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Fine needle biopsy *versus* fine needle aspiration in the diagnosis of immunohistochemistry-required lesions: A multicenter study with prospective evaluation

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ABSTRACT

Objectives: The superiority of EUS–guided fine-needle biopsy (EUS-FNB) over fine-needle aspiration (FNA) remains controversial. This study aimed to compare the efficacy of FNB and FNA in immunohistochemistry (IHC)-required lesions, including, type 1 autoimmune pancreatitis (AIP), neuroendocrine tumor (NET), mesenchymal tumor, and lymphoma.

Methods: In this multicenter study, specimens from all eligible patients who underwent EUS-FNB/FNA with these specific lesions were prospectively evaluated. Demographics, adequacy of specimens for IHC, diagnostic accuracy, and integrity of tissue were analyzed. Subgroup analysis and multivariate logistic regression were also performed to control confounders.

Results: A total of 439 patients were included for analysis. Most lesion types were type 1 AIP (41.69%), followed by NET, mesenchymal tumor, and lymphoma. FNB yielded specimens with better adequacy for IHC (82.41% *vs*. 66.67%, $P < 0.001$) and higher diagnostic accuracy (74.37% *vs*. 55.42%, $P < 0.001$). The superiority of FNB over FNA in adequacy for IHC (odds ratio, 2.786 [1.515–5.291]) and diagnostic accuracy (odds ratio, 2.793 [1.645–4.808]) remained significant after control of confounders including needle size, lesion site, lesion size, and endoscopists. In subgroup analysis, FNB showed higher diagnostic accuracy in AIP and mesenchymal tumor, whereas no statistically significant difference was observed in NET and lymphoma.

Conclusions: FNB was superior to FNA needles in obtaining tissues with better adequacy and integrity. These results suggest that FNB should be considered a first-line modality in the diagnosis of IHC-required lesions, especially AIP and mesenchymal tumor. However, a randomized controlled trial with larger sample size is needed to further confirm our findings.

Keywords: EUS; Fine-needle biopsy; Fine-needle aspiration; Immunohistochemistry; Tissue acquisition

INTRODUCTION

EUS-FNA has been widely used to diagnose lesions in and around the gastrointestinal (GI) tract.[1] Although EUS-FNA is the preferred sampling method, compared with its satisfying performance of acquiring cytological specimens, FNA needles are less capable of obtaining core tissues for histological assessments, especially in the absence of rapid on-site evaluation (ROSE).[2] However, for certain neoplasms such as neuroendocrine tumor (NET) or chronic inflammation, procurement of core tissue is essential for cytological evaluation and performance of immunohistochemistry (IHC) to establish a diagnosis.

In need of acquiring more core tissue for detailed examination and immunostaining, multiple techniques of EUS-FNA were adopted to improve the diagnostic yield but resulted in little success.[3] With the advent of the EUS–fine-needle biopsy (FNB) needles with different tip and side fenestration,[4–6] studies including randomized trials and meta-analyses were conducted to compare the diagnostic yields of FNA and FNB needles, which resulted in conflicting conclusions.[2,7–15] Whether FNB is superior to FNA remains highly controversial. For pancreatic adenocarcinoma and lymph node metastasis, cytology is often adequate for diagnosis, without a significant difference in diagnostic efficiency between FNB and FNA observed.[7,15] Although in other lesions such as NET and lymphoma, consistent results of improved core tissue acquisition and diagnostic yield were observed, a decreased number of needle passes without ROSE was obtained when biopsy needles were compared with conventional

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FNA ones.[16–18] The superior diagnostic yield of FNB over FNA was also confirmed by studies in autoimmune pancreatitis (AIP), where a larger amount of core tissue with preserved architecture may be required for diagnosis.[19–21] Even for pancreatic cancers, a larger quantity of tissue enables molecular profiling and next-generation sequencing, which are vital for risk stratification and targeted therapy or immunotherapy.[14,22]

Current published research comparing FNB needles with FNA in NET or AIP exclusively already explored the advantages of FNB in core tissue acquisition and diagnostic yield. However, most studies mainly focused on diagnostic accuracy and core tissue length, whereas sample quality and tissue adequacy remained as secondary outcomes.[16,17,20,21] Also, the significance of conclusions was limited by sample size. In this context, we aimed to investigate lesions where IHC is necessary to confirm the diagnosis. Especially in cases of uncertain cytological diagnosis, IHC is essential to make a definitive diagnosis. The authors conducted a real-world study with prospective sample evaluation to determine the difference in histologic yield between FNA and FNB needles in 439 patients with IHC-required lesions including AIP, NET, mesenchymal tumor, and lymphoma.

METHODS

Study design

This study was a real-world, multicenter, single-blinded study comparing the efficacy of the FNB and FNA needles in obtaining adequate tissue specimens with prospective sampling evaluation. ROSE was not available during the process. This trial was conducted from April 2015 to July 2022 at Peking Union Medical College Hospital and Tongji Hospital, Tongji Medical College affiliated to Huazhong University of Science and Technology, 2 major tertiary care centers in China. The study was performed in compliance with the Declaration of Helsinki. The protocol was approved by the institutional review boards of each participating center and registered at ClinicalTrial.gov (NCT05565066).

Patients and interventions

Patients who underwent EUS-guided sampling at the 2 centers of this research and were finally diagnosed with either (1) type 1 AIP, (2) NET, (3) mesenchymal tumor, and (4) lymphoma were considered eligible according to the inclusion criteria. All patients with definitive or probable type 1 AIP were diagnosed based on the International Consensus Diagnostic Criteria (ICDC).[23] Patients with NET, mesenchymal tumor, or lymphoma required diagnosis according to histopathological findings (surgical or provided by EUS-FNB/FNA. Exclusion criteria were patient age younger than 18 years, pregnancy, uncorrectable coagulopathy (platelet count <50,000/mm^3, international normalized ratio >1.5), acute pancreatitis in the preceding 2 weeks, severe cardiorespiratory dysfunction precluding endoscopy, and failure to provide informed consent. All consecutive patients provided informed consent.

Participating endoscopists were required to meet the following criteria: (1) have performed more than 100 EUS-guided tissue sampling procedures to date or at least 50 in the last 12 months and (2) willing to comply with the study requirements, including presenting the possibility to participate in the study to all subjects eligible. After confirming the eligibility criteria were fulfilled, investigators would select puncture needle type according to the needles available then and lesion characteristics. Needles used in this study included 19G, 22G, and 25G FNA (either EchoTip Ultra from Cook or Expect needle from Boston Scientific) and 19G, 20G, 22G, and 25G FNB (EchoTip ProCore from Cook or Acquire from Boston Scientific). A more detailed description of intervention procedure is provided in Appendix 1.

Specimen evaluation

The aspirated samples from each pass were expelled onto separate slides with a stylet. After this, 0.1 mL of sterile saline was flushed into the needle and followed with 5 mL of air. The macroscopically visible core tissue was transferred into Eppendorf tubes containing 10% formalin for histological examination and subsequently embedded in paraffin. Specimen sections were cut and stained with eosin and hematoxylin. (Sections of suspected AIP were further stained with IgG4, CD38, and CD138; sections of NET were stained with CgA, Syn, and CD56; sections of suspected mesenchymal tumor were stained with c-kit, CD34, DOG-1, α-SMA, Desmin, and S-100; a section of suspected lymphoma was stained with CD3, CD5, CD19, CD20, CD22, CD30, CD45RO CD79a, PAX5, and BCL2; Supplementary Figure 1, http://links.lww.com/ENUS/A348: Additional IHC markers were stained as needed.) Two pathologists blinded to the type of needles used and clinical information, independently assessed all tissue samples obtained. When the 2 experts made a different diagnosis, the agreement was reached by consulting a third pathologist and carefully discussing the findings.

The tissue integrity for histological analysis was scored from 0 to 5 as follows [Figure 1]: score 5, sufficient material for adequate histological interpretation (core tissue length > 1 × 10 high power field [HPF]); score 4, sufficient material for adequate histological interpretation (core tissue length < 1 × 10 HPF); score 3, sufficient material for limited histological interpretation; score 2, sufficient material for adequate cytological diagnosis; score 1, sufficient material for limited cytological diagnosis (no representativeness); and score 0, inadequate for diagnosis, based on previously reported system.[24] Those cases with histological characteristics resembling AIP, NET, mesenchymal tumor, or lymphoma but without IHC evaluation were excluded from the final diagnosis (not including failed IHC cases).

Outcomes

The 2 primary outcomes of this study were to compare the IHC success rate and diagnostic accuracy of the specimens from FNB needles *versus* FNA ones. Adequate histological core to perform IHC was defined according to the following criteria: (1) adequacy to provide histological diagnosis, and (2) after cutting the sections stained with hematoxylin and eosin, the remaining tissue thickness > (4 × *n*) μm (*n* refers to the number of necessary markers to diagnose the specific disease; each section requires a minimum of 4 μm of thickness). Because specificity was not involved in this study, the diagnostic accuracy was defined as the true positive values divided by the total number of samples. The secondary outcomes were to compare the specimen quality, namely, core tissue length and tissue integrity scores in the samples obtained by FNB and FNA needles. To seek the potential merits of FNB or FNA needles in different situations, we further compared the efficacy of the 2 types of needles in the subgroup analysis (lesion type and lesion size).

Statistical analysis

In this study, the demographic and clinical characteristics of the patients were summarized with mean and SD, and ranked data were

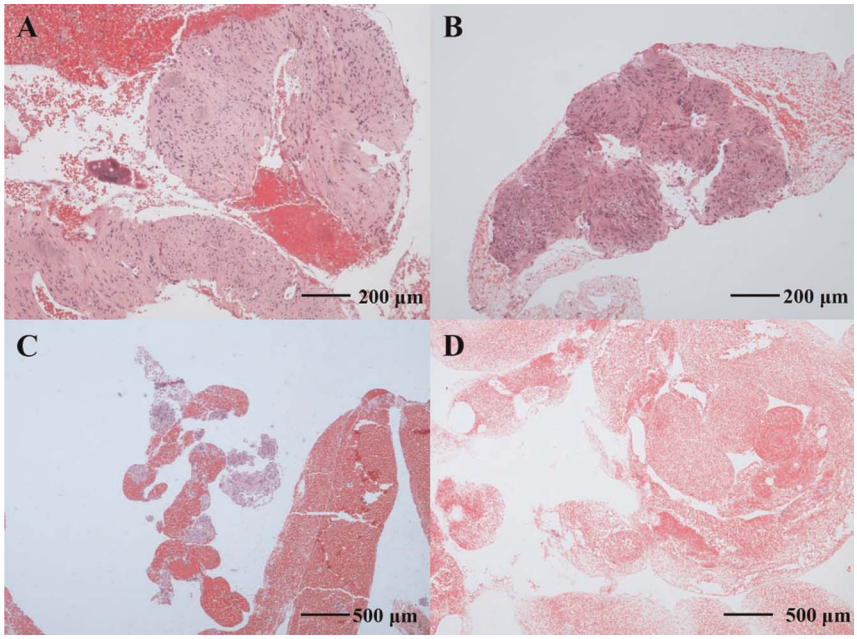

Figure 1. The tissue integrity assessments of specimens (hematoxylin and eosin stained). Example of (A) score 5, sufficient material for adequate histological interpretation (core tissue length > 1 * 10 HPF, original magnification ×100); (B) score 4, sufficient material for adequate histological interpretation (core tissue length < 1 * 10 HPF, original magnification ×100); (C) score 3, sufficient material for limited histological interpretation (original magnification ×40); and (D) score 0, inadequate for diagnosis, based on previously reported system (original magnification ×40). Scores 2 and 3 are measurements of cytological results and are thus not exhibited here.

expressed as median and interquartile range. Categorical parameters including sex, lesion type, lesion site, adverse events, adequacy for IHC, and diagnostic accuracy were expressed in terms of the number of cases and percentage. Qualitative variables were compared using the χ^2 test or Fisher exact test, whereas Student *t* test and the Mann-Whitney *U* test were used for quantitative variables. The effect of FNB or FNA on IHC success rate and diagnostic accuracy was determined using multivariate logistic regression to control the potential confounders, including needle size, lesion site, procedures in different time spans, and different endosonographers. Statistical significance was defined as $P < 0.05$ (2-tailed). All statistical analyses were performed using SPSS V.26.0.

RESULTS

Patient and lesion characteristics

From April 2015 to July 2022, 458 patients were enrolled in this study, and 19 patients were excluded because of suspected pancreatic cancer or lack of definitive diagnosis. Therefore, the remaining 439 patients were analyzed: 199 in the FNB group and 240 in the FNA group. Technical success occurred in all cases [Figure 2]. Table 1 illustrates the baseline clinical characteristics of the recruited patients. There were no significant differences in age, sex ratio, tumor size, or tumor location between groups. Of the 439 patients,

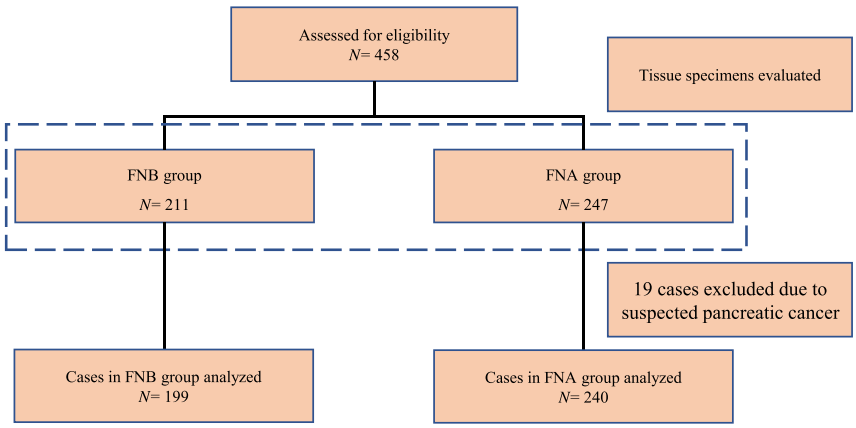

Figure 2. Study flowchart.

Table 1

Baseline characteristics

Variables	FNB (*n* = 199)	FNA (*n* = 240)	*P*
Age, mean (SD), y	56.47 (12.90)	56.42 (12.42)	0.966
Sex, male/female	129/70	153/87	0.816
Lesion size, mean (SD), mm	34.68 (19.11)	33.85 (17.40)	0.675
Puncture site, *n* (%)			0.244
Pancreas head/neck	92 (46.23)	98 (40.83)	
Pancreas body/tail	33 (16.58)	41 (17.08)	
Retroperitoneum	44 (22.11)	51 (21.25)	
Mediastinum	11 (5.53)	27 (11.25)	
GI tract	19 (9.55)	21 (8.75)	
Pelvic cavity	0 (0.00)	2 (0.83)	
Type of lesions, *n* (%)			0.015*
Autoimmune pancreatitis	94 (47.24)	89 (37.08)	
Neuroendocrine tumor	35 (17.60)	64 (26.67)	
Mesenchymal tumor	50 (25.13)	49 (20.42)	
Lymphoma	20 (10.05)	38 (15.83)	
Needle size, *n* (%)			<0.001***
19/20-gauge	1 (0.50)	49 (20.42)	
20-gauge	147 (73.87)	-	
22-gauge	47 (23.62)	181 (75.42)	
25-gauge	4 (2.01)	10 (4.17)	
Adverse events, *n* (%)	2 (1.00)	1 (0.42)	0.592

FNA: fine-needle aspiration; FNB: fine-needle biopsy; GI: gastrointestinal.

*$P < 0.05$.

***$P < 0.001$.

163 (37.13%) were classified as type 1 AIP in final diagnosis, according to the ICDC.[2,3] Two hundred seventy-six patients (99 NET, 99 mesenchymal tumor, 58 lymphoma) were diagnosed based on surgical or EUS-guided tissue sampling histology. Adverse events were minimal (1 minor upper GI hemorrhage after puncture in each group treated with hemostatic clip placement and 1 mild pancreatitis in the FNB group) and not statistically different between the FNB and FNA groups (1.00% *vs*. 0.42%, $P = 0.592$).

Needle and sampling characteristics

Various sizes of needles were used in this study, including 19G, 19G, 19G, and 19G. Sizes such as 20G (73.87%) and 22G (75.42%) were more commonly used in FNB and FNA, respectively. Because of the different compositions of needle sizes, multivariate analysis was subsequently performed to control confounding factors. Despite similar size and location of solid lesions, FNB resulted in fewer total needle passes compared with FNA: median of 3 passes (interquartile range, 3–4) *versus* 4 passes (3–4) ($P < 0.001$). Furthermore, we observed that FNB needles yielded specimens with longer core-tissue length (0.7 [0.5–1.0] *vs*. 0.5 [0.4–0.8], $P = 0.037$) and better adequacy (83.59% *vs*. 69.23%, $P = 0.004$) for histopathological diagnosis (specimens with a tissue integrity score of 3, 4, or 5 were classified as adequate; Table 2, Figure 3). Cumulative data were then stratified by diagnosis (AIP, NET, mesenchymal tumor, and lymphoma). For AIP and mesenchymal tumor, FNB had significantly higher IHC successful rate *versus* FNA (AIP: 75.53% *vs*. 55.06%, OR of 2.520 [95% confidence interval {CI}, 1.344–4.725], $P = 0.0040$; mesenchymal tumor: 86.00% *vs*. 63.27%, OR of 3.567 [95% CI, 1.328–9.577], $P = 0.009$). No statistical differences were observed between FNB and FNA in subgroups of NET and lymphoma. Further stratification analysis based on lesion size revealed a significantly higher rate of successful IHC staining in a subgroup of lesions ≥20 mm (91.25% *vs*. 78.81%; OR, 2.803 [1.148–6.843]; $P = 0.020$), especially among mesenchymal tumors (88.37% *vs*. 64.29%; OR, 4.222 [1.369–13.018]; $P = 0.009$). There were no statistical differences between FNB and FNA in a subgroup of lesions <20 mm (Supplementary Table 1, http://links.lww.com/ENUS/A349).

Diagnostic accuracy

Histopathological diagnosis according to FNB specimens were more consistent with the final diagnosis (for, AIP, being consistent with the final diagnosis refers to the histopathological findings corresponding to lymphoplasmacytic sclerosing pancreatitis level 1 or 2). Compared with FNA, FNB resulted in significantly better diagnostic accuracy (74.37% *vs*. 55.42%; OR, 2.646 [95% CI, 1.739–4.026]; $P < 0.001$; Table 3). In the stratification analysis stratified according to diagnosis, FNB yielded better diagnostic accuracy for AIP (62.77% *vs*. 40.45%; OR, 2.482 [95% CI, 1.369–4.500]; $P = 0.003$), NET (91.43% *vs*. 73.44%; OR, 3.858 [1.044–14.256]; $P = 0.038$), and mesenchymal tumor (82.00% *vs*. 55.10%; OR, 3.712 [1.487–9.269]; $P = 0.004$). There was no significant difference in the diagnostic accuracy of lymphoma between the 2 groups. Furthermore, stratification analysis according to lesion size revealed that, for lesions ≥20 mm, the diagnosis was more accurate based on FNB specimens in NET (100% *vs*. 78.72%; OR, 1.270 [1.095–1.474]; $P = 0.027$) and mesenchymal tumor (83.72% *vs*. 57.14%; OR, 3.857 [1.399–10.637]; $P = 0.009$) subgroups compared with FNA specimens. There were no statistical differences in diagnostic accuracy between FNB and FNA in a subgroup of lesions <20 mm (Supplementary Table 2, http://links.lww.com/ENUS/A350).

Autoimmune pancreatitis

In terms of diagnostic pathological features, according to the ICDC, lymphoplasmacytic sclerosing pancreatitis level 1 or 2 was more commonly found in tissue samples from the FNB group compared with those undergoing FNA (62.77% *vs*. 40.45%; OR, 2.482

Table 2

Specimen adequate for IHC

	FNB (*n* = 199)	FNA (*n* = 240)	OR (95% CI)	*P*
Overall no. adequate sample for IHC, n (%)	164 (82.41)	160 (66.67)	2.343 (1.489–3.685)	<0.001***
AIP	71 (75.53)	49 (55.06)	2.520 (1.344–4.726)	0.004**
NET	33 (94.29)	52 (81.25)	3.808 (0.801–18.106)	0.129
Mesenchymal tumor	43 (86.00)	31 (63.27)	3.567 (1.328–9.577)	0.009**
Lymphoma	17 (85.00)	28 (73.68)	2.024 (0.487–9.862)	0.509

AIP: autoimmune pancreatitis; CI: confidence interval; FNA: fine-needle aspiration; FNB: fine-needle biopsy; IHC: immunohistochemistry; NET: neuroendocrine tumor; OR: odds ratio.

**$P < 0.01$.

***$P < 0.001$.

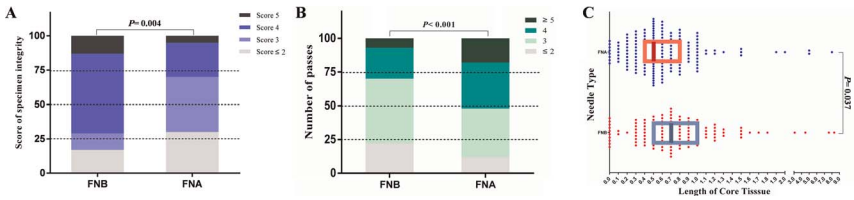

Figure 3. Comparison of specimen quality between FNB and FNA in terms of (A) percentage of adequate specimens, (B) number of passes to acquire adequate specimens, and (C) length of core tissues for all lesions.

[1.369–4.500]; $P = 0.003$). Supplementary Table 3 (http://links.lww.com/ENUS/A351) summarizes the IHC findings. Specimens from patients in FNB group contained >10 CD38/CD138-positive plasma cells per HPF were significantly more than specimens from patients in the FNA group (46.81% *vs*. 25.84%, $P = 0.002$). Likewise, the number of specimens containing IgG4-positive plasma cells >10/HPC from FNB group was significantly higher than in those from the FNA group (30.85% *vs*. 12.36%, $P = 0.002$).

Mesenchymal tumor

The 99 patients diagnosed with mesenchymal tumors consisted of 71 GI stromal tumors (GISTs), 12 schwannomas, 10 leiomyoma/leiomyosarcoma, 3 liposarcomas, 2 sarcomatoid mesotheliomas, and 1 myofibroblastic tumor. In the subgroup analysis for GIST, the c-kit–positive and DOG-1–positive specimens from patients in the FNB group were significantly more than specimens from FNA group patients (c-kit: 85.00% *vs*. 54.84%, $P = 0.005$; DOG-1: 82.50% *vs*. 58.06%, $P = 0.023$; Supplementary Table 3, http://links.lww.com/ENUS/A351).

Neuroendocrine tumor

There were no statistical differences in the number of CgA-positive and Syn-positive specimens between the 2 groups. However, the number of CD56-positive specimens from FNB group patients was significantly higher than specimens from the FNA group (74.29% *vs*. 51.56%, $P = 0.028$).

Multivariate logistic regression

Multivariate analysis was then performed to control needle type, needle size, lesion site, FNA/FNB procedures in different time spans, and different endosonographers as confounding factors. Based on the results of multivariate logistic regression and controlled for the aforementioned variables, needle type was still the significant predictor for higher success rate of IHC (OR, 2.786 [1.515–5.291]; $P = 0.001$) and accurate diagnosis (OR, 2.793 [1.645–4.808]; $P \leq 0.001$; Table 4). However, only in the AIP and mesenchymal subgroup, FNB still resulted in higher diagnostic accuracy after adjusted with all the confounding factors (AIP: OR of 3.861 [1.471–10.870], $P = 0.008$; mesenchymal tumor: OR of 3.802 [1.239–12.500], $P = 0.021$; Table 5), whereas the differences were no longer remarkable in NET and lymphoma.

DISCUSSION

As highlighted in the background, published studies comparing the diagnostic yields between FNB and FNA needles produced conflicting results.[2,7,9,25,26] The current guidelines of endoscopic tissue sampling

Table 3

Comparison of diagnostic rate between FNB and FNA

	FNB (n = 199)	FNA (n = 240)	OR (95% CI)	P
Overall no. cases consistent with final diagnosis, *n* (%)	148 (74.37%)	133 (55.42%)	2.646 (1.739–4.026)	<0.001***
Type 1 AIP	94	89		
No. adequate sample for histopathological classification, *n* (%)	59 (62.77%)	36 (40.45%)	2.482 (1.369–4.500)	0.003**
LPSP level, *n* (%)				0.003**
LPSP level 1[a]	28 (29.79%)	11 (12.40%)		
LPSP level 2[b]	31 (32.98%)	25 (28.09%)		
Unclassifiable	35 (37.23%)	53 (59.55%)		
NET	35	64		
No. cases consistent with final diagnosis, *n* (%)	32 (91.43%)	47 (73.43%)	3.858 (1.044–14.256)	0.038*
Mesenchymal tumor	50	49		
No. cases consistent with final diagnosis, *n* (%)	41 (82.00%)	27 (55.10%)	3.712 (1.487–9.269)	0.004**
Lymphoma	20	38		
No. cases consistent with final diagnosis, *n* (%)	16 (80.00%)	23 (60.53%)	2.609 (0.730–9.328)	0.155

[a]Level 1 LPSP was defined as at least 3 of the following: (i) periductal lymphoplasmacytic infiltrate without granulocytic infiltration, (ii) abundant (>10 cells/HPF) IgG4-positive cells, (iii) storiform fibrosis, and (iv) obliterative fibrosis.

[b]Level 2 LPSP was defined as meeting 2 of the aforementioned criteria.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

AIP: autoimmune pancreatitis; LPSP: lymphoplasmacytic sclerosing pancreatitis; NET: neuroendocrine tumor.

endorsed no particular needle type to improve diagnostic accuracy.[27] However, the guidelines still indicated the advantages of FNB in obtaining more tissues for diagnosis and genetic profiling, especially when ROSE is not available. Histological evaluation in combination with IHC is essential for the diagnosis of AIP, NET, mesenchymal tumor, and lymphoma. IHC provides important information to differentiate neoplastic and nonneoplastic lesions and identify the tumor subtype. Thus, we conducted this real-world, multicenter study to compare the efficacy of FNB and FNA in diagnosing those IHC-required lesions. The results of our analysis demonstrated that FNB needles yielded specimens with better adequacy and quality for IHC. Also, a higher diagnostic accuracy of FNB was observed, potentially due to the superiority of specimen quality.

To date, this multicenter trial remains the largest real-world study to compare the efficacy of EUS-FNB and FNA sampling in IHC-required lesions. Different from previous studies, we collected a large series of patients with multiple types of lesions. Type 1 AIP was the most frequent in this study, followed by NET, mesenchymal tumor, and finally lymphoma. In terms of location, pancreatic lesions (60.55%) accounted for the majority, with the rest being other retroperitoneal sites, GI tract, mediastinum, and pelvic cavity in that order. Twenty-gauge is not available for FNA needles, and 19-gauge is much rarer in FNB needles, which partially explains the different compositions of needle sizes. To make our comparison more reliable, we further performed multivariate logistic regression to eliminate the potential baseline bias, which was unique to our study. Among the 198 NET and mesenchymal patients recruited in this study, 27 patients underwent post-EUS surgical resection based on the histological findings. In the meanwhile, 107 patients were not turned to surgery because of no evidence of malignancy. This again emphasized the importance of EUS for medical decision making.

EUS-FNA seems to be accurate for diagnosing pancreatic cancer or making a preliminary diagnosis for AIP and NET.[17,20] However, it was not satisfying enough to provide personalized management of specific lesions. Especially in cases of AIP, FNA is often incapable of acquiring enough densely fibrotic tissue.[21] For spindle cell lesions, the imperative diagnosis was impossible in the absence of IHC staining.[28] In our study, FNB resulted in specimen cores with better adequacy and quality, which was pivotal for diagnosing the above diseases. In general, 82.41% of specimens from FNB were adequate for IHC staining, significantly higher than the rate of 66.67% from the FNA group ($P < 0.001$). Similar to other studies, we observed that fewer number of passes was required to achieve diagnosis in the FNB group (FNA *vs.* FNB: 4 [3–4] *vs.* 3 [3–4], $P < 0.001$).[9] More importantly, the tissue acquired by FNB had better integrity score (FNA *vs.* FNB: 4 [3–4] *vs.* 3 [0.5–4], $P < 0.001$) and longer core tissue length (FNA *vs.* FNB: 0.5 [0.4–0.8] *vs.* 0.7 [0.5–1.0], $P < 0.001$).[2,9,18] As our previous study indicated, the architecture of tissue was better preserved through FNB obtained.[9] Conversely, the pauci-cellularity nature of FNA led to the tissue collected being distorted or consumed during IHC sectioning.[19,28]

As highlighted in previous studies, the major advantage of FNB in AIP was to decrease cytologically inconclusive cases.[21,29] In our study, based on the ICDC, 37.23% of FNB cases and 59.55% of FNA ones were uninformative ($P = 0.003$). A higher success rate of IgG4 IHC staining accounted for the improvement. We also found IgG4-positive cells >10/HPF in 30.85% of FNB cases compared with 12.36% in FNA ($P = 0.002$), which was reported as 16% to 78% in previous studies.[21,30] The relatively higher rate of uninformative cases could be related to the spans of a long period and the IHC staining of elastic fiber, and the symbol of obliterative phlebitis was not routinely stained in China. High density of fibrosis, as a common feature of AIP tissue, along with the lack of cellular constitution including plasma cells, often leads to an ambiguous diagnosis. Larger tissue cores obtained by FNB are more likely to contain more IgG4-positive plasma cells. A sufficient amount of tissue provides more details to make an accurate diagnosis. After eliminating all confounding factors including needle size and different endoscopists, the differences in IHC rate and diagnostic rate were still significant, indicating that FNB may be a better choice for AIP than conventional FNA.

Table 4

Multivariate logistic regression analysis of overall lesions

	FNB		FNA	
	OR (95% CI)	*P*	OR (95% CI)	*P*
No. cases adequate for IHC				
Crude model	2.427 (1.548–3.861)	<0.001***	1.000 (ref.)	—
Model 1 (needle size)	1.984 (1.215–3.268)	0.007**		
Model 2 (lesion site)	2.457 (1.565–3.922)	<0.001***		
Model 3 (lesion size)	3.413 (1.957–6.211)	<0.001***		
Model 4 (per endosonographer, per year)	2.381 (1.517–3.802)	<0.001***		
Model 5 (all factors)	2.786 (1.515–5.291)	0.001**		
Histopathological diagnostic rate				
Crude model	2.398 (1.600–3.623)	<0.001***	1.000 (ref.)	—
Model 1 (needle size)	2.262 (1.466–3.521)	<0.001***		
Model 2 (lesion site)	2.463 (1.639–3.745)	<0.001***		
Model 3 (lesion size)	3.226 (1.984–5.376)	<0.001***		
Model 4 (per endosonographer, per year)	2.500 (1.658–3.802)	<0.001***		
Model 5 (all factors)	2.793 (1.645–4.808)	<0.001***		

**$P < 0.01$.

***$P < 0.001$.

CI: confidence interval; FNA: fine-needle aspiration; FNB: fine-needle biopsy; IHC: immunohistochemistry; OR: odds ratio.

Table 5

Multivariate logistic regression analysis according to lesion types

	FNB		FNA	
	OR (95% CI)	*P*	OR (95% CI)	*P*
No. cases adequate for IHC				
AIP (model 1[a])	4.444 (1.639–13.514)	0.005**	1.000 (ref.)	—
NET (model 2[b])	5.435 (0.840–111.111)	0.137		
Mesenchymal tumor (model 2)	1.481 (0.347–5.747)	0.576		
Lymphoma (model 2)	1.148 (0.208–7.042)	0.874		
Histopathological diagnostic rate				
AIP (model 1)	3.861 (1.471–10.870)	0.008**	1.000 (ref.)	—
NET (model 2)	4.049 (0.894–29.412)	0.102		
Mesenchymal tumor (model 2)	3.802 (1.239–12.500)	0.021*		
Lymphoma (model 2)	1.515 (0.291–8.197)	0.615		

[a]Model 1 was adjusted for needle size, endosonographer, and operation year.

[b]Model 2 was adjusted for needle size, lesion site, lesion size, endosonographer, and operation year.

*$P < 0.05$.

**$P < 0.01$.

AIP: autoimmune pancreatitis; CI: confidence interval; FNA: fine-needle aspiration; FNB: fine-needle biopsy; IHC: immunohistochemistry; NET: neuroendocrine tumor; OR: odds ratio.

Regarding the mesenchymal tumor, similar to the results of 69.30% to 100% in previous studies,[28,31] our IHC success rate of the FNB group was 86.00%, higher than the rate of 63.72% in FNA ($P = 0.009$). Notably, the diagnostic accuracy of FNB was also significantly higher than that of FNA (82.00% *vs*. 55.10%, $P = 0.004$). GIST was the most common lesion type of mesenchymal tumor in our study. In diagnosis of GIST, c-kit and DOG-1 were commonly used as indicative markers. We further observed a higher rate of c-kit or DOG-1 positivity in the FNB group. Although FNA may be accurate for detecting spindle cell lesions, IHC staining is essential to make differential diagnosis between GIST and leiomyoma/leiomyosarcoma. Specimens obtained by FNB needles could provide a more accurate evaluation of mitotic activity for risk classification,[31] which is important to determine the next-step clinical management. Besides GIST, 5 leiomyoma/leiomyosarcoma, 12 schwannomas, and 11 other types of mesenchymal tumors were also included in this study. Possibly limited by sample size, the rate of α-SMA– or desmin-positive specimen in leiomyoma and the rate of S-100–positive specimen in schwannoma were not statistically different between the 2 groups. However, a tendency indicating the superiority of FNB in Schwannoma (OR, 21.000; $P = 0.067$) was observed. To eliminate the influence of possible confounding factors, multivariate logistic regression was also performed in the analysis of mesenchymal tumors. FNB needles demonstrated a significantly higher diagnostic accuracy than FNA in the mesenchymal tumor subgroup; however, no difference in IHC staining was noted after the regression. As illustrated in previous studies, even if IHC was performed with FNA specimen, limited tissue could not ensure an accurate diagnosis. [28]

In the subgroup analysis of NET and lymphoma, no statistical differences were found in adequacy for IHC between FNB and FNA. Only for NET, the diagnostic accuracy of FNB was higher than FNA (91.43% *vs*. 73.43%, $P = 0.038$). However, after controlling for confounding factors, the differences were not statistically significant, probably because cellular components were much more abundant in NET and lymphoma than in AIP and mesenchymal tumors. Cell crushing was commonly observed in IHC staining of FNA/FNB samples, which may be the cause of insufficient immunostaining in AIP.[29] In contrast, although cell crushing also occurred in NET and lymphoma cases, the abundance of cellular components ensured that a certain number of tumor cells were stained properly. In our study, FNA also yielded a relatively satisfying diagnostic rate of NET. Despite a previous study concluding that FNB yielded higher sensitivity in the diagnosis of NET,[16] the result was not validated by a rigorous statistical comparison but simply the listing of the sensitivity of the 2 methods. Also, in stratification analysis according to lesion size, FNB yielded a higher diagnostic rate in the subgroup of lesion ≥20 mm, whereas no statistical difference was observed in the smaller lesion subgroup. Unfortunately, most of the benign NETs were <20 mm,[32] which is not favorable evidence for FNB application. As for lymphoma, although a tendency indicating the superiority of FNB in the diagnostic rate was present (OR, 2.609; $P = 0.155$), we did not observe a statistical difference possibly due to the small sample size.

Despite being the largest study exclusively evaluating IHC-required lesions, we recognize some major limitations. First, although specimens in this study were again prospectively evaluated uniformly, this is a real-world retrospective study with a lack of randomization and therefore inevitably subjected to selection bias and confounding factors. During the long-time span of this study, the techniques of EUS and endoscopists' skills are constantly being refined, which also gave rise to unquantifiable effects. Similarly, multiple available needle sizes were used as it is a real-world study. In this study, ProCore accounted for a majority of the FNB needles. Given the limited use of other needles, we did not perform the comparative interclass analysis of different products. To eliminate these heterogeneities as much as possible, multivariate logistic regression was performed.

In summary, FNB needles yielded specimens with better adequacy and quality compared with FNA for the IHC-required lesions without ROSE. These results strongly suggest that FNB should be preferably selected in the diagnosis of the IHC-required lesions, especially AIP, mesenchymal tumor, and NET with a size of ≥20 mm. However, larger randomized controlled trials are needed to confirm these findings. The improvement of diagnostic accuracy and classification of IHC-required lesions will certainly help gastroenterologists and surgeons manage challenging situations with more confidence.

Clinical Trial Registration

The protocol was approved by the institutional review boards of each participating center and registered at ClinicalTrial.gov (NCT05565066).

Conflict of Interest

The authors declare that they have no financial conflict of interest with regard to the content of this report.

Ethical Approval

This study was conducted at two tertiary care centers in China, including Tongji Hospital, Tongji Medical College, HUST and Peking Union Medical College Hospital with approval by the hospital ethics board (TJH: TJ-IRB20220647, PUMCH: I-22PJ651).

Author Contributions

All authors contributed to the study concept and design. Material preparation, data collection and analysis were performed by Yuchong Zhao, Dingkun Xiong, and Aruna. The first draft of the manuscript was written by Yuchong Zhao and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Appendix 1

Intervention EUS-FNB/FNA was performed with a curved linear-array echoendoscope (GF-UCT 260; Olympus, Tokyo, Japan). The examinations were performed in a standardized manner with patients under conscious sedation with propofol. After puncturing the lesion, the endoscopist removed the stylet and attached a 5-mL prevacuum syringe for aspiration. Each pass consisted of 20 back-and-forth movements of the needle slowly and steadily within the lesion or diffusely enlarged pancreas, using the fanning technique. To ensure that each patient would receive an accurate diagnosis, additional needle passes were performed until the endosonographer believed a sufficient amount of sample was obtained. At least 1 piece of macroscopically visible tissue (whitish or yellowish, >4 mm in the greatest axis measured) was obtained from each patient. We routinely observed complications and checked the patients' serum amylase for 3 days after EUS-FNB/FNA. All patients were then followed up with telephone calls for 4 to 7 days after the procedure to record any potential adverse events.