult.

CI, confidence interval; PDAC, pancreatic ductal adenocarcinoma.

The ≥ 4 -mm² threshold was an exploratory tissue-quantity benchmark. Firth penalized-likelihood logistic regression was used, with profile penalized-likelihood 95% CIs and penalized likelihood-ratio p values. The main model included the rating group, needle type, and lesion size per 10-mm increase; the extended model additionally included puncture route and operator experience. Reference categories were all other ratings, conventional needle, transgastric puncture, and expert operator. All other ratings comprise easy, indeterminate, difficult, and very difficult. Dashes indicate variables not included in the model.

aOR, adjusted odds ratio; CI, confidence interval; PDAC, pancreatic ductal adenocarcinoma.

Table 1. Patient and lesion characteristics and procedural, diagnostic, and safety outcomes according to puncture performance rating				
	Overall cohort (n=62)	Very easy (n=33)	Easy (n=19)	Indeterminate/difficult/very difficult (n = 10)
Age, years	73 (68–77)	73 (68–78)	72 (67–76)	72 (66–76)
Sex, males	32 (52)	14 (42)	12 (63)	6 (60)
Lesion size, mm	23 (18–30)	24 (19–31)	23 (16–29)	20 (16–23)
Lesion size ≤20 mm	23 (37)	9 (27)	8 (42)	6 (60)
Novel needle	41 (66)	27 (81)	11 (58)	3 (30)
Transduodenal puncture	33 (53)	15 (46)	13 (69)	5 (50)
Trainee Operator	37 (60)	22 (67)	9 (47)	6 (60)
Number of needle passes				
1 pass	19 (31)	10 (30)	5 (26)	4 (40)
2 passes	31 (50)	17 (52)	10 (53)	4 (40)
3 passes	12 (19)	6 (18)	4 (21)	2 (20)
First-pass histological diagnostic accuracy	50 (81)	27 (81)	15 (79)	8 (80)
Overall histological diagnostic accuracy	57 (92)	30 (91)	19 (100)	8 (80)
Any procedure-related AE	1 (2)	0 (0)	0 (0)	1 (10)
First-pass lesion tissue area, mm ²	2.7 (1.5–6.1)	3.5 (1.9–7.5)	2.2 (1.1–3.9)	1.8 (0.9–4.1)
Final diagnosis				
Pancreatic ductal adenocarcinoma	48 (77)	26 (79)	15 (79)	7 (70)
Other pancreatic carcinomas	4 (6)	2 (6)	2 (11)	0 (0)
Pancreatic neuroendocrine neoplasms	3 (4)	0 (0)	1 (5)	2 (20)
Pancreatic metastasis	1 (2)	1 (3)	0 (0)	0 (0)
Lymphoma	1 (2)	1 (3)	0 (0)	0 (0)

Autoimmune pancreatitis	1 (2)	0 (0)	0 (0)	1 (10)
Mass-forming pancreatitis	3 (4)	2 (6)	1 (5)	0 (0)
Benign lymph node enlargement	1 (2)	1 (3)	0 (0)	0 (0)

Table 2A. First-pass tissue acquisition outcomes according to operator-rated puncture performance in the PDAC cohort

Outcome	Very easy (n = 26)	Other ratings (n = 22)	p value
Total specimen area, mm ²	10.65 (8.18–14.6)	7.35 (4.38–11.8)	0.067
Lesion tissue area, mm ²	5.45 (2.05–7.72)	1.90 (0.90–3.88)	0.018
Blood-clot area, mm ²	6.90 (2.68–9.18)	3.95 (1.47–6.78)	0.535

Table 2B. Multivariable linear regression analyses of first-pass lesion tissue area in the PDAC cohort

Variable	Adjusted coefficient, mm ²	95% CI	p value
Very easy rating	1.93	0.12 to 3.74	0.037
Novel needle	−0.19	−2.18 to 1.80	0.845
Lesion size, per 10-mm increase	0.60	0.01 to 1.19	0.046
Transduodenal puncture	−1.15	−2.87 to 0.57	0.184
Trainee operator	−0.06	−1.93 to 1.81	0.950

Table 2C. Firth penalized logistic regression analyses of factors associated with achievement of a first-pass lesion tissue area of ≥4 mm² in the PDAC cohort

Variable	Main model: aOR (95% CI)	p value	Extended model: aOR (95% CI)	p value
Very easy rating	4.44 (1.17–21.1)	0.028	3.82 (1.02–17.4)	0.046
Novel needle	0.60 (0.12–2.64)	0.502	0.68 (0.13–3.23)	0.623
Lesion size, per 10-mm increase	1.85 (1.01–4.50)	0.046	1.77 (0.96–4.25)	0.073
Transduodenal puncture	—	—	0.58 (0.16–2.10)	0.403
Trainee operator	—	—	0.94 (0.23–3.76)	0.927

Data are presented as median (interquartile range). Areas were quantified on histological slides prepared from first-pass specimens. Total specimen area was the sum of lesion tissue and blood-clot areas. Other ratings comprised easy, indeterminate, difficult, and very difficult. Comparisons were performed using the Mann–Whitney U test.

Table 3A. First-pass tissue acquisition outcomes according to operator-rated puncture performance in the overall cohort			
Outcome	Very easy (n = 33)	Other ratings (n = 29)	p value
Total specimen area, mm ²	10.80 (7.90–14.80)	7.80 (4.30–12.50)	0.061
Lesion tissue area, mm ²	3.50 (1.90–7.50)	2.20 (0.80–3.90)	0.044
Blood-clot area, mm ²	6.90 (2.60–9.70)	4.00 (1.60–9.50)	0.489

The model included all variables shown, with lesion size modeled continuously per 10-mm increase. Coefficients represent adjusted absolute differences in first-pass lesion tissue area in mm²; positive coefficients indicate greater area. Reference categories were all other ratings, conventional needle, transgastric puncture, and expert operator. All other ratings comprised easy, indeterminate, difficult, and very difficult.

CI, confidence interval.

Table 3B. Multivariable linear regression analyses of first-pass lesion tissue area in the overall cohort			
Variable	Adjusted coefficient, mm ²	95% CI	p value
Very easy rating	1.34	−0.28 to 2.96	0.103
Novel needle	−0.59	−2.29 to 1.11	0.490
Lesion size, per 10-mm increase	0.78	0.27 to 1.29	0.003
Transduodenal puncture	−0.98	−2.50 to 0.53	0.198
Trainee operator	−0.08	−1.67 to 1.52	0.922

Table 4. Factors associated with a very easy puncture rating in the overall cohort			
Variable	Adjusted odds ratio	95% confidence interval	p value
Novel vs conventional needle	5.60	1.59–19.7	0.007
Transduodenal vs transgastric puncture	0.42	0.13–1.37	0.151
Trainee vs expert operator	1.02	0.31–3.35	0.980
Lesion size >20 vs ≤20 mm	2.93	0.91–9.47	0.073

Adjusted ORs were estimated using a multivariate logistic regression model including all variables shown. The outcome was a very easy puncture-performance rating versus all other ratings (easy, indeterminate, difficult, and very difficult). Reference categories were conventional needle, transgastric puncture, expert operator, and lesion size ≤20 mm.

CI, confidence interval; OR, odds ratio.

Figures

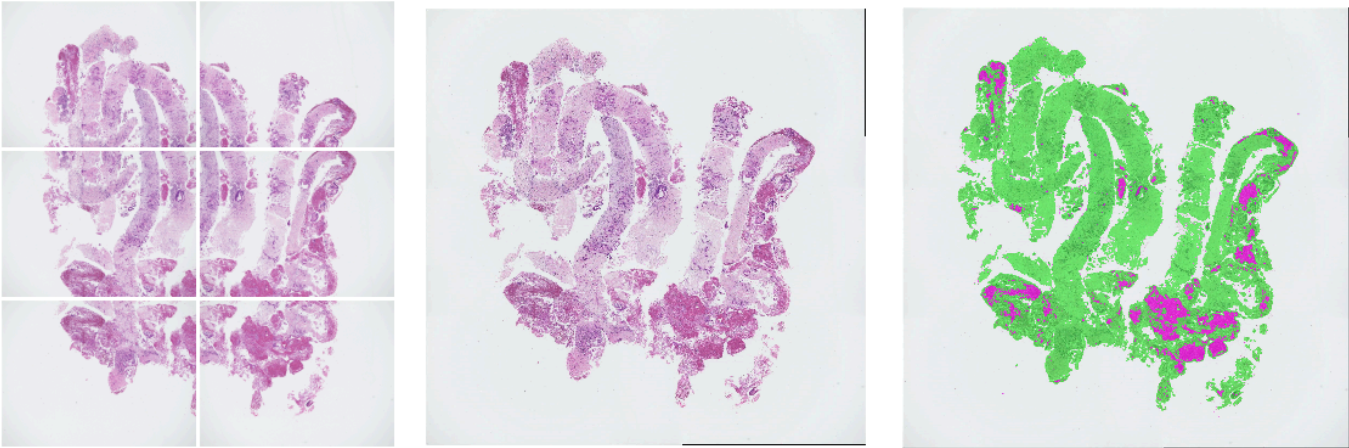


Figure 1

Quantification of lesion tissue and blood-clot areas in a representative first-pass pancreatic ductal adenocarcinoma specimen using a high-performance all-in-one microscope.

- A. Segmented high-resolution images of a hematoxylin and eosin-stained specimen obtained via endoscopic ultrasound-guided tissue acquisition using a Trident needle (original magnification, ×100).
- B. Composite image of the entire specimen generated by stitching the segmented images.

C. Automated color-based selection and area measurement, with the tumor-containing lesion tissue shown in green and blood clot shown in purple.

Figure 2. Relationship between lesion size and first-pass lesion tissue area in the PDAC and overall cohorts

Scatterplots showing the relationship between lesion size and first-pass lesion tissue area in PDAC (n = 48; left) and overall cohorts (n = 62; right). Each point represents one patient and is colored according to the puncture performance rating: the most favorable rating (very easy, blue) or other rating (gray). Black lines represent fitted linear regression lines, and shaded areas indicate 95% confidence intervals. Spearman's rank correlation coefficients (ρ) and corresponding P values are shown in each panel. PDAC, pancreatic ductal adenocarcinoma.

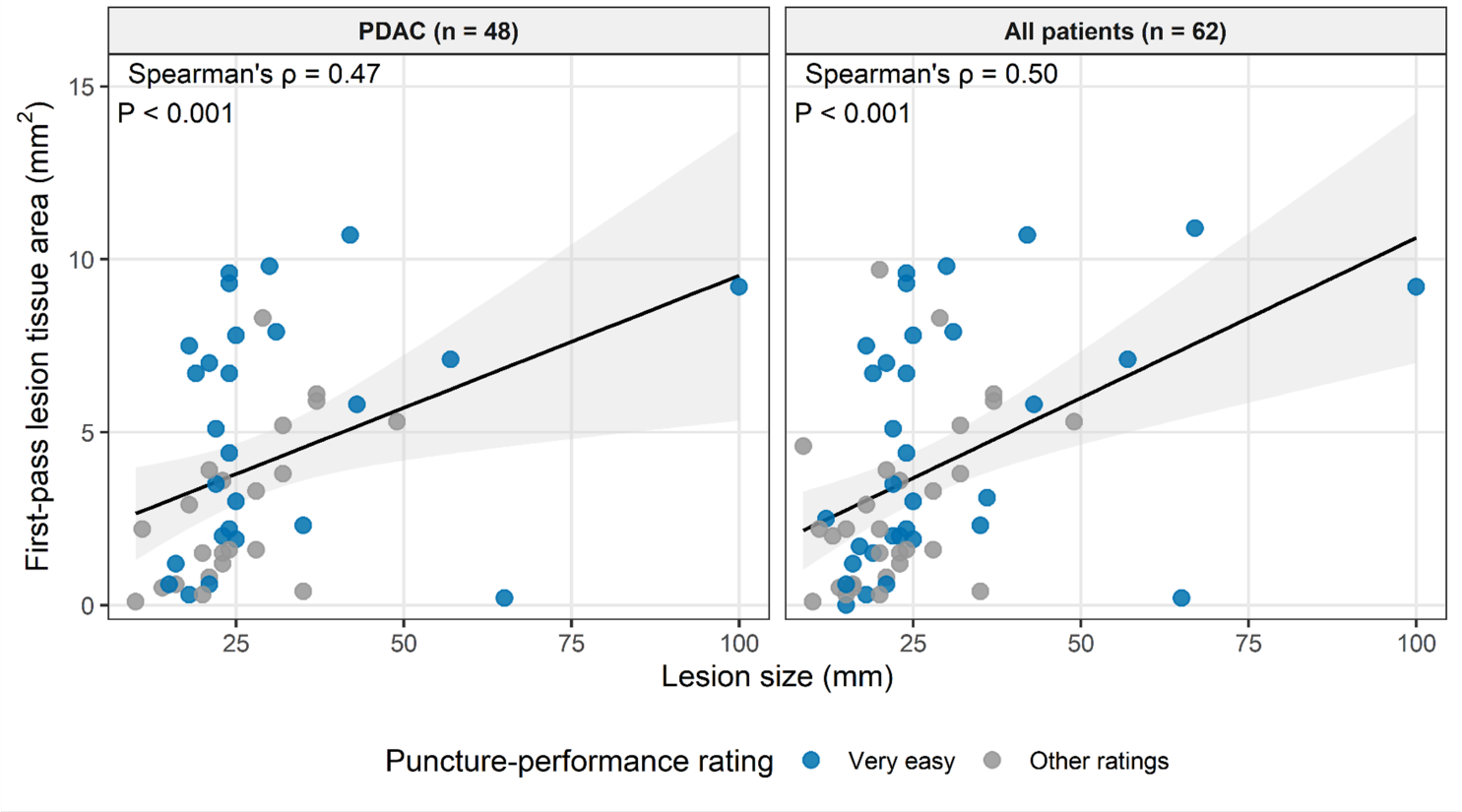


Figure 2

Relationship between lesion size and first-pass lesion tissue area in the PDAC and overall cohorts

Scatterplots showing the relationship between lesion size and first-pass lesion tissue area in PDAC (n = 48; left) and overall cohorts (n = 62; right). Each point represents one patient and is colored according to the puncture performance rating: the most favorable rating (very easy, blue) or other rating (gray). Black lines represent fitted linear regression lines, and shaded areas indicate 95% confidence intervals. Spearman's rank correlation coefficients (ρ) and corresponding P values are shown in each panel. PDAC, pancreatic ductal adenocarcinoma.

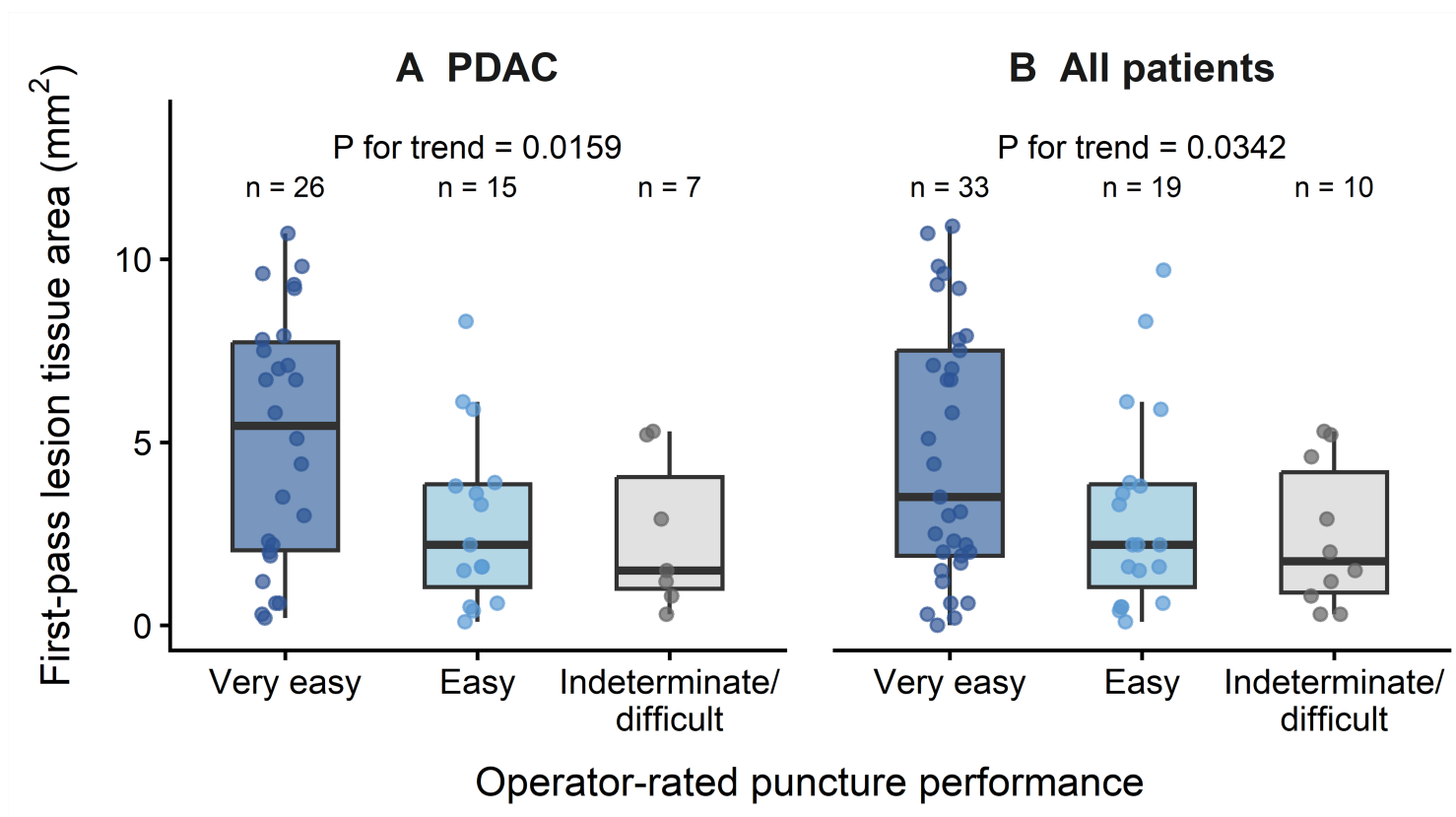


Figure 3

First-pass lesion tissue area across ordered operator-rated puncture-performance categories

(A) Patients with pancreatic ductal adenocarcinoma. (B) Overall cohort. Boxes indicate medians and interquartile ranges, whiskers extend to 1.5 times the interquartile range, and dots represent individual patients. P values for monotonic trends were calculated using the two-sided Jonckheere–Terpstra test.

Abbreviations: PDAC, pancreatic ductal adenocarcinoma.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTable.docx](#)