DETAILS ON LINE

METHODOLOGICAL DESCRIPTION

Patient Inclusion

- ≥19 years of age
- Suspected solid pancreatic mass on computed tomography or magnetic resonance imaging of the abdomen and referred for EUS-guided tissue acquisition
- Written informed consent

Patient Exclusion

- No mass seen at EUS or if the lesion appeared cystic.
- Abnormal coagulation parameters – international normalized ratio (INR) > 1.6 and/or platelets < 80 x 10^9/L

IRB/Registration

- The study was approved by institutional review board of our hospital (No. 889569-12) and registered at ClinicalTrials.gov (NCT02911974).

Main Outcomes

Compare between the two needle types:

- Median area of total tissue
- Median area of tumor
- Presence of desmoplastic fibrosis in patients with neoplasia
- Retained tissue architecture

Other Outcomes

Compare between the two needle types:

- Cell block diagnostic yield
- Ability to perform immunohistochemistry (IHC) studies

Study approach and technique

- *Randomization and Masking*: Computer-generated randomization assignments were obtained prior to study enrollment in a 1:1 sequence using the block randomization method by the statistician. These were then placed in sequentially numbered sealed opaque envelopes and opened by the endoscopy nurse during the procedure when patients met inclusion criteria.
- *Procedural Technique*: All procedures were performed using a linear array echoendoscope with patients in the left lateral decubitus position after administration of propofol. At EUS, the mass was first punctured using the FNB or FNA needle per randomization sequence. To minimize specimen bloodiness, suction was not applied in any of the procedures and tissue acquisition was performed adopting the fanning technique (four strokes at four different locations within the mass). After performing two dedicated passes for cell block for histological analysis using the first (randomized) needle, two additional passes were made for cell block using the alternate needle. EUS-guided sampling was then continued to establish onsite diagnostic adequacy by making the first pass using the first (randomized) needle and then alternating with the second needle until a preliminary diagnosis was established. At least one pass was performed using each needle for onsite specimen evaluation and the procedure was terminated when an onsite diagnosis was achieved with at least one of the needles. The occurrence of any immediate adverse event was noted at the time of the procedure, and late adverse events were documented with follow-up telephone calls one-week post-procedure.
- *Cell block preparation and histological assessment*: Specimens were collected for cell block in a methanol-based preservative solution (CytoLyt, Holgic Inc., Marlborough, MA) that was subsequently concentrated through centrifugation and combined with plasma and thrombin to form a

tissue clot in the laboratory. After forming a tissue clot (cell button), specimens were fixed in formalin, paraffin embedded, sectioned, and stained with hematoxylin and eosin for histological evaluation. To limit subjective interpretation, tissue samples were quantified using image-analyzing software that objectively measures individual tissue components (Nikon DS-Fi2 color camera and NIS-Elements Basic Research Software Version 4.5, Melville, NY). We also compared the proportion of samples in each cohort for the presence of retained histological architecture which is essential for evaluation of morphologically challenging lesions. Immunohistochemistry (IHC) studies were performed for further evaluation of morphologically challenging lesions and molecular biomarker testing was undertaken when required. To ascertain the ability to perform IHC studies, for each cell block, two levels of sectioning were performed (20μm sections into block). Blocks which had 10 or more tumor cells present on the second level (L2 = 40μm deep) were considered sufficient for an extensive IHC panel.

- *Rapid onsite evaluation (ROSE)*: For FNB, the specimens were processed using the touch imprint technique. The surface of the specimen was carefully touched onto the slide, allowing the superficial cells to adhere, and then gently lifted with forceps thereby creating a touch imprint. For FNA, the cytological aspirate was expressed onto slides and smeared. Slides from both techniques were air dried and stained with Diff-Quik for ROSE. All slides were reviewed onsite by a cytopathologist, blinded to needle type and technique, to establish onsite diagnostic adequacy and specimen bloodiness. Wet-fixed smears from both techniques were immediately immersed in 95% ethyl alcohol (rapid fixation) for subsequent Papanicolaou staining in the pathology laboratory.

Study Definitions

- Final diagnosis of malignancy was defined by the presence of one or more of the following criteria: (i) histologic evidence of malignancy in a surgical specimen, (ii) progression of the lesion or presence of metastases on follow-up imaging, (iii) cancer-related death, or (iv) follow-up with the patient's referring physician confirming death or disease progression. Lesions were considered benign if they

met one or more of the following criteria: (i) surgical pathology reported no malignancy, (ii) follow-up imaging reporting a stable mass with no metastases, or (iii) patient well-being at follow-up with the primary care physician. The reference standard for classification of disease included the following: surgical resection, death from disease progression, repeat radiologic, and/or clinical follow-up. Diagnostic adequacy of cell block was defined as the presence of histological tissue from the sampled lesion. Diagnostic accuracy of cell block was defined as the presence of histological tissue that was representative of the findings at final diagnosis. Non-diagnostic cell block was defined as suboptimal or insufficient material that was not representative of the findings at final diagnosis. Onsite diagnostic adequacy for ROSE was defined as a FNA sample of sufficient quality to render a preliminary diagnosis. Non-diagnostic ROSE was defined as the inability to obtain sufficient material to establish an onsite diagnosis.

Data analysis

- *Sample size:* Two-sided sample size calculation was performed at 90% power and type I error rate (α) of 0.05 to detect 1 mm^2 difference in the mean procured core tissue area between needle types. Using standard deviations of 1.81 for FNB and 0.69 for FNA, based on a selection of previously obtained samples (5), sample size was estimated at 41 patients. Recruitment was set at 46 to account for 10% drop-out.
- *Statistical analysis:* Baseline characteristics of patient population, pancreatic mass lesions and procured tissue samples were summarized as means (with standard deviation) and medians (with interquartile range and range) for continuous data and as frequencies and proportions for categorical data. For comparison of categorical data, Chi-square or Fisher's exact test were used; Wilcoxon rank-sum test was used for comparison of continuous data. Statistical significance was determined at $p<0.05$.

DETAILS OF RESULTS

Patient follow-up

At a median follow-up of 232 days (IQR 232-237 days):

- Pancreatic cancer: 15 patients had died, seven had undergone surgery, 14 were receiving chemotherapy/radiation therapy and one patient elected for palliative care.
- Other tumors: Of the two patients with neuroendocrine tumor, one had undergone surgery and the other was receiving chemotherapy. Both the patients with sarcoma and small cell cancer were undergoing chemotherapy and had disease progression.
- Benign disease: All five patients with chronic pancreatitis were clinically well on follow-up with no radiological progression of disease.

Adverse events: None in either cohort.

Supplementary Table 1. Patient details and pancreatic mass characteristics

Age (years):	Mean (SD)	67.9 (14.7)
	Median	70
	IQR	58 - 79
	Range	21 - 93
Gender: n (%)	Female	18 (39.1)
	Male	28 (60.9)
Race: n (%)	Black	9 (19.6)
	Hispanic	4 (8.7)
	White	33 (71.7)
Pancreatic mass size (cm):	Mean (SD)	2.9 (0.8)
	Median	3
	IQR	2.3 - 3.5
	Range	1.5 - 4.3
Pancreatic mass location: n (%)	Head	21 (45.7)
	Uncinate	7 (15.2)
	Neck	10 (21.7)
	Body	2 (4.3)
	Tail	6 (13.0)
Vascular invasion: n (%)		25 (54.3)
Metastases present: n (%)		9 (19.6)
Final diagnosis: n (%)	Adenocarcinoma	37 (80.4)
	Neuroendocrine tumor	2 (4.3)
	Small cell cancer	1 (2.2)
	Sarcoma	1 (2.2)
	Chronic pancreatitis	5 (10.9)

Abbreviations: IQR, interquartile range; SD, standard deviation

Supplementary Table 2. Comparison of procured histological tissue between the two needles

		FNB	FNA	p-value
Total tissue area (mm^2):	Mean (SD)	11.1 (26.6)	0.9 (1.9)	< 0.0001
	Median	6.1	0.28	
	IQR	2.2 - 9.9	0.045 - 0.93	
	Range	0.025 - 181.1	0 - 11.7	
Total tumor area (mm^2):	Mean (SD)	1.7 (2.3)	0.50 (1.86)	< 0.0001
	Median	0.68	0.099	
	IQR	0.23 - 2.8	0.0044 - 0.30	
	Range	0 - 12.4	0 - 11.6	
Desmoplastic fibrosis present: n (%)		33 (84.6)	13 (33.3)	< 0.0001
Area of desmoplastic fibrosis (mm^2):	Mean (SD)	9.8 (28.4)	0.14 (0.45)	< 0.0001
	Median	3.9	0	
	IQR	0.5 - 8.2	0 - 0.11	
	Range	0 - 178.3	0 - 2.7	
Architecture retained: n (%)		43 (93.5)	9 (19.6)	< 0.0001
Suitable for immunohistochemistry studies: n (%)*		41 (100)	28 (68.3)	< 0.0001

Abbreviations: FNA, fine needle aspiration; FNB, fine needle biopsy; IQR, interquartile range; SD, standard deviation

* In 41 patients with neoplastic lesions only

Supplementary Table 3. Comparison of procedural details and outcomes between the two needles

		FNB (n=46)	FNA (n=46)	p-value
ROSE - Diagnostic adequacy: n (%)*		46 (100)	44 (95.7)	0.495
ROSE - Total no. of passes for diagnostic adequacy:†	Mean (SD)	1.15 (0.47)	1.18 (0.58)	0.929
	Median	1	1	
	IQR	1 - 1	1 - 1	
	Range	1 - 3	1 - 4	
	1	41 (89.1)	38 (86.4)	0.778
	2	3 (6.5)	4 (9.1)	
	3	2 (4.3)	1 (2.3)	
	4	0	1 (2.3)	
Specimen bloodiness: n (%)	Mild	12 (26.1)	16 (34.8)	0.736
	Moderate	29 (63.0)	26 (56.5)	
	Severe	5 (10.9)	4 (8.7)	
Cell block - Diagnostic adequacy: n (%)		45 (97.8)	38 (82.6)	0.030
Cell block - Diagnostic accuracy: n (%)‡		43 (93.5)	37 (80.4)	0.063
Adverse events: n (%)		0	0	0.999

Abbreviations: FNA, fine needle aspiration; FNB, fine needle biopsy; IQR, interquartile range; ROSE, Rapid onsite evaluation; SD, standard deviation

*Two patients in the FNA cohort had a non-diagnostic sample on ROSE, which on cell block (both FNB and FNA) were diagnostic for adenocarcinoma and chronic pancreatitis.
† n=44 for calculation of the total no. of passes performed in the FNA group as sample was not diagnostically adequate on ROSE in two patients
‡ FNB cell block was false negative in three patients with adenocarcinoma. FNA cell block was false negative in nine patients, of whom eight had adenocarcinoma and one had chronic pancreatitis.