Thieme

Comparison of endoscopic ultrasound-guided fine-needle aspiration and fine-needle biopsy to generate pancreatic cancer organoids: Randomized trial

Authors

Johannes Roman Wiessner[1], Felix Orben[1], Arlett Schäfer[1], Lisa Fricke[1], Günter Schneider[2,3,4], Maximilian Reichert[1,5,6,7], Alexander Herner[1,8], Ulrich Mayr[1], Veit Phillip[1], Matthias Treiber[1], Guido von Figura[1], Mohamed Abdelhafez[1], Roland M. Schmid[1], Christoph Schlag[8,1]

Institutions

1 Medical Clinic and Polyclinic II, Klinikum rechts der Isar, Technical University of Munich, München, Germany
2 Department of General, Visceral and Pediatric Surgery, University Medical Center Göttingen, Gottingen, Germany
3 Klinikum rechts der Isar, School of Medicine, Technical University of Munich, Chair of Translational Cancer Research and Institute of Experimental Cancer Therapy, München , Germany
4 CCC-N (Comprehensive Cancer Center Lower Saxony), Medizinische Hochschule Hannover, Hannover, Germany
5 Translational Pancreatic Cancer Center, Medical Clinic and Polyclinic II, Technical University of Munich, München, Germany
6 German Cancer Research Center (DKFZ) and German Cancer Consortium (DKTK), Partner Site Munich, DKFZ, Heidelberg, Germany
7 Center for Protein Assemblies (CPA), Technical University of Munich, 85747, Garching, Germany
8 Department of Gastroenterology and Hepatology, University Hospital Zurich, Zürich, Switzerland

Keywords

Endoscopic ultrasonography, Pancreas, Tissue diagnosis, Fine-needle aspiration/biopsy

received 18.7.2023
accepted after revision 19.12.2023

Bibliography

Endosc Int Open 2024; 12: E361–E366
DOI 10.1055/a-2257-3171
ISSN 2364-3722

Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany

Corresponding author

Dr. Johannes Roman Wiessner, Technical University of Munich, Medical Clinic and Polyclinic II, Klinikum rechts der Isar, München, Germany
johannesroman.wiessner@mri.tum.de

ABSTRACT

Background and study aims The prognosis for pancreatic cancer remains poor. Molecular diagnostics and customized therapies are becoming increasingly important in clinical routine. Patient-derived, predictive model systems such as organoids have the potential to substantially increase the depth of information from biopsy material by functional and molecular characterization. We compared the extent to which the use of fine-needle aspiration needles (FNA, 22G) or fine-needle biopsy needles (FNB, 22G) influences the generation of pancreatic cancer patient-derived organoids (PDOs) to establish endoscopic standards of organoid technology.

Patients and methods Endoscopic ultrasound (EUS)-guided punctures by EUS-FNA and EUS-FNB of pancreatic masses highly suspicious for adenocarcinoma (detected by computed tomography and/or magnetic resonance imaging) were prospectively evaluated. Consecutive patients received EUS-FNA and EUS-FNB in a randomized order without the need to exchange the needle shaft (only the inner needle type (FNA/-B) was exchanged) between the passes. With each needle type, the specimens for histological analysis and for PDOs were obtained separately.

Results Fifty patients were enrolled in the study. Histology revealed malignancy in 42 of 50 cases (84%). In total PDOs were generated from 17 patients (34%). Of these, nine were established by FNB only, two by FNA only, and six by both FNA and FNB. Histology revealed malignancy in 13 of 17

PDO cases (76%). In two histologically false-negative cases, PDOs could be established.

Conclusions EUS-FNB was superior to EUS-FNA in terms of successful generation of PDOs, although it failed to show statistical significance.

Introduction

Despite improvements in therapy, the 5-year survival rate for pancreatic ductal adenocarcinoma (PDAC) remains poor at <8% [1, 2]. Because it is mainly diagnosed in advanced stages, only 15% to 30% of patients with PDAC are eligible for resection, the only potentially curative treatment [3, 4]. Moreover, PDAC is resistant to many conventional therapeutic modalities and rapidly metastasizes to other organs [5]. In view of this fact, it is necessary to develop a more profound understanding of the underlying biology of PDACs and also to address potential therapeutic targets. So-called precision medicine aims to deliver treatment options to patients based on genetic profiles, specific biomarkers, and bioinformatics [6]. In the case of PDAC, pancreatic cancer patient-derived organoids (PDOs) might be able to make a significant contribution to precision medicine in the future. Organoids are cells growing in a 3-dimensional (3D) structure, generated from primary tissues and with the capacity to expand into ex vivo organ-like structures [7, 8], thereby allowing for evaluation of potential diagnostic biomarkers, drug testing, and identification of therapeutic vulnerabilities [9, 10]. Organoids can primarily be obtained from surgical specimens and then have the potential to be used for personalized treatment.

A major problem regarding a clinical benefit is that, as mentioned previously, the majority of patients with PDAC are not eligible for surgical resection because they are usually in a palliative or neoadjuvant setting at the time of diagnosis. Organoids at the time of initial tumor diagnosis, therefore, are crucial.

Our group as well as others were able to show that successful organoid creation is also possible with endoscopic ultrasound (EUS) material [8, 10, 11]. Recent studies have shown superior results with EUS-fine-needle biopsy (EUS-FNB) in comparison with EUS-fine-needle aspiration (EUS-FNA) regarding histological tissue acquisition and diagnostic accuracy with fewer needle passes [12, 13, 14, 15]. However, with regard to the generation of PDOs, there are very little data about which needle type has potential to produce the best results. The aim of this study was to compare the extent to which the use of FNA needles (22G) or FNB needles (22G) influences the generation of pancreatic cancer PDOs to establish endoscopic standards for organoid technology.

Patients and methods

Patient selection

Eligible patients for this study were those aged >18 years with written informed consent and pancreatic masses highly suspicious for PDAC (detected by computed tomography and/or magnetic resonance imaging) and an indication for EUS-guided puncture of these lesions such as planned neoadjuvant or palliative chemotherapy. Exclusion criteria were patients without written informed consent, patients with pregnancy, an international normalized ratio >1.5, a platelet count <50 × 10^9/L or who were medically insufficiently stable to undergo sedation for EUS. The study was approved by the Ethics Committee of the University Hospital of the Technical University of Munich, Klinikum rechts der Isar. The study was performed in accordance with the principles of the Declaration of Helsinki.

Study design and outcomes

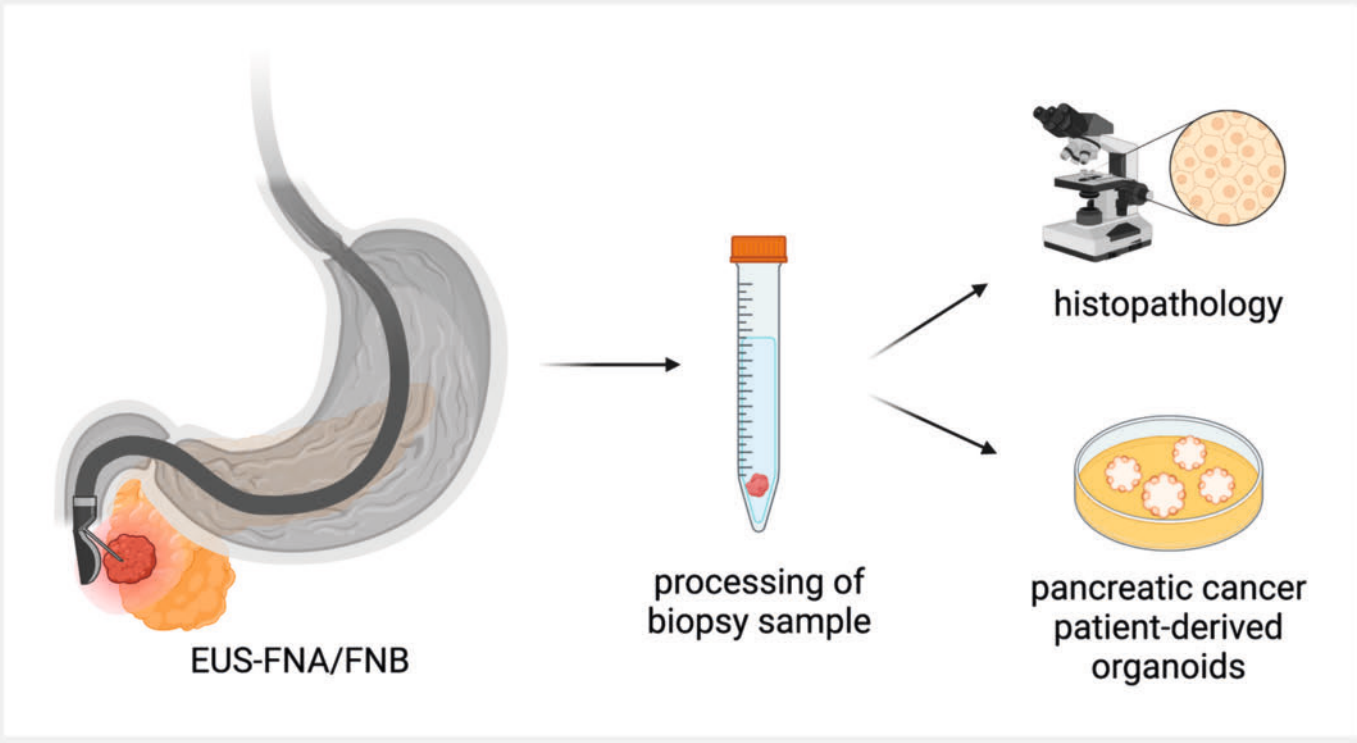
This prospective study was conducted at Klinikum rechts der Isar, a high-volume university endoscopy center with >1000 EUS procedures per year by experienced endosonographers (U. M., M.T., G.F., M.A., C.S.). The primary outcome was successful generation of pancreatic organoids, defined as reaching passage 5 (P5). Secondary outcomes included diagnostic performance (sensitivity, specificity, and accuracy of FNA/FNB).

Procedure details

EUS punctures were carried out under sedation with propofol (Braun, Melsungen, Germany). All procedures were performed by using a linear array echoendoscope (PENTAX, EG-3870UTK, PENTAX Medical, Tokyo, Japan). Each patient received EUS-FNA (Beacon 22G, Medtronic, Minneapolis, Minnesota, United States) and EUS-FNB (SharkCore 22G, Medtronic, Minneapolis, Minnesota, United States) in a randomized order for histology and PDOs without the need to exchange the needle shaft (only the inner needle [FNA/-B] was exchanged) between the passes. All punctures were carried out using the “slow pull” method. Per needle, at least one passage for histology and one passage for PDOs were performed, with a limit of two passages for each needle and purpose (PDOs or histology). Therefore, a maximum of eight passages in total were possible. The adequacy of the specimen was estimated by the endosonographer after each passage and a second passage was only performed in case of non-adequate material. Obtained specimens for PDOs were immediately transferred into a minimal organoid media (10 mL DMEM-F12 cell culture media containing 1% penicillin/streptomycin and 0.2% Primocin) and transported to the organoid facility for further processing and organoid generation. The specimens for histology were placed into formalin and paraffin embedded for standard histological analysis in the Pathology Department.

Organoid generation

The isolation of organoids out of FNA/FNB was performed as previously described [16]. Briefly, the sample was centrifuged (1000 rpm, 4°C, 5 minutes) and the supernatant was discarded. The biopsy was cut into small pieces and transferred into a new 15-mL falcon filled with cold phosphate-buffered saline (PBS)



▶ **Fig. 1** Schematic representation of the workflow and PDO generation. Created with BioRender.com [rerif].

(#14190144 Thermo Fisher Scientific) supplemented with 0.1% bovine serum albumin (#11930 Serva). After a second centrifugation and discarding of the supernatant, the sample was incubated with red blood cell lysis buffer (#A1049201 Thermo Fisher Scientific) for 3 to 15 minutes. PBS was added and the flask was centrifuged. The supernatant was discarded and the sample was digested with TrypLE (#12604039 Thermo Fisher Scientific) for 5 to 10 minutes at 37°C. The flask was filled up with PBS and centrifuged again. After discarding the supernatant, the sample was finally resuspended in 50 µL of Matrigel/well (#354230 Corning Life Sciences). (One FNA or FNB sample was usually used to generate two to four wells.) After 20 minutes, 500 µL of organoid media (DMEM-F12 (#11320033 Thermo Fisher), 5 mg/mL D-glucose (#G8270 Sigma-Aldrich), 0.5% ITS Premix (#354350 Fisher Scientific), 5 nM 3,3,5-Triiodo-L-thyronine (#T0821 Sigma-Aldrich), 1 µM dexamethasone (#D175 Sigma-Aldrich), 100 ng/mL cholera toxin (#C9903 Sigma-Aldrich), 1% penicillin/streptomycin (#15140122 Thermo Fisher Scientific), 5% NU-Serum IV (#355500 Fisher Scientific), 25 µg/mL bovine pituitary extract (#P1167 Sigma-Aldrich), 10 mM nicotinamide (#N3376 Sigma-Aldrich), 100 µg/mL Primocin (#ant-pm05 Invivogen), 0.5 µm A83–01 (#2939 Tocris), 10% RSPO1-conditioned medium (R-spondin-1 overexpressing cell line HEK293T, provided by the Hubrecht Institute (Uppsalalaan 8, 3584 CT Utrecht, Netherlands), 100 ng/mL recombinant human geregulin-1 (#100–03 Peprotech), and 10 µM Rho kinase inhibitor (#TB1254-GMP Tocris) were added per well.

Statistical analysis and sample size calculation

Statistical analyses were performed using Prism for MacOS (Version 9.1.1 GraphPad Software, San Diego, California, United States). For diagnostic performance analysis of needle types, Fisher´s exact test was used. Statistical significance was set at P <0.05. We estimated a difference of at least 40% of growth rates of the organoid depending on the needle type. To achieve a statistical power of 80% at a significance level of P <0.05, at least 46 patients had to be included in the study. Block randomization was used to ensure balanced group sizes. Thus, a total of 25 initial punctures were performed using FNA and 25 using FNB.

Definitions

True positive was defined when malignancy was proven by histology. We considered a lesion to be benign if there were no signs for malignancy on histology and interval stability in radiological imaging.

Successful establishment of an organoid culture was defined by the ability to reach P5 and sustain at least one freeze-thaw cycle. Any molecular characterization was not the subject of the study.

Results

Between July 2019 and October 2020, 50 consecutive patients were included (26 men, 24 women) with a median age of 74.5 years (range 49–88 years). Median body mass index was 23.9 kg/m^2 (range 18.6–33.2 kg/m^2). About one-fifth (22%) of

the patients were diagnosed with type 2 diabetes mellitus. The majority of the lesions were located in the pancreatic body region (60%), with a median size of 32 mm (range 15–110 mm). ▶ **Table 1** shows the characteristics of the tumors. ▶ **Table 2** shows patient demographics. No serious adverse events were observed, and in particular, no post-procedure pancreatitis or bleeding.

EUS-guided puncture of pancreatic lesions was followed by histological analysis and PDO generation. Histologically, 42 lesions (84%) were malignant (▶ **Table 3**). FNA had sensitivity, specificity, and accuracy of 61%, 100% and 61%, respectively, whereas FNB had sensitivity, specificity, and accuracy of 84%, 100% and 84%, respectively ($P = 0.18$). The primary outcome, successful generation of PDOs, was achieved in 17 of 50 patients (34%; 95% confidence interval [CI], 21%–47%). Of these, nine PDOs were generated by FNB only (53%), two by FNA only (12%), and six by both FNA and FNB (35%) (▶ **Fig. 1** and ▶ **Fig. 2**). To summarize, 15 of 50 PDOs were generated by FNB (30%; 95% CI, 17%-43%) and eight of 50 PDOs by FNA (16%; 95% CI 6%-26%), respectively.

Discussion

Because of the complex biology of pancreatic cancer with a high number of affected genes and pathways [17], which vary in each case, there is a need for individualized treatment of each patient with PDAC. Precision medicine describes the concept of using patient-specific information (e. g. genomic or proteomic) to help clinicians make tailored diagnostic or therapeutic decisions. Due to the limited treatment options for pancreatic cancer, this has particular importance. Next-generation sequencing is a primary example of precision medicine in pancreatic cancer. It allows the assessment of millions of segments of the genome and detection of various genetic alterations or point mutations [18].

PDOs are a promising new tool for precision medicine and translational research in pancreatic cancer. They represent a miniature tumor model of a human PDAC and can be rapidly generated beside surgical resections from EUS-guided punctures [11, 19, 20]. EUS-guided sampling has a low risk of complications and can be repeatedly performed at any stage of pancreatic cancer, therefore allowing for evaluation of the treatment response to ongoing therapy.

Recently published data show that regarding pancreatic masses, FNB may be superior to FNA in terms of histological core tissue [21], accuracy [14], and the number of passages required for an adequate specimen [16]. The accuracy of 84% in our study with FNB was similar to the current data for the same FNB needle [14], as well as the FNA needle with inferior accuracy performance of 61%. Experience with other FNB needles, such as the 22G Acquire needle (Boston Scientific Natick, Massachusetts, United States) confirms similar accuracy (87%), and thus, the superiority of the FNB needle [15].

Because the establishing organoids is a challenging and not-yet-standardized technique, there are very little data regarding optimal endoscopic tissue acquisition for it. To the best of our knowledge, this is the first prospective, randomized study com-

▶ **Table 1** Characteristics of newly diagnosed PDAs.

Tumors (n = 50)	
Localization, n (%)	
Head/uncinate	15 (30)
Body	30 (60)
Tail	5 (10)
Size of mass, (IQR), mm	32 (15–110)
Average number of needle passes for positive histological diagnosis (range)	
FNA	1.08 (1–2)
FNB	1.02 (1–2)

PDA, pancreatic ductal adenocarcinoma; IQR, interquartile range; FNA, fine-needle
Aspiration; FNB, fine-needle biopsy.

▶ **Table 2** Demographics of patients newly diagnosed with PDA.

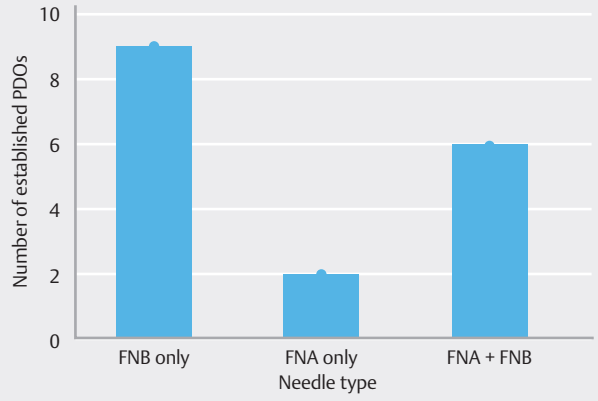
Patient demographics (n = 50)	
Median age, years (range)	74.5 (49–88)
Sex, n (%)	
Female	24 (48)
Male	26 (52)
Median BMI kg/m^2 (range)	23.9 (18.6–33.2)
Diabetes mellitus, type 2, n (%)	11 (22)
Smoker n, (%)	13 (26)

PDA, pancreatic ductal adenocarcinoma; BMI, body mass index.

▶ **Table 3** Diagnostic performance of needle types.

Diagnostic performance	
Sensitivity	
FNA	61% (95% CI 47%–73%)
FNB	84% (95% CI 71%–91%)
Specificity	
FNA	100% (95% CI 5%–100%)
FNB	100% (95% CI 5%–100%)
Accuracy	
FNA	61% (95% CI 51%–71%)
FNB	84% (95% CI 77%–91%)

FNA, fine-needle aspiration; CI, confidence interval; FNB, fine-needle biopsy.



▶ **Fig. 2** Overview of established organoids per needle type.

paring EUS-FNA and EUS-FNB with respect to establish pancreatic cancer organoids. Tiriac et al. [11] were able to show for the first time that establishing organoids through EUS-guided tissue acquisition is generally possible. However, the organoids in their work came exclusively from FNB after malignancy was proven by EUS-FNA using rapid on-site evaluation (ROSE). Only patients from whom adequate cellular material was obtained were included in their study. In the current study, we successfully created pancreatic cancer organoids by means of EUS-FNA and EUS-FNB in patients at the time they were first diagnosed with their tumor. Our results suggest that EUS-FNB is superior to EUS-FNA regarding PDO generation and histologic specimen sampling of pancreatic tumors.

One limitation of our study is the relatively low rate of established organoids (34%). Overall, culture and media conditions for PDOs are continuously improving; however, to generate comparable results within our cohort, the original protocol was followed. The studies by other groups with higher rates of established organoids ranging between 60% and 76% incorporated a step of ROSE to select for higher-quality biopsy material, potentially influencing subsequent organoid culture [11, 22, 23]. For general diagnosis of pancreatic cancer and prerequisite for chemotherapy treatment, histology is still the current diagnostic gold standard. Because FNB is already established as having the best diagnostic performance, future research should be performed to identify the FNB needle that offers the best histological as well as molecular analysis capabilities. However, PDOs as functional biomarkers may complement diagnostics in clinical practice in the future.

Conclusions

In conclusion, our study shows that EUS-FNB is superior to EUS-FNA in terms of successful generation of PDOs as well as in diagnostic performance, although statistical significance was not observed. EUS-FNB has already become the standard for histological assessment and it also may be advantageous for PDO acquisition.

Conflict of Interest

Christoph Schlag received lecture and consulting fees from Olympus, Boston Scientific and Medtronic. The remaining authors have no conflict of interest to declare.

Funding Information

Deutsche Forschungsgemeinschaft
http://dx.doi.org/10.13039/501100001659
SFB1321 (Project-ID 329628492), RE 3723/4-1

Deutsche Krebshilfe
http://dx.doi.org/10.13039/501100005972
Max-Eder Program 111273 and 70114328

Clinical trial

ClinicalTrials.gov (http://www.clinicaltrials.gov/)
NCT03990675
Type of Study: Prospective randomized

References

[1] Roser M, Ritchie H. Cancer. Our World Data.https://ourworldindata.org/cancer

[2] Hidalgo M. Pancreatic cancer. N Engl J Med 2010; 362: 1605–1617 doi:10.1056/NEJMoa1304369

[3] Strobel O, Neoptolemos J, Jäger D et al. Optimizing the outcomes of pancreatic cancer surgery. Nat Rev Clin Oncol 2019; 16: 11–26 doi:10.1038/s41571-018-0112-1

[4] Khorana AA, Mangu PB, Berlin J et al. Potentially curable pancreatic cancer: American Society of Clinical Oncology clinical practice guideline. J Clin Oncol 2016; 34: 2541–2556 doi:10.1200/JCO.2016.67.5553

[5] Ma J, Jemal A. The rise and fall of cancer mortality in the USA: why does pancreatic cancer not follow the trend? Future Oncol 2013; 9: 917–919

[6] Schmidt KT, Chau CH, Price DK et al. Precision oncology medicine: The clinical relevance of patient-specific biomarkers used to optimize cancer treatment. J Clin Pharmacol 2016; 56: 1484–1499 doi:10.1002/jcph.765

[7] Moreira L, Bakir B, Chatterji P et al. Pancreas 3 D organoids: Current and future aspects as a research platform for personalized medicine in pancreatic cancer. Cell Mol Gastroenterol Hepatol 2018; 5: 289–298 doi:10.1016/j.jcmgh.2017.12.004

[8] Dantes Z, Yen HY, Pfarr N et al. Implementing cell-free DNA of pancreatic cancer patient-derived organoids for personalized oncology. JCI Insight 2020; 5: doi:10.1172/jci.insight.137809

[9] Huang L, Holtzinger A, Jagan I et al. Ductal pancreatic cancer modeling and drug screening using human pluripotent stem cell- and patient-derived tumor organoids. Nat Med 2015; 21: 1364–1371 doi:10.1038/nm.3973

[10] Peschke K, Jakubowsky H, Schäfer A et al. Identification of treatment-induced vulnerabilities in pancreatic cancer patients using functional model systems. EMBO Mol Med 2022; 14: e14876 doi:10.15252/emmm.202114876

[11] Tiriac H, Bucobo JC, Tzimas D et al. Successful creation of pancreatic cancer organoids by means of EUS-guided fine-needle biopsy sampling for personalized cancer treatment. Gastrointest Endosc 2018; 87: 1474–1480 doi:10.1016/j.gie.2017.12.032

[12] Kandel P, Tranesh G, Nassar A et al. EUS-guided fine needle biopsy sampling using a novel fork-tip needle: a case-control study. Gastrointest Endosc 2016; 84: 1034–1039 doi:10.1016/j.gie.2016.03.1405

[13] DiMaio CJ, Kolb JM, Benias PC et al. Initial experience with a novel EUS-guided core biopsy needle (SharkCore): results of a large North American multicenter study. Endosc Int Open 2016; 4: E974–979

[14] Oppong KW, Bekkali NLH, Leeds JS et al. Fork-tip needle biopsy versus fine-needle aspiration in endoscopic ultrasound-guided sampling of solid pancreatic masses: a randomized crossover study. Endoscopy 2020; 52: 454–461 doi:10.1055/a-1114-5903

[15] Karsenti D, Palazzo L, Perrot B et al. 22G Acquire vs. 20G Procore needle for endoscopic ultrasound-guided biopsy of pancreatic masses: a randomized study comparing histologic sample quantity and diagnostic accuracy. Endoscopy 2020; 52: 747–753 doi:10.1055/a-1160-5485

[16] Tian L, Tang AL, Zhang L et al. Evaluation of 22G fine-needle aspiration (FNA) versus fine-needle biopsy (FNB) for endoscopic ultrasound-guided sampling of pancreatic lesions: a prospective comparison study. Surg Endosc 2018; 32: 3533–3539 doi:10.1007/s00464-018-6075-6

[17] Jones S, Zhang X, Parsons DW et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 2008; 321: 1801–1806 doi:10.1126/science.1164368

[18] Wei S, Lieberman D, Morrissette JJD et al. Using 'residual' FNA rinse and body fluid specimens for next-generation sequencing: An institutional experience. Cancer Cytopathol 2016; 124: 324–329 doi:10.1002/cncy.21666

[19] Boj SF, Hwang CI, Baker LA et al. Organoid models of human and mouse ductal pancreatic cancer. Cell 2015; 160: 324–338 doi:10.1016/j.cell.2014.12.021

[20] Juiz N, Elkaoutari A, Bigonnet M et al. Basal-like and classical cells coexist in pancreatic cancer revealed by single-cell analysis on biopsy-derived pancreatic cancer organoids from the classical subtype. FASEB J Off Publ Fed Am Soc Exp Biol 2020; 34: 12214–12228

[21] Asokkumar R, Yung Ka C, Loh T et al. Comparison of tissue and molecular yield between fine-needle biopsy (FNB) and fine-needle aspiration (FNA): a randomized study. Endosc Int Open 2019; 7: E955–E963

[22] Ishida Y, Tsunoda T, Hamada Y et al. Standardized methods using EUS-guided fine-needle biopsy and a minimal medium creates three pancreatic cancer organoids. Anticancer Res 2022; 42: 4103–4109 doi:10.21873/anticanres.15908

[23] Lacomb JF, Plenker D, Tiriac H et al. Single-pass vs 2-pass endoscopic ultrasound-guided fine-needle biopsy sample collection for creation of pancreatic adenocarcinoma organoids. Clin Gastroenterol Hepatol 2021; 19: 845–847 doi:10.1016/j.cgh.2020.02.045