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Standardisation of EUS-guided FNB technique for molecular profiling in pancreatic cancer: results of a randomised trial

Ji Young Bang [1], Nirag Jhala,[2] Anjali Seth,[3] Konrad Krall,[1] Udayakumar Navaneethan,[1] Robert Hawes,[1] Charles Melbern Wilcox,[1] Shyam Varadarajulu [1]

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[1]Digestive Health Institute, Orlando Health, Orlando, Florida, USA
[2]Department of Pathology, Temple University, Philadelphia, Pennsylvania, USA
[3]Pathology, Temple University, Philadelphia, Pennsylvania, USA

Correspondence to
Dr Shyam Varadarajulu, Digestive Health Institute, Orlando Health, Orlando, Florida, USA;
svaradarajulu@yahoo.com

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MESSAGE

As clinically actionable genomic lesions are found in almost 30% of pancreatic cancers that can potentially impact management, there has been increased focus on molecular profiling. Although tissue acquisition under endoscopic ultrasound (EUS) guidance is an established diagnostic method, procedural outcomes for comprehensive molecular profiling (CMP) have been variable. In a randomised trial, we found that performing two dedicated passes using the 22-gauge Franseen needle, adopting the fanning and stylet-retraction manoeuvres, yielded optimum specimen from which adequate RNA and DNA could be extracted for CMP in almost 95% of patients with pancreatic cancer.

IN MORE DETAIL

Given the poor outcomes of traditional chemotherapy, there is increased focus on molecular profiling to personalise pancreatic cancer treatment. Recently, CMP tests using next-generation sequencing (NGS) were approved for commercial use. These tests provide clinically relevant information on gene alterations that include both actionable and resistant mutations thereby enabling selection of chemotherapy tailored to individual patients. While the National Comprehensive Cancer Network (NCCN) guidelines recommend EUS as the modality of choice for establishing pathological diagnosis in suspected pancreatic cancer, it is unclear if the technique is also suited for tissue procurement to conduct CMP.[1] This is because the volume of tissue obtained using 22-gauge (G) needles is small, viability of DNA extracted from formalin-fixed paraffin-embedded (FFPE) tissue is unknown and RNA extraction from pancreatic tissue is more cumbersome and difficult due to enzymatic degradation.[2 3] To determine the optimal technique by which adequate DNA and RNA can be procured for CMP studies, we conducted a randomised trial in patients undergoing EUS-guided fine needle biopsy (FNB) of suspected pancreatic cancer.

Patients with pancreatic mass proven to have adenocarcinoma by rapid onsite evaluation at EUS were randomised intra-procedurally to undergo two or three dedicated FNB passes for CMP. Tissue was procured using the 22G Franseen needle (Boston Scientific) adopting evidence-based practices (fanning technique and stylet-retraction manoeuvre) that yield highly cellular tissue.[4 5] Genomic DNA and total RNA were simultaneously extracted from FFPE cell blocks (QIAGEN AllPrep).[6] The pathology laboratory assessing specimens for CMP was blinded to the randomisation assignment. Main outcome was the proportion of specimens from which adequate DNA and RNA could be extracted for CMP and the sample size was estimated at 16 per group (total sample size of 33 to account for a 5% drop out rate).

Thirty-three patients diagnosed to have pancreatic adenocarcinoma at EUS-FNB were randomised to undergo two (n=17) or three (n=16) FNB passes (online supplemental figure 1, table 1). While sufficient DNA was extracted from all 33 (100%) FFPE cell blocks, adequate RNA was extracted from 15 of 16 FFPE cell blocks in the three pass (93.8%) versus 16 of 17 in the two pass cohort (94.1%) (p=0.99) with no significant difference in the median concentration of DNA (two pass 9.6 ng/µL (IQR 2.8–16.6) vs three pass 7.8 ng/µL (IQR 5.0–10.8); p=0.228) or RNA (two pass 36.5 ng/µL (IQR 11.4–52.5) vs three pass 30.5 ng/µL (IQR 15.2–39.8); p=0.374) between groups (table 2). While DNA mutations were identified in all 33 specimens, 12.1% of the study cohort had clinically relevant or actionable mutations that included BRCA1 in one, KRAS G12C mutations in two and somatic oncogene RNA fusion (LDAH-ETV1) in one (online supplemental table 1).

COMMENTS

The NCCN guidelines recommend that germline testing be considered in all patients diagnosed with pancreatic cancer and molecular analysis in those patients with locally advanced or metastatic disease.[1] Germline testing and molecular profiling carry important implications for screening and treatment. Molecular profiling enables better selection of chemotherapy regimens tailored to individual patients thereby improving treatment outcomes while at same time avoiding unnecessary treatment in patients in whom chemotherapy may be ineffective.

Although NGS can sequence multiple genes, acquisition of sufficient tissue is a mandatory requisite for testing. While surgical biopsies can yield larger specimens, only 20% of patients with pancreatic cancer are potential surgical candidates.

Table 1 Patient demographics and procedure details

		Two passes (n=17)	Three passes (n=16)	P value
Age: (years)	Mean (SD)	76.2 (7.6)	77.9 (7.9)	
	Median	77	80	0.471
	IQR	72–80	72–83	
	Range	61–88	60–92	
Gender: n (%)	Female	9 (52.9)	11 (68.7)	0.353
	Male	8 (47.1)	5 (31.3)	
Ethnicity: n (%)	Black	3 (17.6)	1 (6.3)	0.584
	Caucasian	12 (70.6)	14 (87.5)	
	Hispanic	2 (11.8)	1 (6.3)	
Pancreatic mass size (cm):	Mean (SD)	3.8 (0.9)	3.6 (1.1)	
	Median	3.7	3.6	0.649
	IQR	3.0–4.0	2.8–4.9	
	Range	2.0–5.5	1.8–5.0	
Mass location: n (%)	Head/uncinate	14 (82.4)	8 (50.0)	0.071
	Neck/body/tail	3 (17.6)	8 (50.0)	
Distant metastasis: n (%)*		4 (23.5)	4 (25.0)	0.999
Technical success: n (%)		17 (100)	16 (100)	0.999
Adverse events: n (%)†		1 (5.9)	1 (6.3)	0.999

*Distant metastasis: Two pass group—peritoneal carcinomatosis (n=1), liver (n=3). Three pass group—liver (n=4).
†Adverse events: one patient in the two pass group died from underlying cancer in hospice care 1 week after the procedure; one patient in the three pass group had a duodenal perforation during Endoscopic Retrograde Cholangiopancreatography (ERCP) for biliary stricture and underwent surgical repair.

Therefore, several studies have explored surrogacy of EUS procured samples for surgically resected specimens in NGS. These studies have shown modest concordance with reported adequacy ranging from 60% to 100%.[7] Given lack of knowledge on optimum needle gauge, tailored procedural manoeuvres, requisite number of passes, specimen processing methods and their correlation to extracted DNA and RNA, the debate on effectiveness of EUS-guided tissue acquisition for molecular profiling persists. Addressing these questions will enable reliable procurement of adequate tissue for CMP, expedite oncological care and minimise financial loss due to suboptimal sampling as state-of-the-art NGS testing can cost upward of US $4000.

Table 2 Details on specimen procured and molecular profiling

		Two passes (n=17)	Three passes (n=16)	P value
Diagnostic adequacy on onsite evaluation: n (%)		17 (100)	16 (100)	0.999
Diagnostic adequacy on cell block: n (%)		17 (100)	16 (100)	0.999
Specimen bloodiness: n (%)	Low	8 (47.1)	7 (43.7)	0.999
	Moderate	8 (47.1)	8 (50.0)	
	High	1 (5.9)	1 (6.3)	
Adequate DNA extracted: n (%)		17 (100)	16 (100)	0.999
DNA concentration: ng/µL	Mean (SD)	10.7 (7.1)	7.9 (4.4)	
	Median	9.6	7.8	0.228
	IQR	2.8–16.6	5.0–10.8	
	Range	0.87–20.9	0.68–16.0	
Adequate RNA extracted: n (%)		16 (94.1)	15 (93.8)	0.999
RNA concentration: ng/µL	Mean (SD)	37.1 (26.5)	28.9 (13.2)	
	Median	36.5	30.5	0.374
	IQR	11.4–52.5	15.2–39.8	
	Range	3.6–97.0	9.9–49.0	

How are the findings of the present study important and why is it relevant? One, our study has proposed a specific needle gauge, identified procedural manoeuvres and determined the finite number of biopsies required for reliable procurement of tissue for CMP (figure 1, online supplemental video 1). This study showed that performing two passes at EUS-FNB yielded samples that were adequate for CMP, with no significant difference between two and three passes, likely due to the tumour becoming significantly bloody after two passes that then led to suboptimal extraction of RNA and DNA. Two, FFPE cell blocks are commonly used worldwide for specimen processing and the present study demonstrates that CMP testing can be performed satisfactorily from these cell blocks. Three, although we were able to create 16–20 FFPE cell blocks with tissue obtained from two dedicated FNB passes, only 50–100 ng of RNA or DNA were required for conducting assays which could be derived from 10 or fewer cell blocks. Therefore, we believe that by adopting our proposed method, testing for CMP can be undertaken using any commercially available NGS product that may require a larger volume of DNA or RNA. Four, 12% of the study cohort had clinically relevant or actionable mutations that impacted management. While DNA mutations were identified in all specimens, one with BRCA1 positivity warranted oxaliplatin-based chemotherapy, two with rare G12C mutation could be targeted using sotorasib or adagrasib and presence of LDAH-ETV1 somatic oncogene RNA fusion in one patient indicated desmoplastic stromal expansion and metastatic progression of pancreatic cancer denoting futility of aggressive chemotherapy.

There are a few limitations to this study. One, we evaluated only the Franseen FNB needle and therefore the outcomes may not be applicable to other FNB needle types. Two, the sample size was estimated to examine the difference between two and three FNB passes and not just a single pass because we believe that a single pass may not procure adequate tissue for NGS testing when using some commercially available tests such as FoundationOne CDx that evaluates for nearly 300 mutations.

In conclusion, we propose an evidence-based EUS technique that can standardise reliable procurement of tissue for comprehensive molecular profiling in pancreatic cancer. This development is likely to further advance the role of EUS in the oncological management of patients.

TRIAL REGISTRATION NUMBER

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Contributors JYB: Study design, endoscopist performing procedures in the study, statistical analysis, interpretation of data, drafting of manuscript, critical revision of manuscript. SV: Study concept and design, endoscopist performing procedures in the study, interpretation of data, drafting of manuscript, critical revision of manuscript. RH: Endoscopist performing procedures in the study, critical revision of manuscript. UN: Endoscopist performing procedures in the study, critical revision of manuscript. CMW: Critical revision of manuscript. NJ: Cytopathologist performing molecular profiling, drafting of the manuscript, critical revision of manuscript. AS: Cytopathologist performing molecular profiling, critical revision of manuscript. KK: Cytopathology technician preparing slides and cell blocks in the study, critical revision of manuscript.

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Competing interests JYB: Consultant for Olympus America and Boston Scientific Corporation. SV: Consultant for Boston Scientific, Olympus America and Medtronic. RH: Consultant for Boston Scientific, Olympus America and Medtronic. UN: Consultant for Janssen, Pfizer, Takeda, AbbVie, Bristol Myers Squibb and GIE Medical. NJ, AS, KK and CMW have no disclosures to declare.

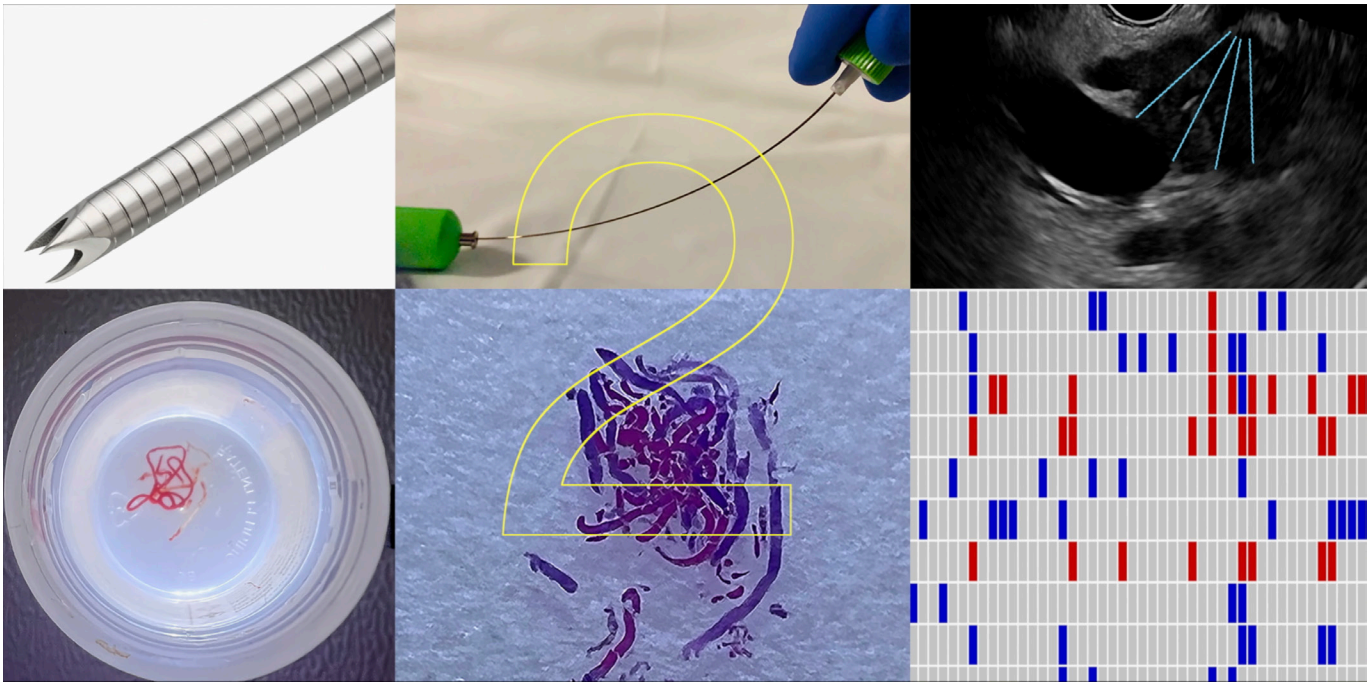

Figure 1 Graphical abstract demonstrating, Franseen-tip needle, stylet-retraction technique and fanning manoeuvre (upper panel, left to right); pancreatic cancer tissue collected at endoscopic ultrasound in 10% formalin, formalin-fixed paraffin embedded cell block and molecular profiling (lower panel, left to right), can be achieved with two dedicated fine needle biopsy passes.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval The study was approved by the Orlando Health Institutional Review - Approval Notice 1741103. Participants gave informed consent to participate in the study before taking part.

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ORCID iDs
Ji Young Bang http://orcid.org/0000-0001-9351-3267
Shyam Varadarajulu http://orcid.org/0000-0002-0047-0016

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SUPPLEMENTARY FILE to manuscript:

Standardization of EUS-guided FNB technique for molecular profiling in pancreatic cancer: Results of a randomized trial

Ji Young Bang MD MPH, Nirag Jhala, MD, Anjali Seth, MD, Konrad Krall CT (ASCP),
Udayakumar Navaneethan MD, Robert Hawes MD, C. Mel Wilcox MD,
Shyam Varadarajulu MD

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IRB/Registration

The study was approved by the institutional review board of the hospital (IRB No. 1741103) and registered at ClinicalTrials.gov (NCT05043532).

Specimen processing

After specimen procurement and formation of formalin-fixed paraffin-embedded cell blocks, the purified DNA and RNA were eluted separately to quantify nucleic acid. NGS was performed (Archer Panels) for analyzing 69 gene DNA mutations (single nucleotide variants, insertions, and deletions) and 53 RNA somatic oncogenic gene fusions.

Randomization and Masking

Computer-generated randomization assignments were provided by the statistician using a block randomization method (block randomization of 4) and placed in sequentially numbered, sealed, opaque envelopes. Patients with pancreatic mass proven to have adenocarcinoma by rapid onsite evaluation at EUS were randomized equally (1:1 allocation) to two or three dedicated passes for comprehensive molecular profiling.

Sample size calculation and Statistical analysis

A two-sided sample size calculation was performed based on the proportion of specimens with adequate tissue to enable molecular profiling. Assuming presence of adequate tissue to allow molecular profiling in 50% of patients in the two-pass group and in 90% in the three-pass group[1-3], the sample size was estimated at 16 per group (total sample size of 33 to account for a 5% drop out rate), at 80% power and two-sided alpha of 0.05.

Continuous data were summarized as means with standard deviation or medians with interquartile range and range, and were compared using the Student's t-test or Wilcoxon rank-sum test as indicated.

Categorical data were summarized as frequencies with percentages and were compared using the chi-square or the Fisher's exact test as indicated.

Additional Comments

Germline testing is important in pancreatic cancer because it is associated with hereditary syndromes involving multiple generations. For such families, screening is recommended. However, screening does not detect all persons at risk. The Memorial Sloan Kettering IMPACT study that conducted germline testing of more than 1,000 patients with cancer revealed a high incidence (17%) of mutations in the pancreatic cancer subset, of whom 42% had no family history of cancer and would not have met current screening recommendations.[4] Although the main driver mutation is KRAS, there are numerous other potentially actionable mutations that can be identified using molecular profiling.[5] Also, from a diagnostic standpoint, there is growing evidence that when cytology and histology are inconclusive, molecular testing can aid diagnosis.[3]

In a recently published study in which 25G needles were used and 2 passes were performed for tissue procurement, only 84% of FFPE specimen yielded adequate DNA.[6] In two previous randomized trials, we have shown that fanning the needle and performing stylet-retraction when using the Franseen needle yielded best cellularity.

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Supplemental Table 1. Details of the mutations identified in 33 pancreatic ductal adenocarcinoma patients from comprehensive molecular profiling

Randomization-FNB passes	RNA Concentration	DNA Concentration	Variants Detected 1	Mutations 1	Variants Detected 2	Mutations 2	Variants Detected 3	Mutations 3	Notes
three	25.7	7.56	KRAS	p.Gly12Val	RB1	p.Arg320Ter	TP53	p.Thr155Pro	TERT G373A
two	3.57	0.874	KRAS	p.Gly12Asp					
two	3.98	2.79	KRAS	p.Gly12Asp	KRAS	p.Gln61His			
three	10.2	2.47	KRAS	p.Gln61Leu					
three	35.2	8.07	KRAS	p.Gly12Val					
two	10.4	2.76	KRAS	p.Gly12Asp					TERT G373A
three	13.7	16	KRAS	p.Gly12Asp	PIK3CA	p.Glu545Ala	TP53	p.Arg175His	
two	TOO LOW	8.35	none detected	TERT G49C					
two	72	20	KRAS	p.Gly12Asp	TP53	p.Arg175His			TERT G349C
three	24	6.99	KRAS	p.Gly12Asp					
three	9.9	8.94	KRAS	p.Gly12Asp	TP53	p.Tyr163Cys			
two	47.3	13	KRAS	p.Gly12Asp	TP53	p.Arg196Ter			
three	38.3	13.8	KRAS	p.Gly12Arg					
two	40.1	7.21	KRAS	p.Gly12Asp	GNAS	p.Arg201His			
two	32.8	11.9	KRAS	p.Gly12Val					
three	46.8	6.23	KRAS	p.Gly12Val	TP53	p.Tyr205His			
two	5.8	2.6	KRAS	p.Gly12Val	TP53	p.Cys242Phe			
two	12.3	2.4	KRAS	p.Gly12Cys					
three	49	14	KRAS	p.Gly12Asp	TP53	p.Arg175His			
three	39.8	2.56	KRAS	p.Gly12Asp	TP53	p.Arg273Cys			
three	40.1	9.69	KRAS	p.Gly12Val	TP53	p.Arg175His			
two	53	20.3	KRAS	p.Gly12Val					
three	36.5	11.9	KRAS	p.Gly12Val	TP53	p.Arg282Trp			
two	32.5	8.29	KRAS	p.Gly12Val	TP53	p.Cys176Ser			
two	52	20.9	TERT	T349C					
three	TOO LOW	0.68	KRAS	p.Gly12Val	SMAD4	p.Arg445Ter			
two	57	16.6	KRAS	p.Gly12Asp	TERT	T349C			
two	27.2	9.64	KRAS	p.Gln61His	TP53	p.Tyr163Asn			
three	15.2	4.2	KRAS	p.Gly12Val					
three	17.9	8.07	KRAS	p.Gly12Arg	TP53	p.Cys176Arg			
three	30.5	5.69	none detected	NONE DETECTED					RNA FUSION POSITIVE
two	47.2	14.3	TP53	p.Pro278Arg	TERT	T349C	BRCA1	p.Gln356Ter	
two	97	20.5	KRAS	p.Gly12Val					

Supplemental Figure 1. CONSORT flow diagram of patient enrollment