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*Clinical Study Protocol*

# Diagnosing Pancreatic Tumors Using Contrast-enhanced Harmonic Endoscopic Ultrasonography with Sonazoid

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**Contrast-enhanced harmonic endoscopic ultrasonography (CH-EUS) with contrast agent enabled us to assess the hemodynamics closely, despite limited data in pancreatic tumors. We have initiated a prospective, single arm, and non-randomized study to clarify the accuracy and safety of CH-EUS with Sonazoid and time-intensity curve (TIC) analysis for diagnosing benign or malignant pancreatic tumors. A total of 200 patients will undergo CH-EUS and TIC analysis. Receiver operating characteristic (ROC) analysis will be used to determine the optimal parameter cutoff values for TIC analysis. This will clarify whether CH-EUS and TIC can further improve the diagnosis of pancreatic tumors over conventional EUS.**

**Key words:** CH-EUS, pancreatic tumor, TIC

It is widely accepted that endoscopic ultrasonography (EUS) is useful for the diagnosis of pancreatic diseases [1, 2] because EUS is superior to any other current modality with respect to spatial resolution [3]. Therefore, EUS is one of the most reliable modalities for the detection of detailed features in pancreatic tumors. However, even with EUS, the differential diagnosis of pancreatic tumors remains a problem. EUS also has limitations in evaluating dynamic perfusion imaging of pancreatic tumors. Doppler mode EUS with contrast agents cannot adequately detect signals from microbubbles in very slow flowing microscopic vessels and in parenchymal perfusion [4], and the results are often accompanied by artifacts such as blooming.

Recently, due to advances in ultrasonographic technology and the clinical application of second-generation intravenous contrast agents such as Sonazoid (Daiichi-Sankyo, Tokyo, Japan), contrast-enhanced harmonic EUS (CH-EUS) has enabled us to be able to assess the microvasculature without Doppler-related artifacts and to evaluate the hemodynamics of the target lesion in real-time. Close observation of the hemodynamics may be useful in the diagnosis of pancreatic tumors. Moreover, echo intensity changes can be measured and a time-intensity curve (TIC) can be obtained, we can quantitatively analyze the blood flow in the tumor microvasculature.

Unfortunately, there are limited data on the dif-

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ferential diagnosis of solid and cystic pancreatic tumors using CH-EUS and there are few reports on the differential diagnosis of a pancreatic tumor using CH-EUS with TIC analysis [5]. The aim of this study is to analyze the accuracy of CH-EUS with intravenous Sonazoid and TIC analysis for diagnosing benign or malignant pancreatic tumors by exploring the optimal cutoff values for several relevant parameters, such as the vascularity pattern of pancreatic tumors in comparison with the surrounding pancreatic parenchyma. Our finding could provide information as to whether CH-EUS and TIC analysis can aid the differential diagnosis of pancreatic tumors detected by conventional EUS.

## Endpoints

***Study Design and endpoints.*** A single-arm, prospective, non-randomized, open label trial will be conducted to evaluate the usefulness and safety of CH-EUS and TIC analysis in the differential diagnosis of pancreatic tumors. The primary outcome parameter is the diagnostic ability of CH-EUS and whether CH-EUS can aid in the differential diagnosis of pancreatic tumors detected by conventional EUS. Secondary outcome parameters include the diagnostic ability of CH-EUS compared to conventional B-mode EUS and multidetector-row computed tomography (MDCT), and the dignostic ability of CH-EUS with time-intensity curve analysis in differentiating pancreatic tumors, and the safety of CH-EUS with Sonazoid.

## Eligibility Criteria

All patients who meet the main inclusion and exclusion criteria will be invited. The inclusion and exclusion criteria are listed in Table 1.

The study is approved by our institutional review board (No. 1845), and we will obtain informed consent from all patients.

Table 1 Patient eligibility

| |
|---|
| Inclusion criteria |
| Patients with pancreatic tumors detected by fundamental EUS |
| Aged 20 years or older |
| Witten informed consent |
| Exclusion criteria |
| Allergy to eggs and Sonazoid |
| Karnofsky performance status (KPS) less than 50% |
| Risk of bleeding as defined below: platelets less than 50,000/$\mu$L, Prothrombin time less than 50% |
| Severe complication in other organs, such as heart failure, hepatic failure and so on |
| Without written informed consent |
| Physician judged improper to entry this trial |

## Treatment Methods

***EUS and CH-EUS imaging.*** Before undergoing CH-EUS, all patients will undergo fundamental B-mode EUS for pancreatic tumor evaluation. EUS examinations and ultrasonography imaging analyses will be performed with a GF-UE260-AL5 device (Olympus, Tokyo, Japan) and an ALOKA ProSound SSD $\alpha$-10 device (Aloka, Tokyo, Japan), respectively. The bolus of Sonazoid contrast agent (Daiichi Sankyo, Tokyo, Japan) will then be administered intravenously in order to evaluate the blood flow of the tumor microvasculature. The tumor will then be observed continuously for 120 sec in order to compare its enhancement with that of the surrounding pancreatic parenchyma.

Safety is evaluated by the onsite investigators. Blood pressure, heart rate and saturation pulse oximetry are measured before, during, and immediately after the examination. If they are abnormal, clinical chemical parameters are assessed within 24 h. Thus, patients are observed for adverse events.

***Time-intensity curve analysis.*** The digital CH-EUS data that is generated will be stored on the hard drive of the ultrasonography imaging system. Two circular regions of interest (ROI) will be placed on the pancreatic tumor and the normal pancreatic parenchyma. The echo intensity in the ROI will be quantified, and the time-intensity curve will be calculated using the software program built into the ultrasonography imaging system.

We will quantitatively analyze the blood flow in the tumor microvasculature from several aspects using time–intensity curve analysis. The following parameters will be measured (Fig. 1): (i) echo intensity change from baseline to peak, (ii) time to contrast enhancement peak, (iii) velocity of contrast imaging from baseline to peak, (iv) echo intensity reduction rate from peak to 120 sec after injection, and (v) nodule/pancreatic parenchyma contrast ratio. These parameters will be compared between pancreatic tumor types

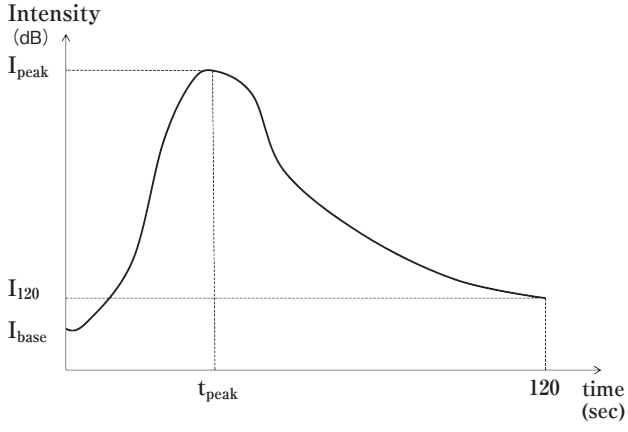


Fig. 1 A representative schematic of a time-intensity characteristic peak showing how the parameters will be measured. $I_{peak}-I_{base}$: echo intensity change from baseline to peak; $t_{peak}$: time to contrast enhancement peak; $(I_{peak}-I_{base})/t_{peak}$: velocity of contrast imaging from baseline to peak; $(I_{peak}-I_{120})/I_{peak}$: echo intensity reduction rate; ($I_{peak}-I_{base}$, of nodule)/($I_{peak}-I_{base}$, of parenchyma) : nodule/pancreatic parenchyma contrast ratio.

to identify any significant differences.

***Outcome measures.*** The primary outcome is the determination of diagnostic accuracy of TIC analysis for malignant or benign tumor. The Youden-index, a summary measure from receiver operating characteristic (ROC) analysis, will be utilized to determine the optimal cutoff values of the echo intensity change, echo intensity reduction rate, and nodule/pancreatic parenchyma contrast ratio in order to obtain the best combination of sensitivity/specificity values and to classify patients into those with benign and those with malignant tumors. Final diagnoses will be based on the histology of the resected specimens or the histology and cytology of samples obtained by EUS-fine needle aspiration (EUS-FNA).

## Statistical Consideration

Statistical analysis will be performed using the JMP software program version 8.0 (SAS Institute, Cary, NC, USA). Continuous values will be presented as the median and interquartile range and the two populations will be compared using the Mann-Whitney $U$ test. $P$ values $< 0.05$ will be considered to be statistically significant.

The diagnostic accuracy of conventional EUS was 76% from the retrospective study in our institution. We estimated that 20% elevation of diagnostic accuracy is expected by using CH-EUS with Sonazoid. The historical control data are based on the histology of the resected specimens or the histology and cytology of samples obtained by EUS-FNA. We consider the lower limit of interest to be 10%. We don't have relevant data regarding the additional expected effect of CH-EUS, so instead simply assumed that a 20% or more increase in diagnostic ability of pancreatic tumors would be clinically meaningful. The sample size of this study was estimated to be 120 patients, with a 1-sided $\alpha = 0.05$ and 1-$\beta = 0.8$. However, it is sometimes difficult hold target images continuously with EUS during this procedure due to respiratory movement and patient's body motion, which makes it difficult to measure TIC. We experience such case in one-in-three patient. Therefore, we aim for accumulating 200 patients.

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