Fork-tip needle biopsy versus fine-needle aspiration in endoscopic ultrasound-guided sampling of solid pancreatic masses: a randomized crossover study

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ABSTRACT
Background A novel fork-tip fine-needle biopsy (FNB) needle has recently been introduced for endoscopic ultrasound (EUS)-guided sampling. The aim of this study was to compare the performance of fork-tip FNB histology and standard fine-needle aspiration (FNA) cytology in the diagnosis of solid pancreatic masses.

Methods A randomized crossover study was performed in patients referred for EUS-guided sampling. Three passes were taken with each needle in a randomized order. Only samples reported as diagnostic of malignancy were considered positive. The primary end point was the sensitivity of diagnosis of malignancy. Secondary end points included the amount of sample obtained, ease of diagnosis, duration of tissue sampling, pathologist viewing time, and cost.

Results 108 patients were recruited. Median age was 69 years (range 30–87) and 57 were male; 85.2% had a final diagnosis of malignancy. There were statistically significant differences in sensitivity (82% [95% confidence interval (CI) 72% to 89%] vs. 71% [95% CI 60% to 80%]), accuracy (84% [95% CI 76% to 91%] vs. 75% [95% CI 66% to 83%]), proportion graded as a straightforward diagnosis (69% [95% CI 60% to 78%] vs. 51% [95% CI 41% to 61%]), and median pathology viewing time (188 vs. 332 seconds) (P<0.001) between FNB and FNA needles, respectively. There was no significant difference in cost between an FNB or FNA strategy.

Conclusion The diagnostic performance of the fork-tip FNB needle was significantly better than that of FNA; it was associated with ease of diagnosis, shorter pathological viewing times, and was cost neutral.

Introduction
Tissue acquisition by means of endoscopic ultrasound (EUS)-guided fine-needle aspiration (FNA) is the standard of care for diagnosing pancreatic malignancy. Systematic reviews and meta-analyses [1,2] have reported pooled sensitivity of 86.8% and 85%, respectively. However, reported sensitivity varies and some studies that have used strict cytological criteria and accounted for inadequate samples have documented less im-
pressive diagnostic performance [3–5]. In an international sur-
vey, only one third of endosonographers reported a sensitivity
for the diagnosis of solid mass lesions of >80% [6]. Needle
size, aspiration technique (suction/no-suction, slow stylet
pull), and the use of rapid onsite evaluation (ROSE) [7] have all
been investigated as means of improving diagnostic perform-
ance, without consistent benefit. EUS-guided fine-needle biopsy
(FNB) with acquisition of tissue cores for histological assess-
ment offers the theoretical benefit of preserved tissue architec-
ture, improved sample adequacy, and therefore better diagnos-
tic performance. However, the majority of studies with the first-
generation reverse bevel needle (ProCore; Cook Medical, Bloom-
ington, Indiana, USA) have found no diagnostic benefit [8,9].

Recently, a new core biopsy needle with a novel fork tip
(SharkCore FNB needle; Medtronic, Watford, UK) has been in-
troduced. Initial case series have suggested improved diagnos-
tic performance compared with FNA [10,11] and first-genera-
tion core biopsy [12] needles. Prospective randomized studies
to investigate this new fork-tip needle vs. FNA needles are lack-
ing. Therefore, the aim of our study was to address this ques-
tion in a randomized crossover study.

Methods
Study design and patient population

The study recruited consecutive patients attending for EUS-
guided tissue sampling of solid pancreatic masses in a tertiary
pancreatic cancer center between May 2017 and June 2018.
Written informed consent was obtained from all patients for
the procedure. The study was carried out in accordance with
the Declaration of Helsinki and was approved by the North
East-Newcastle and North Tyneside 1 Research Ethics Committ-
tee (reference 17/NE/001). The study is registered at Clinical-
Trials.gov (NCT03532347), and is reported according to the
Standards for Reporting Diagnostic Accuracy Studies (STARD)
and the Consolidated Standards of Reporting Trials (CONSORT)
statements (see the online-only Supplementary material).

Adult patients (≥18 years) with a suspicion of pancreatic
mass based on prior cross-sectional imaging were eligible. Ex-
clusion criteria included cystic lesions without a significant so-
lid component, and any contraindication to pancreatic biopsy.
The presence of a self-expandable metal stent in patients with
a mass in the head of the pancreas was an initial exclusion crite-
rion, but this was removed by protocol amendment part way
through the study.

EUS-guided sampling

All procedures were performed under conscious sedation using
combinations of intravenous midazolam and pethidine, with
standard cardiorespiratory monitoring. Pentax echoendo-
scopes (EG3270UK and EG3870UTX; Pentax, Slough, UK) and
Hitachi ultrasound workstations (Preirus; Hitachi Medical Sys-
tems, Wellingborough, UK) were used. A single Beacon EUS de-
ivery device (Medtronic Ltd., Watford, UK) was used with 25-
gauge and 22-gauge needles for transduodenal and transgas-
tric biopsy, respectively. For all procedures, 10 mL suction and
fanning were used. The needle was moved backward and for-
ward within the lesion for a minimum of 10 throws. Suction
was stopped prior to withdrawal of the needle.

Needles were used in a randomized order. Patients were ran-
domly allocated to an initial three passes with the FNA needle
(Beacon FNA needle; Medtronic) or FNB needle (SharkCore FNB
needle; Medtronic) and then crossed over to the other needle.
Randomization was via a computer-generated list of allocations
produced in advance of the study and referred to after partici-
pant recruitment. After the initial three passes, the first needle
was discarded and the other needle was affixed to the delivery
device, which remained attached to the echoendoscope, and
a further three passes were made. Procedures were timed using a
stopwatch.

Samples were expelled from the needles in a standardized
manner. The stylet was first introduced and then the needle
was flushed with a few milliliters of normal saline. For FNA, a
drop from the needle was expelled onto a glass slide and a sin-
gle spread slide was made from each pass. The remaining aspir-
ate was expelled into a container with approximately 5 mL of
BD CytoRich Red Preservative solution (BD; Bioscience Health-
care, Nottingham, UK). The air-dried slides, including the draw
slide, were fixed in Reastain Quick Diff fix solution (Gamidor
Technical Services, Southmead Park UK) to be stained using
the Reastain Quick Diff stains. In the laboratory, any clots or mi-
crobiospies present in the CytoRich solution were removed and
placed in formalin and subsequently embedded in paraffin. A
single 4-μm section was cut and stained with hematoxylin and
eosin (H&E).

For all specimens, the remaining CytoRich specimen was
processed for staining by SurePath Papanicolaou staining
method (Bioscience Healthcare).

All samples from the FNB needle were placed in a single con-
tainer of 10% neutral buffered formalin. Samples were subse-
quently embedded in paraffin. A single 4-μm section was cut and
stained with H&E.

Additional stains were performed at the discretion of the
pathologist if further information for diagnosis was required.

Follow-up

All procedures were performed as day cases. Patients were ob-
served in the endoscopy recovery area as per routine recovery
protocol until fully awake and allowed home if asymptomatic.
Patients were contacted by a research nurse 7 days after the
procedure to ascertain the occurrence of any post-procedure
adverse events. Serious adverse events were defined as compli-
cations resulting in a physician visit or an episode of hospitaliza-
tion or extension of an existing hospital stay, significant disabil-
ity or death [13].

Pathology

The FNA samples were reported by one of two specialist cyto-
pathologists and the FNB by one of two pancreatic histopathol-
ogists. The pathologists were blinded to the report of the other
sampling method. All samples were reported with a prose re-
port describing the findings, with an interpretation and final di-
agnostic category: inadequate, benign, atypical, suspicious of
malignancy or malignant. The pathologist timed microscope
interpretation using a stopwatch (including for any subsequent immunostaining); only the time spent examining slides was measured. A novel, simple three-tier score to allow comparison of cytology and histology samples with regard to amount of diagnostic material and ease of diagnosis was used (see Supplementary material).

Definitions and end points
The primary end point was diagnostic performance of the fork-tip FNB needle compared with the standard FNA needle. The primary end point was assessed using “strict” criteria as follows; samples reported as benign, atypical, and suspicious were categorized as negative for malignancy and only samples reported as malignant were categorized as positive for malignancy. For the purposes of the study, low grade malignant tumors (i.e. gastrointestinal stromal tumors, lymphoma, and neuroendocrine tumors) were considered malignant. Specimens that contained inadequate material were not excluded from primary analysis and were considered as negative for malignancy. A secondary “less strict” analysis was also performed excluding inadequate samples and categorizing suspicious samples as diagnostic of malignancy.

A conventional “malignancy” analysis was performed purely for the differentiation of malignancy from benign (i.e. correct classification as benign or malignant was the only requirement). A “specific diagnosis” analysis was also performed; for this, a specific tissue diagnosis that matched the final diagnosis was required. Diagnostic performance was expressed using standard performance parameters including sensitivity, specificity, and overall accuracy. Secondary end points were duration of sampling time (in seconds), duration of pathologist viewing time (in seconds), amount of diagnostic material, ease of diagnosis, and cost analysis.

The final gold standard diagnosis for malignancy required either unequivocal malignant pathology obtained by EUS sampling, surgical resection or alternative biopsy. For nonoperated patients with nondiagnostic tissue sampling, clinical and radiological disease progression consistent with malignancy at 6-month follow-up was required.

Benign classification required nonmalignant and nonsuspicious tissue sampling and follow-up of at least 6 months with a consistent clinical picture and no evidence of malignancy on interval imaging.

Statistical analysis
Sample size
The sample size was calculated in order to show a difference in sensitivity for the diagnosis of malignancy of 14% between groups based on prior data from our unit (92% FNB, 78% FNA). The percentage of patients having discordant results for the two needles was estimated to be 22%. Using a 5% significance level and 80% power, we calculated that 86 malignant cases were required and estimated that malignant cases would account for 85% of all recruited cases. It was also assumed that there would be an attrition rate of 5% due to loss of patients during follow-up. After inflating the sample size to account for both factors, we calculated that 107 patients were required. To allow for a balanced design the target sample size was set at 108.

Data analyses
Baseline characteristics of the patient population, pancreatic mass lesions, technical details, and procedure outcomes were summarized as means with standard deviation (SD) or medians with interquartile range (IQR) for continuous data, and as frequencies and proportions for categorical data. The data analysis allowed for the fact that each patient had two results, and that two outcomes from the same patient are likely to be more similar than two results from different patients. The analysis also factored in that outcomes in the first period (i.e. first needle used) may be different from those in the second period. All analyses were performed using multilevel logistic regression or multilevel linear regression (STATA version 15.1; StataCorp, College Station, Texas, USA). Two-level models were used, with individual results nested within patients. Fixed terms were included in the model for the period (first or second needle) and the needle used (FNA or FNB), while patient was used as a random factor.

An initial analysis examined whether the differences between needles varied depending on whether they were from the first or second period (needle by period interaction) as this could indicate a carryover effect from the first to second period. If this interaction was not statistically significant, the interaction term was omitted, and the differences between needles were assessed using data from both periods.

Analysis of diagnostic performance, amount of material, and ease of diagnosis was performed. For amount of material and ease of diagnosis, two different outcomes were considered: first, the proportion scored as 3, and second the proportion scored as 2 or 3. Tissue sampling duration and pathology viewing time were compared. All tests were two-sided and P values <0.05 were considered statistically significant.

Cost analysis
The cost of an FNB sampling strategy was compared with an FNA sampling strategy. The cost was based on the England National Health Service (NHS) 2017/18 tariff for an EUS tissue sampling procedure (£639). This tariff includes the cost of any of the accessories used, pathological processing, and reporting. The standard NHS cost was €187 for the FNA needle and €263 for the fork-tip needle. To better reflect the cost of needle choice to the health economy for the purposes of the study, the “cost” of the procedure was assumed to be the NHS tariff plus the cost of needle.

If a nondiagnostic result was obtained, it was assumed that a single repeat procedure would be performed for that patient using the same type of needle. Bootstrap methods were used to obtain the confidence interval (CI) for the cost difference between needles; 100 bootstrap samples were used in the calculations.
Results
A total of 171 patients were assessed for eligibility, 63 were excluded, and 108 (57 men, 51 women) with a median age of 69 years (range 30–87) were randomized (▶Fig. 1). Five patients had a self-expandable metal stent in situ. Lesion characteristics and final diagnoses are shown in ▶Table 1. ▶Table 2 shows the pathological diagnostic category. There was no difference between the FNB and FNA needles in the proportion of malignant cases reported as unsatisfactory (3.3 % vs 4.3 %, respectively); however, there was a difference in the proportion of malignant cases categorized as suspicious (6.5 % vs. 18.5 %, respectively).

Diagnostic performance
The results (▶Table 3) suggested that for all diagnostic outcomes, there was no significant needle by period interaction. Therefore, the needle effect was quantified using the data from both periods combined.

The needles were found to vary significantly in terms of accuracy for specific diagnosis. Accuracy was 79 % (95 %CI 70 % to 86 %) for FNB compared with 64 % (95 %CI 54 % to 73 %) for FNA. The odds of an accurate specific diagnosis were 4 times higher with the FNB needle compared with the FNA needle (OR 4.79, 95 %CI 1.67 to 13.7).

Analysis using less strict criteria
There was no significant difference in diagnostic performance for malignancy or specific diagnosis when the “less strict” criteria were used (Table 1s).

Duration of sampling and pathology viewing
▶Table 4 summarizes the results. The sampling time was found to be significantly shorter for the FNB needle compared with the FNA needle: mean 710 (SD 171) seconds vs. 759 (SD 144) seconds (P=0.001). Pathology viewing times were also significantly shorter for FNB compared with FNA needles: median 188 seconds (IQR 56–330) vs. 332 seconds (IQR 212–411) (P<0.001).
The results for amount of material and ease of diagnosis are summarized in Table 5. A significantly higher proportion of FNB cases were categorized as 3 (abundant for diagnosis): 58% (95% CI 48% to 68%) vs. 44% (95% CI 35% to 54%) for FNA; however, this was associated with a significant needle by period interaction. There was no difference when only data from the first period were analyzed. For ease of diagnosis, a significantly higher proportion of FNB cases were graded as 3 (straightforward diagnosis): 69% (95% CI 60% to 78%) vs. 51% (95% CI 41% to 61%) for FNA. There was no significant difference in cost: mean €1044 (SD 330) vs. €1033 (SD 359) for FNB vs. FNA, respectively. The mean difference was 12 (95% CI –5 to 95; P = 0.74).

### Adverse events

Serious adverse events occurred in four patients within 7 days (3.7%): two developed cholangitis (one of whom underwent endoscopic retrograde cholangiopancreatography immediately following EUS tissue sampling, and the other had a pre-existing plastic biliary stent), one patient developed pancreatitis, and one patient was hospitalized for 24 hours with severe abdominal pain.

### Discussion

In this prospective, randomized, crossover study comparing the fork-tip FNB and FNA needles in the sampling of solid pancreatic masses, the FNB needle was more sensitive than the FNA needle.
dle for the diagnosis of pancreatic malignancy (82% vs. 71%) when using strict criteria. Additionally, FNB had significantly shorter sampling and pathology viewing times, as well as greater ease of diagnosis reported by the pathologists. Under “less strict” criteria, with suspicious samples categorized as diagnostic of malignancy, the difference in diagnostic performance was eliminated. However, a suspicious diagnosis is not acceptable by most oncologists for initiation of chemotherapy and such patients would not be eligible for recruitment into chemotherapy trials.

EUS core biopsy needles have been designed to provide larger tissue samples with preserved tissue architecture that are adequate for histological assessment. If achieved, this would enable speedier, more accurate diagnosis, in addition to the potential to conduct the more detailed histological subtyping and genetic analysis of tumors required for personalized therapies. Histological assessment should also facilitate the definitive diagnosis of benign conditions such as autoimmune pancreatitis and chronic pancreatitis, which are challenging to make with FNA.

The reverse bevel needle was the first widely used core biopsy needle; however, this has not shown unequivocal superiority to FNA. A recent large prospective study [14] reported significantly higher diagnostic yield with the reverse bevel needle compared with FNA for pancreatic lesions; however, it is unclear whether this equated to better diagnostic accuracy or rather more frequent diagnostic histological samples. In an earlier study by Vaniervliet et al., in addition to finding no difference in diagnostic performance, the authors reported better overall sample quality with the FNA needle [15]. Two meta-analyses [8, 16] reported no significant difference in sensitivity or acquisition of cores, while another [17] reported the core needles to have better specimen adequacy and diagnostic accuracy.

The SharkCore needle has a fork tip, opposing bevel design with six cutting surfaces designed to acquire architecturally intact tissue and minimize tissue disruption. Initial retrospective studies documented high pathological yield with fewer passes [10, 11] and the needle significantly outperformed the reverse bevel with an adjusted OR of 7.93 [12]. A further retrospective study found no difference in diagnostic performance between the fork-tip needle and FNA needle, although the fork-tip needle yielded a significantly higher proportion of tissue that was adequate for histological evaluation and ancillary diagnostic techniques [18]. A recent multicenter prospective study using the 25-gauge fork-tip needle for sampling solid pancreatic masses reported the presence of cores in 67% of samples [19]. In a large retrospective study of 3020 patients, 71.3% of whom were undergoing sampling of a pancreatic mass using FNA or second-generation core biopsy needles (either Franseen–Acquire, Boston Scientific Corp – or fork-tip), the median number of passes required for diagnostic adequacy on ROSE was significantly lower for FNB compared with FNA, and diagnostic yield on cell block was superior with FNB [20]. The superior performance of FNB over FNA was observed for both pancreatic and nonpancreatic lesions. A multicenter retrospective study of 22-gauge fork-tip and 22-gauge FNA needles in patients with solid pancreatic mass lesions found no significant difference in diagnostic performance but a significantly reduced number of passes to obtain diagnostic tissue [21]; ROSE was available for all procedures in this study. Another retrospective study of primarily pancreas and gut wall lesions without on-site cytopathology also reported fewer needle passes with the fork-tip needle [22]; there was, again, no significant difference in sensitivity (fork-tip 89.9% vs. FNA 81.0%). The diagnostic performance of Franseen and fork-tip needles appears to be similar; a recent randomized trial [23] found no significant difference between the two needles in tissue yield, with >90% of diagnostic cell blocks.

Conventionally, the diagnostic performance of EUS tissue sampling in pancreatic masses is considered in a binary manner – cancer/noncancer. This reflects the clinical scenario in which the pre-test probability of malignancy is high, and a diagnosis of malignancy will trigger major surgery and/or chemotherapy. However, a significant minority of cases (15% in the present study) will be benign and a definitive histological diagnosis in this context is of value. First, by enhancing the benign diagnosis beyond one of just “nonmalignant,” confidence in the accuracy
of the diagnosis is increased, thereby lessening the likelihood of repeat sampling or inappropriate surgery. Second, a specific histological diagnosis (e.g., autoimmune pancreatitis) will ensure appropriate therapy is initiated promptly.

This study therefore also reported diagnostic performance in terms of requiring a specific tissue diagnosis (malignant or benign) that matched the final diagnosis. Using this threshold, a benign case (e.g., chronic pancreatitis) where the pathology report stated benign/noncancer without establishing a diagnosis of chronic pancreatitis would be classified as inaccurate. Use of this higher threshold resulted in an even greater difference in diagnostic performance between the needles, with an OR of 4.79.

The results reflect the twin benefits of greater amount of tissue and preserved tissue architecture with the FNB needle. Sampling time was also significantly shorter, and reflects the differences in in-room preparation; FNB samples were expelled directly into formaldehyde whereas for each FNA pass, a slide was prepared and the remaining material expelled into a container. However, though statistically significant, this small difference in sampling time is unlikely to be clinically relevant.

The strengths of the present study include its prospective, randomized, crossover design and the blinding of the reporting pathologists to the results with the paired needle. Potential weaknesses include the inability to blind the endosonographers to the needle type; however, adherence to strict protocols in terms of number of passes, throws within the lesion, and use of the fanning technique should have mitigated this bias. The use of separate histopathologists and cytopathologists is another possible weakness as this prevented blinding to the needle type. However, both sets of pathologists had extensive expertise in the reporting of pancreatic histology and cytology, respectively, and due to the very different nature of the pathological processing it would have been impossible for pathologists to be blinded to the nature of the sample they were reporting. The different processing techniques of the samples does raise the possibility that this, and not only the needle, was a factor in the data differences identified; however, any clots or microbiopsies obtained with the FNA needle were processed in the same way as samples from the FNB needle. Additionally, the amount of material and ease of diagnosis were as-

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### Table 5  Tissue amount and ease of diagnosis.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>FNB</th>
<th>FNA</th>
<th>NxP interaction</th>
<th>Effect of needle type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n/N</td>
<td>% (95%CI)</td>
<td>n/N</td>
<td>% (95%CI)</td>
</tr>
<tr>
<td><strong>Amount of material classification</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Too scanty for diagnosis – 1</td>
<td>11/108</td>
<td>10 (5 to 17)</td>
<td>18/108</td>
<td>17 (10 to 25)</td>
</tr>
<tr>
<td>Sufficient for diagnosis – 2</td>
<td>34/108</td>
<td>31 (23 to 41)</td>
<td>42/108</td>
<td>39 (30 to 49)</td>
</tr>
<tr>
<td>Abundant for diagnosis – 3</td>
<td>63/108</td>
<td>58 (48 to 68)</td>
<td>48/108</td>
<td>44 (35 to 54)</td>
</tr>
<tr>
<td><strong>Ease of diagnosis classification</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Challenging/impossible – 1</td>
<td>9/108</td>
<td>8 (4 to 15)</td>
<td>12/108</td>
<td>11 (6 to 19)</td>
</tr>
<tr>
<td>Some difficulty – 2</td>
<td>24/108</td>
<td>22 (15 to 31)</td>
<td>41/108</td>
<td>38 (29 to 48)</td>
</tr>
<tr>
<td>Straightforward – 3</td>
<td>75/108</td>
<td>69 (60 to 78)</td>
<td>55/108</td>
<td>51 (41 to 61)</td>
</tr>
<tr>
<td><strong>Statistical comparison</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion graded 3</td>
<td>63/108</td>
<td>58 (48 to 68)</td>
<td>48/108</td>
<td>44 (35 to 54)</td>
</tr>
<tr>
<td>Proportion graded 3(^1)</td>
<td>27/53</td>
<td>51 (37 to 65)</td>
<td>31/55</td>
<td>56 (42 to 70)</td>
</tr>
<tr>
<td>Proportion graded 2 or 3</td>
<td>97/108</td>
<td>90 (83 to 95)</td>
<td>90/108</td>
<td>83 (75 to 90)</td>
</tr>
</tbody>
</table>

FNB, fine-needle biopsy; FNA, fine-needle aspiration; NxP, needle by period; CI, confidence interval; OR, odds ratio.

\(^1\) Analysis to examine whether the differences between needles varied depending on whether they were used first or second.

\(^2\) OR given as odds for FNB relative to odds for FNA.

\(^3\) Results based on data from first period only.
sessed using a novel nonvalidated scoring system, as existing scoring systems were not suitable for this purpose.

In conclusion, we have demonstrated in a prospective randomized study that the EUS fork-tip biopsy needle yielding a histology specimen delivers substantially better diagnostic performance, better sample amount, ease of reporting, and shorter sampling and pathological viewing times than a standard FNA needle yielding a cytology specimen, and this strategy was cost neutral. This finding provides the first prospective evidence to support the contention that such EUS core biopsy needles should be regarded as the standard of care for tissue acquisition from solid pancreatic masses.

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The study was presented as a poster at Digestive Disease Week, San Diego, May 2019, and the British Society of Gastroenterology annual meeting, Glasgow, June 2019.

Competing interests

Dr. Oppong has received a research grant, travel grant, and speaker fees from Medtronic. Dr. Bekkali has received a travel grant from Boston Scientific. Drs. Nayar and Haugk have received research grants and speaker fees from Medtronic. Drs. Johnson, Leeds, and Darne have received research grants from Medtronic. Dr. Bassett has no conflict of interest.

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