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Evaluation of malignant pancreatic tumor using artificial acoustic radiation force ultrasonography in patients undergoing pancreatectomy

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To evaluate pancreatic elasticity and tumor diagnosis, we preoperatively investigated the elasticity calculated by a new ultrasonography (US) technique, acoustic radiation force impulse (ARFI). Materials and Methods: We examined ARFI in non-tumorous and tumor regions by push pulse of probe in 30 patients with pancreatic malignancies undergoing pancreatectomy. Measurement of stiffness was indicated as the Vs (mm/sec). Results: The Vs in the non-tumor region was measured in the pancreas head in 17 and in the body in 13 patients. The Vs of pancreatic tumors was measured in 14 of 22 patients (64%). The Vs in pancreatic tumors (2.17±0.95 m/sec.) was significantly higher than that in the non-tumorous pancreas (1.41±0.47 m/sec.) ($p<0.01$). The Vs of the non-pancreatic regions in the pancreas head and body were not significantly different. The Vs of the non-tumorous pancreas was not associated with patient demographics, laboratory data, the hardness of the pancreatic tissue, or postoperative morbidity. The Vs of the resected pancreatic tumors was not associated with any tumor-related parameters. Conclusions: ARFI imaging elastography can be used to evaluate pancreatic malignant tumor lesion in comparison with the non-tumor lesion.

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Introduction

Pancreatic resection is the only curable treatment for pancreatic tumors (1, 2). In the surgical procedure, post-treatment morbidity can be a problem and mortality is typically a result of pancreatic fistula and related complications such as intra-abdominal infections or bleeding (1, 3). It is important to predict such morbidity. Pancreatic elasticity or fibrosis is closely related to this complication (4-6). It is difficult to accurately evaluate this elasticity by conventional blood test or imaging diagnostic tools such as computed tomography or ultrasonography. A few investigators have examined pancreatic stiffness using magnetic resonance imaging but this technique has not been clinically applied because of the high cost and special apparatus requirements (7). Pancreatic stiffness is usually evaluated using palpation during operation or it can be histologically diagnosed in specimens. Using these classical diagnostic procedures, an objective evaluation of hardness or elasticity of the pancreas is difficult and can be influenced by the surgeon's experience.

Preoperative ultrasonography (US) is an essential tool to identify tumor location in the pancreas (8). Because of recent advances in US function in the field of hepatic diagnosis, hepatic elasticity has been extensively evaluated using FibroScan® (Echosens Co., Paris, France), which was developed recently (9, 10). Hepatic elasticity detected in the procedure is well correlated with liver fibrosis, which is non-invasive and does not require liver biopsy. While, the acous-

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tic radiation force impulse (ARFI) imaging US (Siemens AG, Munich, Germany) is a new ultrasound-based modality that has been integrated into conventional US systems and is able to non-invasively evaluate the stiffness of deep tissues (11). Virtual Touch Tissue Quantification applications are not greatly influenced by the examiner's ability. The converged ultrasonic pulse sound without a press US probe forces pressure to the liver parenchyma and the shear wave velocity in the region of interest (ROI) (12). If stiffness of pancreatic parenchyma is correlated with pathology, it is useful for diagnosing pancreatic malignancies because pancreatic solid tumors are harder than surrounding tissue. To our knowledge, investigations regarding ARFI US has been gradually reported in the field of diagnosis for pancreatic diseases (13-15).

We hypothesized that ARFI US would be applied for evaluating pancreatic stiffness or diagnosing pancreas tumor, and applied for predicting post-operative morbidity. Our aim in this preliminary study was to clarify this aspect of the new functional US, and we examined the findings of ARFI US in 30 patients with peri-pancreatic diseases who underwent pancreatectomy. The feasibility or limitations of this method were considered as a supportive diagnostic modality in pancreatic surgery.

Patients and Methods

A total of 30 patients with peri-pancreatic tumorous diseases admitted to the Division of Surgical Oncology at Nagasaki University Graduate School of Biomedical Sciences (NUGSBS) between September 2009 and August 2013 were consecutively examined. These pancreatic tumors included invasive ductal carcinoma of the pancreas (PC) in 16 patients, non-invasive intraductal papillary mucin-producing carcinoma (IPMC) in four, pancreatic neuroendocrine tumor in one, pancreatic metastasis originated from the renal cell carcinoma in one, lower bile duct carcinoma in five, and ampullar carcinoma, duodenal carcinoma and tumor-forming pancreatitis in one patient each. The mean age for the patients at the time of surgery was 54 ± 13 years (range, 40 - 80 years). Fourteen patients were male, and 16 were female. The operative procedures included standard pancreaticoduodenectomy (PD) in eight patients, subtotal stomach-preserving PD in eight, distal pancreatectomy in 12, and probe laparotomy in two.

The study protocols were approved by the Human Ethics Review Board of our institution. Informed consent for the ultrasonic examination and data collection was obtained from each patient. The examination with ARFI US was preoperatively performed by radiologists (Dr. A.S., I.S. and H.H.) using an ACUSON S2000™ Ultrasound System (Siemens AG, Munich, Germany) and a convex probe (4C1, 1-4.5 MHz, Siemens). The ultrasound probe automatically produces an acoustic "push" pulse that generates shear waves, which propels into the pancreas. The speed, measured in meters/second (m/s), is displayed on the screen (16). The eSie Touch™ elasticity imaging uses gentle compression to provide a high resolution elastogram, and the Virtual Touch™ tissue imaging is an application for ARFI technology for evaluation of deep tissues. Virtual Touch quantification uses an acoustic push pulse followed by detection pulses to calculate shear wave speed. The acoustic push pulse applied adjacent to the ROI and, however, size or range of ROI was not clearly established in this study. The tracking beams (sensitive to greater than 1/00 the wavelength of sound) are applied adjacent to the acoustic push pulse. The time between the generation of the shear wave and the passing of the shear wave peak at an adjacent location is utilized to compute the shear wave velocity. The force impulse (F) is calculated by the formula below:

$$F = 2 * \alpha \text{ (attenuation constant)} * I \text{ (time mean intensity)} / C \text{ (sonic speed)}$$

We used this apparatus to examine the measurement of ARFI as mean stiffness (Vs [millimeter /second]) in three times per lesion of pancreas, and the mean value was determined because of reproducibility could not be always maintained. The value of the Vs was compared with the patient demographics, laboratory data, histological findings, surgical records, and postoperative complications. The hardness of the resected pancreatic specimens was measured using a muscle durometer (Neutone) (TDM-Z1(RB); TRY-ALL Co., Chiba, Japan). The tumor-node-metastases (TNM) classifications for biliary and pancreas carcinoma were according to *Classification of Pancreatic Carcinoma (3rd English Edition)* (17).

All continuous data were expressed as mean ± SD. The data for the different groups were compared using one-way analysis of variance (ANOVA), which was examined by Student's t-test and the Scheffe's multiple comparison test. The correlation of the continuous data was tested by Spearman's rank correlation test, and its correlation coefficient (r) was determined. A two-tailed P value < 0.05 was considered significant. All the statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, version 18.0 (IBM, Chicago, IL, USA).

Results

The Vs in the non-tumor region was measured in the pancreas head in 17 of 30 cases (57%) and in the body in 13 (43%) cases. The Vs of the intra-pancreatic tumor was measured in 14 of 22 pancreatic tumors (64%). The Vs in the pancreatic tumors was 2.17 ± 0.95 m/sec., which was significantly higher than that in the pancreas (1.41 ± 0.47 m/sec.) (p=0.002). The Vs of the non-pancreatic regions in the pancreas head (1.47 ± 0.49 m/sec.) and body (1.30 ± 0.26 m/sec.) were not significantly different (p=0.25).

The relationship between the Vs in each region and the clinicopathological parameters were examined (Table 1). Age or body surface area (m^2), calculated by the patient's

Table 1. Relationship between ARFI-Vs in the pancreas and clinical or surgical parameters.

	Non-cancer region	p value
Age	r=0.133	0.516
Gender		
Male (n=14)	1.39 ± 0.39	0.612
Female (n=16)	1.40 ± 0.44	
Body surface area (/m^2)	r=-0.309	0.244
Background pancreas		
Normal (n=20)	1.39 ± 0.28	
Fatty (n=1)	1.23	ns
Pancreatitis (n=9)	1.44 ± 0.63	
Diabetes		
No (n=15)	1.32 ± 0.28	0.377
Yes (n=15)	1.47 ± 0.50	
Smoking		
No (n=16)	1.33 ± 0.28	0.530
Yes (n=14)	1.48 ± 0.53	
Alcohol		
No (n=15)	1.30 ± 0.20	0.652
Yes (n=15)	1.49 ± 0.53	
History of chronic pancreatitis		
No (n=23)	1.37 ± 0.29	0.980
Yes (n=7)	1.48 ± 0.69	
Laboratory parameter		
Hemoglobin (g/dl)	r=0.160	0.588
Creatinine (mg/dl)	r=-0.128	0.526
Albumin (g/dl)	r=-0.006	0.976
Amylase (IU/mL)	r=0.246	0.217
Total cholesterol (mg/dl)	r=-0.145	0.531
Blood sugar (mg/dl)	r=0.052	0.799
Hemoglobin A1c (%)	r=-0.011	0.959
Pancreatic function diagnostant test (%) (n=5)	r=-0.436	0.249
Blood loss (ml)	r=-0.042	0.834
Palpation of pancreas		
Soft (n=21)	1.37 ± 0.28	0.694
Hard (n=9)	1.46 ± 0.62	
Hardness of pancreatic specimens (Neutone)		
Non-tumorous region (n=13)	r=0.126	0.682
Postoperative morbidity		
Pancreatic fistula		
No (n=20)	1.34 ± 0.35	0.514
Yes (n=9)	1.39 ± 0.26	
Intra-abdominal infection		
Yes (n=21)	1.35 ± 0.34	0.856
No (n=8)	1.37 ± 0.27	
Long-term ascites		
No (n=24)	1.33 ± 0.31	0.731
Yes (n=5)	1.47 ± 0.36	

height (cm) and weight (kg), were not correlated with Vs in non-tumorous pancreatic region and the Vs in the pancreas was not significantly different between males and females. The Vs of the pancreas was not associated with background pancreas, patient co-morbidity, or habits. On the examination of preoperative laboratory data, the Vs in the pancreas was not significantly correlated with any parameters. The Vs in the pancreas was not significantly associated with the hardness of the pancreatic tissue. The Vs in the pancreas was not significantly associated with postoperative morbidity.

Table 2 shows the relationship between the Vs of the resected pancreatic tumors and the tumor-related factors. Tumors included solid pancreatic carcinomas, other pancreatic solid tumors, and IPMN in 14 patients. The Vs of the tumor was not significantly associated with tumor markers, macroscopic findings, tumor size, tumor stage, or histological differentiation of the resected pancreas.

Table 2. Relationship between ARFI-Vs and resected tumor-related parameters.

	Pancreatic tumor
Pancreatic tumor (n=12)	
Invasive pancreatic carcinoma (n=10)	2.14 ± 0.72
Other pancreatic solid tumor (n=2)#	2.59 ± 1.68
IPMC (n=2)*	2.36 ± 1.92
Carcinoembryonic antigen (ng/ml)	r=-0.139
CA19-9 (U/ml)	r=-0.141
DUPAN-2 (U/ml) (n=8)	r=-0.424
Macroscopic findings of pancreas carcinoma (n=14)	
Nodular (n=4)	2.34 ± 1.31
Invasive (n=8)	2.25 ± 0.76
Cystic (n=2)	2.36 ± 1.92
Tumor size (cm)	r=0.121
Japan TNM stage of pancreatic carcinoma (n=14)**	
I, II, III(n=4)	2.33 ± 1.67
IVa (n=3)	2.28 ± 0.36
IVb (n=7)	2.39 ± 1.07
Histological differentiation (n=14)	
Papillary (n=2)	2.26 ± 1.52
Well (n=4)	2.45 ± 1.00
Moderately (n=8)	2.08 ± 0.83

IPMN; intraductal papillary mucin-producing neoplasm, DUPAN-2; pancreatic cancer associated antigen

#; pancreatic neuroendocrine tumor in one and pancreatic metastasis

*; Intraductal papillary mucin-producing carcinoma with mucin production and solid component

**; According to *the Classification of Pancreatic carcinoma* (17)

Discussion

Recent ultrasonic technology has allowed for precise measurement of organ stiffness (8-12). By applying this modality, the diagnostic accuracy of fibrosis can be examined without invasive biopsy. In recent years, elastography has been widely used for diagnosing stiffness or tumors in solid organs (18-20), and ultrasonic elastography to diagnose pancreas stiffness or pancreatic tumor has been increasingly applied (13-15, 21-23). Göya et al. reported the usefulness of ARFI for diagnosis of acute pancreatitis (24).

Three modalities of ultrasonic elastography have been primarily reported, and each mechanism of measuring stiffness is different, with specific advantages (9-11, 19). A selection of ARFI imaging in the pancreas is clinically useful (13-15). Determination of the measuring point was important in this procedure, and we examined the Vs of ARFI in the target ROI in the pancreatic head or body regions in this study. ARFI imaging may allow for examining deep tissues. In the present series, the Vs of the pancreas was similar to that in previous reports (13). Determination could not be fixed in a specific part of the pancreas because of physical conditions, such as the thickness of the pancreas or disturbances by intestinal air. This is a limitation to determine control or reference data of ARFI-Vs in the pancreas. By comparing the Vs between the pancreas head and body, there was no significant difference; however, the gap of the mean value was 0.17m/sec. based on our results. A standard method for measuring the pancreas itself is necessary. Even with this limitation, diagnostic sensitivity of acute or chronic pancreatitis was high in comparison with conventional US or other imaging modalities (14, 15) We examined several factors, including patient demographics, status of background pancreas, preoperative laboratory data, pancreatic function, surgical records, and outcomes after pancreatectomy in the present study. However, there were no significant correlations between the Vs in the pancreas and these factors. Although we hypothesized that the Vs would be correlated with the hardness or stiffness of the pancreas by palpation or measurement using a durometer, the Vs was not correlated at all, in contrast to previous reports (14, 15). Harada et al. or Lee et al. reported that ARFI was useful to estimate pathological fibrosis and related pancreatic fistula (23, 25). However, on the other hand, postoperative mortality was not correlated with the Vs in the present study and our aim to predict morbidity would be difficult at this stage. As the Vs was correlated with histological fibrosis in liver by our previous study (26), it is necessary to examine in a larger number of patients in the next step to clarify correlation with between the Vs and pancreatic fibrosis.

An intra-pancreatic solid mass lesion hypothetically increases stiffness or decreases elasticity. Previous studies on elastography clarified the usefulness of this method for diagnosing pancreatic tumor regions (21, 26-28). Park et al. recently reported the diagnostic usefulness to define malignant solid pancreatic lesion using ARFI as well (29). The Vs of intrahepatic solid malignancies would increase in comparison with non-cancerous pancreas, and the present result showed a significant increase of Vs in the tumors. By comparing pancreatic carcinoma, neuroendocrine tumor, metastasis, and IPMN, the Vs was not significantly different. D'Onofrio et al. also reported the Vs as measured by ARFI was increased in highly mucinous cystic tumors of the pancreas and, however, reliable value of the VS might not be always obtained. (30-32). In the present study, although the high Vs was measured in both two cases of IPMN because of solid component with mucin production, which was more increased in comparison with the non-tumorous lesion. In general, however, it is still difficult to accurately measure the reliable Vs in various cystic tumor of the pancreas as above previous reports (30-32). The Vs of the all tumor lesion was not correlated with tumor-related factors. We also applied ARFI-Vs to diagnose the existence of pancreatic solid tumors; however, this was not useful to determine malignant behaviors of the pancreatic carcinomas.

Endoscopic ultrasonography-elastography has recently been applied for diagnosing pancreatitis or pancreas tumors (19, 27, 33). As this procedure may be more sensitive for determining pancreatic elasticity or tumor locations, we plan to apply this latest technique in future studies. Furthermore, Yin et al. reported the usefulness of magnetic resonance imaging elastography (34). In the future, it will be important to compare other modalities to measure organ elasticity.

In conclusion, we demonstrated the usefulness of a newly developed elastography, ARFI image ultrasonography, in patients with peri-pancreatic diseases. Measuring the Vs of intra-pancreatic malignancies may allow to define malignant tumor lesion in the pancreas. We found that measuring the Vs in non-tumorous pancreas does not reflect the pancreatic function or status, and that the Vs of the pancreas tumor cannot be used for the differential diagnosis of tumors or malignant behaviors of tumors. This modality is only useful to diagnose pancreatic tumors.

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