"Study Protocol for the Comparison of Endoscopic Ultrasound and Ultrasound-Guided Pancreatic Biopsy in the Diagnosis of Focal Pancreatic Diseases: A Retrospective, Multicenter, Propensity Score Analysis".

August 17 2025

Background

The acquisition of pancreatic tissue is a critical step in the diagnosis and treatment of pancreatic diseases and has been included in several international guidelines for pancreatic disease management. Commonly used techniques include open or laparoscopic biopsy, image-guided percutaneous biopsy, and endoscopic ultrasound-guided biopsy. It is essential to establish a pathological diagnosis of the tumor before initiating non-surgical or neoadjuvant therapy. Currently, endoscopic ultrasound (EUS) and ultrasound (US)-guided pancreatic biopsies are recommended as first-line methods for obtaining samples from focal pancreatic lesions. Both methods demonstrate excellent diagnostic performance and are associated with a low incidence of adverse events. EUS-guided pancreatic biopsy, including fine needle aspiration (FNA) and fine needle biopsy (FNB), has become the preferred technique due to its high patient tolerance and low risk of complications. In contrast, image-guided percutaneous biopsy techniques, such as FNA or core needle biopsy (CNB), can obtain more histological specimens for molecular biological testing and genetic testing. Currently, there are no randomized controlled trials directly comparing the effectiveness and safety of these two methods, and most of the previous related studies are single-center, retrospective studies with low evidence strength. Therefore, it is imperative to conduct large-scale, multicenter clinical studies to provide high-quality evidence for the clinical application of both techniques, thereby offering a research basis for formulating individualized pancreatic biopsy protocols.

Objective

It is proposed to conduct a retrospective, multicenter, propensity score-matched study to compare the differences in diagnostic inaccuracy, complication rates, and the risk of repeat biopsy between EUS and US-guided pancreatic biopsy techniques for focal pancreatic diseases.

Protocol

Study Period: January 1, 2017, to December 31, 2023, retrospective study. Study Location: Seven tertiary Grade A hospitals in China: The First Affiliated Hospital of Zhejiang University School of Medicine, Zhejiang Cancer Hospital, Taizhou Hospital of Zhejiang Province, West China Hospital of Sichuan University, The Third Affiliated Hospital of Sun Yat-sen University, The First Affiliated Hospital of China Medical University, and The First

Affiliated Hospital of Xinxiang Medical University. Inclusion Criteria: All patients who underwent biopsy for focal pancreatic lesions, including US-guided FNA or CNB, EUS-guided FNA or FNB, to obtain cytological or histological specimens of the pancreas for pathological examination. Exclusion criteria: ① Patients with incomplete or missing case data (imaging, laboratory tests, pathological examinations); ② Patients with less than 12 months of clinical follow-up, lost to follow-up, or incomplete follow-up data; ③ Patients who underwent intraoperative biopsy; ④ Patients who underwent fluid aspiration procedures.

All EUS-FNA/FNB procedures were performed using a linear array echoendoscope (GF-UCT240, Olympus Medical Systems, Tokyo, Japan) and an ultrasound biopsy needle (Echotip, Cook Medical, Bloomington, USA). The biopsy needles included 25G, 22G, and 19G models, with the 22G FNA or FNB needle being the most widely used in our study. Under intravenous general anesthesia, the tip of the echoendoscope was inserted through the oropharynx into the gastric fundus, pylorus, and duodenum. After locating the target pancreatic lesion, the tip of the echoendoscope was fixed. For transgastric wall puncture, 25G, 22G, and 19G needles can be selected based on the type of lesion, surrounding tissue structure, and the operator's experience. When performing transduodenal wall puncture, considering the flexibility of the needle, 22G or 25G needles are chosen. Under endoscopic ultrasound guidance, if the FNA technique is used, the needle is inserted into the target lesion, and tissue or cells are obtained using the "slow pull" technique and negative pressure aspiration. The aspirated cell clusters are smeared and fixed with 95% alcohol for smear cytology examination; or placed in a liquid-based cell vial for liquid-based cytology examination. Simultaneously, the aspirated tissue is fixed in 10% formalin solution and sent for histopathological examination. When employing the FNB technique, the biopsy needle is inserted into the target lesion, and the lesion is excised using a specially shaped needle tip to collect tissue into the hollow core of the needle. After withdrawing the biopsy needle, the tissue is fixed in a 10% formalin solution. In this study, all cases utilized the Macroscopic On-Site Quality Evaluation (MOSE) technique, where the operator conducts an initial visual assessment of the extracted specimen. If the amount of tissue or cells obtained is insufficient for pathological evaluation, the biopsy procedure can be repeated until the specimen meets the pathological requirements. Preoperative and intraoperative use of Sonazoid (16ul/2ml, GE, USA) for contrast-enhanced ultrasound aids in assessing the blood supply within and around the target lesion. Sonazoid contrast agent is provided in lyophilized powder form and reconstituted with 2 ml of a specific solution to form a uniform microbubble suspension. A 2 ml bolus of this suspension is administered, followed by a 5 ml saline flush. The arterial phase enhancement process begins immediately after aortic enhancement, lasting 10 to 30

seconds, followed by the venous phase, which lasts 30 to 120 seconds. Routine archiving of imaging data is performed.

The ultrasound systems (Mindray, Shenzhen, China; Aloka, Tokyo, Japan; Esaote, MyLab 9, Florence, Italy) were equipped with ultrasound transducers (3.5 MHz convex array probes) for guiding and monitoring percutaneous pancreatic biopsy. The procedure was performed using CNB or FNA techniques. If the CNB technique was employed for tissue sampling, an automated core biopsy needle (18G and 16G, Magnum Bard, Covington, GA, USA) was used directly or guided by a coaxial needle; in FNA, a negative pressure aspiration biopsy needle (18G and 21G, HAKKO medical, Japan) was utilized. After preparing the skin, disinfecting, and draping at the puncture site, 1% lidocaine is used for layer-by-layer infiltration anesthesia at the intended puncture area. Under ultrasound guidance, an 18G fully automatic core biopsy needle is advanced to the anterior edge of the target pancreatic lesion, with the path options including direct, transgastric, or transhepatic routes. After activating the biopsy device, the specimen is retrieved and placed on filter paper, then promptly fixed in 10% formalin solution for histopathological examination. Typically, a single biopsy specimen measures 19mm in length and 1mm in width. If the sample is insufficient or the tissue appearance is inconsistent with pancreatic tumor, additional puncture biopsies can be immediately performed. If the FNA technique is used for sampling, an 18G or 21G puncture needle is inserted into the target pancreatic lesion. First, create negative pressure by pulling the device, then move the puncture needle up and down multiple times to obtain the specimen. The obtained cell clusters are smeared, then fixed in 95% alcohol for smear cytology examination. Meanwhile, the aspirated tissue strips are fixed in 10% formalin solution for histopathological examination. The operator conducts a preliminary macroscopic assessment of the retrieved specimens. If the specimen quantity is insufficient or its appearance does not match the disease, additional puncture biopsies can be performed until the specimen quantity meets the pathological requirements as assessed macroscopically. After puncture, routine examination of the puncture site and needle tract is necessary to early identify puncture-related complications. If signs of bleeding in the needle tract are observed, gelatin sponge can be inserted through the coaxial needle, or immediate compression hemostasis can be performed. Preoperative and intraoperative use of SonoVue (59 mg/vial, Bracco, Milan, Italy) or Sonazoid (16ul/2ml, GE, USA) for contrast-enhanced ultrasound (CEUS) aids in evaluating the blood supply within and around the target lesion. SonoVue contrast agent is provided in lyophilized powder form and reconstituted with 5 ml saline to form a uniform microbubble suspension. In use, 2.4 ml of this suspension is injected as a bolus, followed by a 5 ml saline flush. Sonazoid contrast agent is provided in the form of

lyophilized powder and is reconstituted with 2 ml of a specific solution to form a homogeneous microbubble suspension. A 0.5 ml bolus of this suspension is administered, followed by a 5 ml saline flush. The arterial phase enhancement process begins immediately after aortic enhancement, lasting for 10 to 30 seconds, followed by the venous phase, which persists for 30 to 120 seconds. Routine archiving of imaging data is performed.

The baseline data of enrolled patients were collected from the electronic medical record system, including the patients' names, genders, hospital admission numbers, examination numbers, puncture dates, and body mass index (BMI). Collect all imaging data (CT, MR, ultrasound, PET, and MRCP, etc.) of the patient on the integrated imaging system, and evaluate and record the imaging characteristics of focal pancreatic lesions. The indicators include the location of the lesion, which is divided into the head, uncinate process, neck, body, and tail; the size of the lesion, measured by the puncture operator and assistant on the maximum cross-section of the lesion displayed on the ultrasound image, with the average value recorded; and the exophytic retrograde morphology of the lesion, assessed by CT or MR. This term refers to the lesion morphology originating from the innermost edge of the pancreas and extending posteriorly beyond the pancreas, or presenting as a perivascular soft tissue sheath surrounding the superior mesenteric artery or celiac trunk, rather than a distinct mass. This is an atypical feature of common pancreatic lesions and is therefore easily overlooked. Information related to pancreatic puncture, including previous puncture history, imaging guidance method, puncture technique, needle gauge, puncture path, auxiliary techniques (contrast-enhanced ultrasound or coaxial technique), operator, post-puncture pathological results, complication status, and repeat puncture status, is collected and recorded by an assistant through the electronic medical record system.

After the puncture, follow-up education is provided to the patient, including a 12-month clinical follow-up. The follow-up encompasses surgical pathology results, repeat puncture pathology results, imaging examination results (CT, MRI, and ultrasound), laboratory test results (complete blood count, liver and kidney function, blood biochemistry, tumor markers, blood and urine amylase), and case records (outpatient and inpatient records).

The primary endpoint of this study was the inaccuracy of EUS- and US-guided pancreatic biopsy in diagnosing focal pancreatic lesions. Cases were classified as true positive results if the histopathological diagnosis post-biopsy was pancreatic ductal adenocarcinoma (PDAC), pancreatic neuroendocrine tumor (PNET), or other definitive malignant or benign pancreatic tumors, and if these findings were consistent with the results of at least

12 months of clinical follow-up (including medical history, laboratory tests, imaging studies, and pathological examinations). When post-puncture histopathological diagnosis indicates inflammation or an inconclusive result, further confirmation of the disease nature is required. If the biopsy results suggest inflammation or are inconclusive, but clinical and imaging evidence strongly indicate malignancy, a repeat biopsy or sampling is recommended. The necessity for repeated sample collection is determined through multidisciplinary discussion. For patients without indications for repeat biopsy, at least 12 months of clinical dynamic monitoring is required, including medical history, imaging (CT or MR), and tumor marker tests. Cases where the lesion size remains stable (diameter increase $\leqslant$20%), exhibit imaging characteristics of benign lesions, and show no change in tumor markers are classified as benign. Cases with a diameter increase >20% and an absolute increase of more than 5 mm, along with an increase in tumor markers, are classified as malignant. Based on clinical follow-up of at least 6 months post-procedure, cases ultimately diagnosed as benign were considered true negative results, while those ultimately diagnosed as malignant were considered false negative results. The probability of diagnostic inaccuracy was the percentage of the sum of false positive and false negative cases out of the total number of cases. The secondary study endpoints were the incidence rates of severe complications, mild complications, and repeat biopsy after EUS and US-guided pancreatic biopsy. Post-procedure complications were assessed and graded according to the clinical practice guidelines of the Society of Interventional Radiology. Adverse events related to puncture occurring within 30 days post-operation fall under the category of complications. These include immediate post-puncture bleeding or hematoma, vagal reflex, infection, puncture-related pancreatitis, organ rupture (duodenum or colon), arteriovenous fistula, and arterial dissection.

Normally distributed measurement data are expressed as mean and standard deviation, while non-normally distributed measurement data are expressed as median and interquartile range (IQR). Categorical data are presented as percentages. For intergroup comparison of normally distributed measurement data, the t-test is used to assess differences; for non-normally distributed data, non-parametric tests are employed. Differences in intergroup categorical data are evaluated using the chi-square test or Fisher's exact test. Missing BMI values, which account for 0-12.7% of all data, are estimated using a random forest-based imputation technique. The propensity score matching (PSM) method was employed to control for confounding factors between the two groups, ensuring a balance of variables across them. PSM utilized a nearest-neighbor 1:1 matching approach with a caliper value set at 0.02. Before and after PSM matching, differences in study endpoints between the EUS-FNA/FNB and US-FNA/CNB groups were compared using chi-square or Fisher's exact tests. In addition to

analyzing the entire population, subgroup analyses were conducted based on lesion size, location, and morphology. A P-value of <0.05 was considered statistically significant. Statistical analysis was conducted using SPSS software (version 26.0; IBM SPSS) and R software (version 4.2.3; R Development Core Team).

All records regarding the identity of enrolled patients are kept confidential and will not be disclosed outside the scope permitted by relevant laws and/or regulations.