

Contrast-enhanced harmonic endoscopic ultrasonography with time-intensity curve analysis for intraductal papillary mucinous neoplasms of the pancreas

Naoki Yamamoto¹, Hironari Kato¹, Takeshi Tomoda¹, Kazuyuki Matsumoto¹,
Ichiro Sakakihara¹, Yasuhiro Noma², Shigeru Horiguchi¹, Ryo Harada¹,
Koichiro Tsutsumi¹, Keisuke Hori², Takehiro Tanaka³, Hiroyuki Okada²,
Kazuhide Yamamoto¹

¹ Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan

² Department of Endoscopy, Okayama University Hospital, Okayama, Japan

³ Department of Pathology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan

Short title: Contrast-enhanced harmonic EUS with time-intensity curve analysis for diagnosis of pancreatic IPMNs

Corresponding author

Hironari Kato, MD

Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences

2-5-1 Shikata-cho, kita-ku

Okayama 700-8558

Japan

Fax: +81-86-223-5991

Email: katou-h@cc.okayama-u.ac.jp

Submitted: 7 October 2014 Accepted after revision: 13 July 2015

Copy editor: [Your initials] / Deadline at publishers / Typesetting deadline

In brief

This retrospective study of 30 patients with intraductal papillary mucinous neoplasm of the pancreas used time–intensity curve analysis to quantify contrast enhancement of mural nodules during endoscopic ultrasonography. Three of the five time–intensity parameters analyzed differed significantly between nodules with or without high grade dysplasia or cancer and there was a strong correlation between echo intensity change and the tumor microvessel density found on pathologic examination.

Background and study aims: Preoperative diagnosis of the pathological grade of intraductal papillary mucinous neoplasms (IPMNs) is difficult. This study aimed to evaluate the accuracy of contrast-enhanced harmonic endoscopic ultrasonography (CH-EUS) with time–intensity curve analysis in differentiating between low or intermediate grade dysplasia (LGD/IGD) and high grade dysplasia or invasive carcinoma (HGD/invasive carcinoma) in IPMNs and to assess correlation between the time–intensity curve parameters and tumor microvessel density.

Patients and methods: Data from 30 patients with resected IPMNs (14 LGD/IGD, 16 HGD/invasive carcinoma) who underwent CH-EUS with time–intensity curve analysis were evaluated retrospectively. Time–intensity curve parameters and the microvessel density of the mural nodule were compared between the HGD/invasive carcinoma and LGD/IGD groups; the diagnostic accuracy of the time–intensity curve parameters was evaluated.

Results: The echo intensity change and echo intensity reduction rate of the mural nodule, and the nodule/pancreatic parenchyma contrast ratio were significantly higher in the HGD/invasive carcinoma group than in the LGD/IGD group ($P < 0.05$); the accuracies of these parameters were 80%, 86.7%, and 93.3%, respectively. The microvessel density of the mural nodule was significantly higher in the HGD/invasive carcinoma group ($P = 0.002$). There was a strong positive, linear correlation between the echo intensity change of the mural nodule and the microvessel density ($r = 0.803$, $P < 0.001$).

Conclusions: CH-EUS with time–intensity curve analysis is potentially useful for quantitatively evaluating the blood flow of IPMN microvasculature, and for differentiating between HGD/invasive carcinoma and LGD/IGD.

Introduction

Intraductal papillary mucinous neoplasms (IPMNs) of the pancreas include a wide spectrum of pathological grades ranging from low grade dysplasia (LGD) and intermediate grade dysplasia (IGD) to high grade dysplasia (HGD) and invasive carcinoma. The prognosis of IPMNs with LGD or IGD is favorable, whereas IPMNs with HGD are at significant risk of malignant transformation [1,2]. IPMNs with invasive carcinoma have a poor prognosis [3]. Therefore, IPMNs with HGD or invasive carcinoma are an indication for surgery, whereas IPMNs with LGD or IGD are not. However, there are no accurate methods to preoperatively differentiate between those two groups. As a result, many patients who undergo surgical resection are overtreated, particularly patients with neoplasms of branch duct type [4].

Angiogenesis is essential for sustained tumor growth, invasion, and metastasis [5]. Microvessel density in tumor tissue has been shown to be an important prognostic factor associated with survival in patients with pancreatic ductal adenocarcinoma [6,7]. It has also been reported that neovascularization plays an important role in the tumorigenesis of invasive IPMNs of the pancreas [8].

Contrast-enhanced harmonic endoscopic ultrasonography (CH-EUS) is a new method that enables assessment of tumor microvasculature; it has been shown to be useful for differentiating between solid pancreatic lesions [9–13]. Moreover, echo intensity changes over time can be measured, and a time–intensity curve can be obtained. CH-EUS with analysis of the time–intensity curve has been used to differentiate solid pancreatic lesions; however, similar data are not available for IPMNs [13].

The aim of this study was to evaluate the diagnostic accuracy of CH-EUS with time–intensity curve analysis in differentiating between LGD/IGD and HGD/invasive carcinoma in IPMNs and to assess correlation between the time–intensity curve parameters and tumor microvessel density. This study was reported according to the Standards for Reporting of Diagnostic Accuracy (STARD) guidelines [14].

Patients and methods

Patients and study design

Eligibility criteria included: (i) presence of IPMNs with mural nodules for which CH-EUS and time–intensity curve analysis had been performed, and (ii) a pathologically confirmed IPMN grade based on evaluation of surgically resected specimens. We excluded IPMNs that were suspected to be malignant on computed tomography or magnetic resonance imaging, although we did not exclude IPMNs only on the basis of their size. A database search for the period between December 2009 and November 2013 was performed at the Department of Gastroenterology and Hepatology at the Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, and 30 patients were enrolled in this study (**Fig. 1**). IPMNs were subclassified into main-duct, mixed, and branch-duct type according to the international consensus guidelines established in 2012 [15]. At our institution, main-duct and mixed type IPMNs are considered to be indications for surgery, as well as branch-duct type IPMNs that have mural nodules. For all patients, endoscopic, radiological, and clinical data were retrospectively reviewed from clinical records.

In this study, IPMN patients with high grade dysplasia (i.e., noninvasive carcinoma) and with IPMN-associated invasive carcinoma were classified into the “HGD/invasive carcinoma” group, while patients with low grade dysplasia and with intermediate-grade dysplasia were classified into the “LGD/IGD” group.

The study was approved by the review board of our institution, and we obtained informed consent with regard to CH-EUS with time–intensity curve analysis from all patients.

CH-EUS imaging

Before undergoing CH-EUS, all patients underwent fundamental B-mode EUS to determine the largest diameter of the cyst, the presence or absence of a mural nodule, the size of the nodule, and the largest diameter of the main pancreatic duct. EUS examinations and ultrasonography imaging analyses were performed with a GF-UE260-AL5 device (Olympus, Tokyo, Japan) and an ALOKA ProSound SSD α -10 device (Aloka, Tokyo, Japan), respectively.

For CH-EUS, the extended pure harmonic detection mode was used, which combines the filtered fundamental and second-harmonic component frequencies with a transmitting frequency of 4.7 MHz. The mechanical index was 0.2 and the frame rates were 10–11 Hz. The focus was set on the distal side of the target lesion. If a mural nodule was identified upon tumor imaging using fundamental B-mode EUS, then the contrast agent Sonazoid (Daiichi Sankyo, Tokyo, Japan) was intravenously administered to evaluate the blood flow of tumor microvasculature. Sonazoid is a second-generation ultrasonography contrast agent composed of perfluorobutane microbubbles with a median diameter of 2–3 μ m. One vial of Sonazoid (16 μ L as perfluorobutane) was suspended in 2 mL of distilled water and administered via bolus injection at 0.015 mL/kg. The nodule was then observed for 120 s continuously in order to compare its enhancement with that of the surrounding pancreatic parenchyma.

Time–intensity curve analysis

The digital CH-EUS data generated for 120 s continuously after the injection of contrast agents were stored on the hard drive of the ultrasonography imaging system. Later, these data were retrieved from the hard drive and analyzed.

Two circular regions of interest (ROI) were placed, on the mural nodule and normal pancreatic parenchyma, respectively. In most cases, we put the ROI on the base of the nodule. If there were multiple nodules in IPMNs, we performed time–intensity curve analysis for the largest nodule. In order to avoid an incorrect setting, the location of the ROI was decided by three experienced endoscopists when we performed CH-EUS. These three endoscopists had no knowledge of the final diagnosis.

The position of the ROI was manually corrected for the patient's respiratory movements. The size of the ROI did not change with respect to all evaluated cases. We performed time–intensity curve analysis only once for a single ROI in all cases. The echo intensity in the ROI was quantified, and the time–intensity curve was calculated using the software program built into the ultrasonography imaging system.

We could quantitatively analyze the blood flow in the tumor microvasculature from several aspects using time–intensity curve analysis. The following parameters were measured from the time–intensity curve (**Fig. 2**):

- (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 s after injection; and
- (v) nodule/pancreatic parenchyma contrast ratio.

These parameters were compared between the HGD/invasive carcinoma and LGD/IGD groups to identify any statistically significant differences. Histopathological examination findings from the resected specimen provided the reference standard.

Assessment of microvessel density

Immunohistochemical staining of surgically resected tissue samples was performed with 4- μ m thick sections of formalin-fixed and paraffin-embedded tissue specimens. To quantify angiogenesis, microvessel density was assessed by immunostaining with an anti-CD31 antibody (Dako Japan, Tokyo, Japan), which labels vascular endothelial cells [16]. The sections were counterstained with hematoxylin and then were independently examined by a board-certified pathologist (T.T.) blinded to the study information.

The tissue sections were screened under low magnification ($\times 40$), and the three most vascularized areas with the greatest number of tumor microvessels were selected. To determine microvessel density, the areas containing CD31-positive microvessels were quantified under high magnification ($\times 100$) using Adobe Photoshop (version CS6; Adobe Systems), as the proportion of the CD31-stained area in the tumor sections. The mean value of the microvessel densities of these three selected areas was calculated and used as the microvessel density value for the tumor. The microvessel density values were compared between the malignant and benign groups in order to identify any significant differences.

Statistical analysis

Statistical calculations were performed using the JMP software program version 8.0 (SAS Institute, Cary, North Carolina, USA). Categorical values were compared using

Fisher's exact test. Continuous values are presented as the median and interquartile range and were compared using the Mann–Whitney *U* test.

For diagnosis based on the time–intensity curve, the cutoff values for the echo intensity change, echo intensity reduction rate, and nodule/pancreatic parenchyma contrast ratio were determined by a receiver operating characteristic (ROC) analysis, using the Youden index calculation to show the best combination of sensitivity and specificity values for diagnosing patients as having LGD/IGD or HGD/invasive carcinoma.

The relationship between echo intensity change and microvessel density was assessed using the Spearman rank correlation analysis.

For all analyses, *P* values <0.05 were considered to be statistically significant. This was an exploratory study; hence no correction for multiple hypothesis testing was applied.

Results

The clinical characteristics of the 30 patients are presented in **Table 1**. All patients underwent surgical resection, and the final diagnoses were based on the pathological findings. A total of 16 patients were categorized into the HGD/invasive carcinoma group and 14 patients into the LGD/IGD group.

No significant differences with respect to the clinical and morphologic factors analyzed in this study were found between these two groups.

No significant adverse events were attributed to CH-EUS.

Time–intensity curve parameters of IPMNs

Typical findings and time–intensity curves are shown in **Fig. 3** for the LGD/IGD group and in **Fig. 4** for the HGD/invasive carcinoma group. The echo intensity change, echo

intensity reduction rate, and nodule/pancreatic parenchyma contrast ratio were significantly higher in the HGD/invasive carcinoma group than in the LGD/IGD group (**Table 2**). No significant differences between the two groups were observed in the times to peak contrast enhancement and in the velocities of contrast imaging (**Table 2**). The areas under the ROCs (AUROCs) for the echo intensity change, echo intensity reduction rate, and nodule/pancreatic parenchyma contrast ratio were 0.8, 0.9, and 0.89, respectively (**Fig. e5**, available online only). The cutoff values determined by ROC analysis were: echo intensity change, 14.7; echo intensity reduction rate, 0.48; and nodule/pancreatic parenchyma contrast ratio, 0.98. The diagnostic accuracies of echo intensity change, echo intensity reduction rate, and nodule/pancreatic parenchyma contrast ratio are shown in **Table 3**.

There was no association between the size of the nodule and the parameters of the time–intensity curves.

Microvessel density of IPMNs

There was extensive anti-CD31 antibody staining on the surface of both the endothelial cells in the tumor and the normal pancreatic parenchyma (**Fig. 6**). Intratumoral microvessel density was assessed on the basis of CD31 staining of endothelial cells. The microvessel density values in the mural nodule were significantly higher in the HGD/invasive carcinoma group than in the LGD/IGD group, with median (interquartile range [IQR]) values of 1.82 (1.17–2.17) versus 0.71 (0.65–0.95), respectively, $P = 0.002$ (**Fig. e7**, available online only).

There was a strong, positive linear correlation between the mural nodule echo intensity change and the microvessel density ($r = 0.803$, $P < 0.001$) (**Fig. e8**, available online only).

Discussion

Cytological examination of pancreatic juice [17] or the measurement of biochemical and tumor markers in cyst fluid obtained using an EUS-guided fine needle aspiration technique have been developed as procedures to establish diagnosis of IPMNs [18–22]. However, it is still difficult to diagnose the pathological grade of IPMNs using these procedures.

According to international consensus guidelines [15], the criteria for IPMN resection include obstructive jaundice, an enhanced solid component within the cyst, and a main pancreatic duct size of more than 10 mm. These parameters, however, do not allow accurate diagnosis of the pathological grade of IPMNs. This holds true also for other predictive factors, including age, IPMN type (main-duct or branch -duct), tumor size, main pancreatic duct size, or presence of mural nodules [23–25]. CH-EUS has been established as a new diagnostic imaging method, in which the influx and washout of the contrast agent can be continuously monitored. The echo intensity changes also allow a more detailed assessment of both morphological features and the blood flow in tumor microvasculature. In addition, the echo intensity changes over time can be evaluated objectively and quantitatively using time–intensity curve analysis.

For IPMNs, the morphological diagnosis of mural nodules using CH-EUS has been reported to be a useful method for the identification of malignant lesions [9]. In transabdominal contrast-enhanced ultrasonography using the first-generation intravenous ultrasonography contrast agent Levovist (Bayer Schering Pharma, Osaka, Japan), the quantitative evaluation of the echo intensity change of the IPMN septum was reported to provide a parameter for the differential diagnosis of benign and

malignant tumors [26]. Although there are limited data on the differential diagnosis of solid pancreatic masses using CH-EUS with time–intensity curve analysis [13], to our knowledge, the usefulness of CH-EUS with time–intensity curve analysis for the detection of malignant IPMNs has not been reported previously.

In this study, we used time–intensity curve analysis to quantitatively evaluate the pattern of echo intensity changes in mural nodules of IPMNs, as shown by CH-EUS with the use of Sonazoid, in order to diagnose the pathological grade of IPMNs; this helped us to differentiate between IPMNs with LGD/IGD and with HGD/invasive carcinoma. We found that the mural nodule echo intensity change, echo intensity reduction rate, and nodule/pancreatic parenchyma contrast ratio were significantly greater for IPMNs with HGD/invasive carcinoma compared with IPMNs with LGD/IGD. Among these three parameters, the nodule/pancreatic parenchyma contrast ratio was the most accurate marker.

In general, the incidence of malignancy is higher in main-duct or mixed IPMNs than in branch-duct IPMNs. However, in the current study, the incidence of malignancy in branch-duct IPMNs was comparable to that of main-duct IPMNs and mixed IPMNs. This is because we chose branch-duct IPMNs with mural nodules as the main subject for analysis, which led to the increased incidence of malignancy among the branch-duct IPMNs. A mural nodule in an IPMN is considered to be a “high-risk stigmata” according to the 2012 guidelines [15].

The size of mural nodules of IPMNs has been correlated with malignant potential in several reports [27]; however, in the current study, the median diameter of the nodules in the HGD/invasive carcinoma group was not statistically different from that in the LGD/IGD group. One reason for this finding may be that the sample size was too small.

Microvessel density is a reliable marker for tumor angiogenesis [6], and immunohistochemical staining for microvessel density markers has been shown to be an important prognostic factor [28]. Microvessel density shows a continuous increase from normal pancreatic tissue through chronic pancreatitis to malignant tissue [29,30], reflecting its important role in tumor growth. In the present study, an immunohistochemical analysis of CD31 expression revealed that intratumoral microvessel density was greater in HGD/invasive carcinoma than in LGD/IGD IPMNs. However, this analysis does not allow intratumoral microvessel densities to be assessed preoperatively. Our data showed a strong positive linear correlation between the mural nodule echo intensity change and microvessel density ($\gamma = 0.803, P < 0.001$), indicating that the echo intensity becomes higher as the microvessel density increases. Therefore, CH-EUS with time–intensity curve examination may be a useful noninvasive method for preoperatively evaluating the microvessel density of the tumor.

This study has several limitations. First, this is a retrospective case study with a small cohort, and all CH-EUS procedures were performed at a single institution. Owing to the small sample size, no definitive recommendations can be made; further investigation with more cases from multiple institutions is needed to establish the role of CH-EUS with time–intensity curve analysis in the management of IPMNs. Second, it was sometimes difficult to obtain target images continuously with EUS during this procedure, which in turn made it difficult to measure the time–intensity curve. Third, the echo intensity is dependent on depth, and to take this into account, both the ROI of the tumor and the pancreatic parenchyma must be as near to the same depth as possible. Fourth, as mentioned above, the features of the time–intensity curve differed between the LGD/IGD group and the HGD/invasive carcinoma group. However, nodules may have components of both LGD/IGD and HGD/invasive carcinoma, which occurs with

multiple nodules spreading widely or with large nodules, especially in main-duct IPMNs; in such cases it is difficult to correctly identify the area of HGD/invasive carcinoma when we decide the location of the ROI. In addition, the differentiation is also difficult if HGD or invasive carcinoma is located in a wall or septum of IPMNs, except for nodules with LGD or IGD. In this study, there was only one case in which IGD was located in the wall of the IPMN despite the presence of LGD in the nodule, although the group categorization between LGD/IGD or HGD/invasive carcinoma did not change. However, we might have to omit widely spreading or large nodules, such as those seen in main duct IPMNs, from the objectives of time–intensity curve analysis. Fifth, we always attempted to perform microvessel analysis in the same location where the time–intensity curve analysis was done. However, there is no proof that both parameters were measured in precisely the same region of the tumor. This study was a pilot study, and our results regarding the performance characteristics of time–intensity curve parameters should be validated in the future in an independent dataset.

In conclusion, the blood flow in the microvasculature of mural nodules in IPMN patients, as assessed with CH-EUS with time–intensity curve analysis, was significantly associated with tumor microvessel density and pathological grade. CH-EUS with time–intensity curve analysis is potentially useful for differentiation between IPMNs with LGD/IGD and with HGD/invasive carcinoma.

Acknowledgements

This study received no financial support.

Competing interests: All authors declare that they have no competing interests in association with this study.

References

- 1 D'Angelica M, Brennan MF, Suriawinata AA et al. Intraductal papillary mucinous neoplasms of the pancreas: an analysis of clinicopathologic features and outcome. *Ann Surg* 2004; 239: 400–408
- 2 Nakagohri T, Kinoshita T, Konishi M et al. Surgical outcome of intraductal papillary mucinous neoplasms of the pancreas. *Ann Surg Oncol* 2007; 14: 3174–3180
- 3 Maire F, Hammel P, Terris B et al. Prognosis of malignant intraductal papillary mucinous tumors of the pancreas after surgical resection: comparison with pancreatic ductal adenocarcinoma. *Gut* 2002; 51: 717–722
- 4 Correa-Gallego C, Do R, Lefemina J et al. Predicting dysplasia and invasive carcinoma in intraductal papillary mucinous neoplasms of the pancreas: development of a preoperative nomogram. *Ann Surg Oncol* 2013; 20: 4348–4355
- 5 Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 1996; 86: 353–364
- 6 van der Zee JA, van Eijck CH, Hop WC et al. Angiogenesis: a prognostic determinant in pancreatic cancer? *Eur J Cancer*. 2011; 47: 2576–2584
- 7 Yoshitomi H, Kobayashi S, Ohtsuka M et al. Specific expression of endoglin (CD105) in endothelial cells of intratumoral blood and lymphatic vessels in pancreatic cancer. *Pancreas* 2008; 37: 275–281

- 8 Tachezy M, Reichelt U, Melenberg T et al. Angiogenesis index CD105 (endoglin)/CD31 (PECAM-1) as a predictive factor for invasion and proliferation in intraductal papillary mucinous neoplasm (IPMN) of the pancreas. *Histol Histopathol* 2010; 25: 1239–1246
- 9 Ohno E, Hirooka Y, Itoh A et al. Intraductal papillary mucinous neoplasms of the pancreas: differentiation of malignant and benign tumors by endoscopic ultrasonography findings of mural nodules. *Ann Surg* 2009; 249: 628–634
- 10 Kitano M, Kudo M, Yamao K et al. Characterization of small solid tumors in the pancreas: the value of contrast-enhanced harmonic endoscopic ultrasonography. *Am J Gastroenterol* 2012; 107: 303–310
- 11 Napoleon B, Alvarez-Sanchez M.V, Gincoul R et al. Contrast-enhanced harmonic endoscopic ultrasound in solid lesions of the pancreas: results of a pilot study. *Endoscopy* 2010; 42: 564–470
- 12 Gincul R, Palazzo M, Pujol B et al. Contrast-harmonic endoscopic ultrasound for the diagnosis of pancreatic adenocarcinoma: a prospective multicenter trial. *Endoscopy* 2014; 46: 373–379
- 13 Matsubara H, Itoh A, Kawashima H et al. Dynamic quantitative evaluation of contrast-enhanced endoscopic ultrasonography in the diagnosis of pancreatic disease. *Pancreas* 2011; 40: 1073–1079
- 14 Bossuyt PM, Reitsma JB, Bruns DE et al. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. *Clin Chem* 2003; 49: 1–6

- 15 Tanaka M, Carlos F, Volkan A et al. International consensus guidelines 2012 for the management of IPMN and MCN of the pancreas. *Pancreatology* 2012; 12: 183–197
- 16 Guo J1, Higashi K, Ueda Y et al. Microvessel density: correlation with 18F-FDG uptake and prognostic impact in lung adenocarcinomas. *J Nucl Med* 2006; 47: 419–425
- 17 Yamaguchi T, Shirai Y, Ishihara T et al. Pancreatic juice cytology in the diagnosis of intraductal papillary mucinous neoplasm of the pancreas. *Cancer* 2005; 104: 2830–2836
- 18 Paris SA, Attasaranya S, Leblanc JK et al. Role of endoscopic ultrasound in the diagnosis of intraductal papillary mucinous neoplasms: correlation with surgical histopathology. *Clin Gastroenterol Hepatol* 2007; 5: 489–495
- 19 Frossard JL, Amouyal P, Amouyal G et al. Performance of endosonography-guided fine needle aspiration and biopsy in the diagnosis of pancreatic cystic lesions. *Am J Gastroenterol* 2003; 98: 1516–1524
- 20 Marie F, Voitot H, Aubert A et al. Intraductal papillary mucinous neoplasms of the pancreas: performance of pancreatic fluid analysis for positive diagnosis and the prediction of malignancy. *Am J Gastroenterol* 2008; 103: 2871–2877
- 21 Lim LG, Lakhtakia S, Ang TL et al. Factors determining diagnostic yield of endoscopic ultrasound guided fine-needle aspiration for pancreatic cystic lesions: a multicenter Asian study. *Dig Dis Sci* 2013; 58: 1751–1757

- 22 Thornton GD, McPhail MJ, Nayagam S et al. Endoscopic ultrasound guided fine needle aspiration for the diagnosis of pancreatic cystic neoplasms: a meta-analysis. *Pancreatology* 2013; 13: 48–57
- 23 Ohno E, Itoh A, Kawashima H et al. Malignant transformation of branch-duct type intraductal papillary mucinous neoplasms of the pancreas based on contrast-enhanced endoscopic ultrasonography morphological changes. *Pancreas* 2012; 41: 855–862
- 24 Lee JH, Lee KT, Park J et al. Predictive factors associated with malignancy of intraductal papillary mucinous pancreatic neoplasms. *World J Gastroenterol* 2010; 16: 5353–5358
- 25 Ohtsuka T, Kono H, Nagayoshi Y et al. An increase in the number of predictive factors augments the likelihood of malignancy in branch duct intraductal papillary mucinous neoplasm of the pancreas. *Surgery* 2012; 151: 76–83
- 26 Itoh T, Hirooka Y, Itoh A et al. Usefulness of contrast-enhanced transabdominal ultrasonography in the diagnosis of intraductal papillary mucinous tumors of the pancreas. *Am J Gastroenterol* 2005; 100: 144–152
- 27 Uehara H, Ishikawa O, Katayama K et al. Size of mural nodule as an indicator of surgery for branch duct intraductal papillary mucinous neoplasm of the pancreas during follow-up. *J Gastroenterol.* 2011; 46: 657–663
- 28 Zhuang H, Yang ZG, Chen HJ et al. Time-intensity curve parameters in colorectal tumours measured using double contrast-enhanced ultrasound: correlations with tumour angiogenesis. *Colorectal Dis* 2012; 14: 181–187

- 29 Kuehn R, Lelkes PI, Bloechle C et al. Angiogenesis, angiogenic growth factors, and cell adhesion molecules are upregulated in chronic pancreatic disease: angiogenesis in chronic pancreatitis and in pancreatic cancer. *Pancreas* 1999; 18: 96–103
- 30 Banerjee SK, Zoubine MN, Mullick M et al. Tumor angiogenesis in chronic pancreatitis and pancreatic adenocarcinoma: impact of K-ras mutations. *Pancreas* 2000; 20: 248–255

Fig. 1 Contrast-enhanced harmonic endoscopic ultrasonography (CH-EUS) with analysis of time–intensity curves in patients with intraductal papillary mucinous neoplasms (IPMNs): study flow diagram. CT, computed tomography; MRI, magnetic resonance imaging.

Fig. 2 Schematic of time-intensity characteristic showing the measured parameters.

$I_{\text{peak}} - I_{\text{base}}$, echo intensity change from baseline to peak

t_{peak} , time to contrast enhancement peak

$(I_{\text{peak}} - I_{\text{base}})/t_{\text{peak}}$, velocity of contrast imaging from baseline to peak

$(I_{\text{peak}} - I_{120})/I_{\text{peak}}$, echo intensity reduction rate

$(I_{\text{peak}} - I_{\text{base}} , \text{ for nodule}) / (I_{\text{peak}} - I_{\text{base}} , \text{ for parenchyma})$, nodule/pancreatic parenchyma contrast ratio

Fig. 3 Branch-duct intraductal papillary mucinous neoplasm (IPMN) with intermediate grade dysplasia of the pancreatic head. **a** Contrast-enhanced computed tomography image. **b** Fundamental B-mode endoscopic ultrasonography (EUS) revealed a mural nodule. **c** A pre-enhancement image. **d** EUS image at the peak of contrast enhancement. The yellow and light blue circles show the regions of interest (ROIs) in the mural nodule and pancreatic parenchyma, respectively, both of which were enhanced.

e EUS image obtained 120 s after injection, revealing continued enhancement in both the mural nodule (yellow circle) and the pancreatic parenchyma (blue circle). **f** Histological appearance of the mural nodule. **g** Time–intensity characteristics of the mural nodule (yellow line) and pancreatic parenchyma (light blue line). At 120 s after injection, the enhanced echo intensity was unchanged in the pancreatic parenchyma but in the mural nodule it had decreased slightly from the peak value. (“Level” value $\times 0.173$ = value in decibels [dB].)

Fig. 4 Branch duct intraductal papillary mucinous neoplasm (IPMN) with invasive carcinoma of the pancreatic head. **a** Contrast-enhanced computed tomography image. **b** Fundamental B-mode endoscopic ultrasonography (EUS) revealed a mural nodule. **c** A pre-enhancement image. **d** EUS image at the peak of contrast enhancement. The yellow and light blue circles show the regions of interest (ROIs) in the mural nodule and pancreatic parenchyma, respectively, both of which showed enhancement. **e** EUS image obtained 120 s after injection showed reduced enhancement in the mural nodule (yellow circle), while it persisted in the pancreatic parenchyma (blue circle). **f** Histological appearance of the mural nodule. **g** Time–intensity curves of the mural nodule (yellow line) and pancreatic parenchyma (light blue line). At 120 s after injection, the enhanced echo intensity of the pancreatic parenchyma was slightly reduced, whereas that of the mural nodule was markedly decreased from peak intensity. (“Level” value $\times 0.173$ = value in decibels [dB]).

Fig. e5 Contrast-enhanced harmonic endoscopic ultrasonography (CH-EUS) of intraductal papillary mucinous neoplasms (IPMNs). Area under the receiver operating characteristic (AUROC) for time–intensity curve parameters:
a echo intensity change; **b** echo intensity reduction rate;
c nodule/pancreatic parenchyma contrast ratio.

Fig. 6 Immunohistochemical staining with anti-CD31 antibodies: **a** normal pancreatic parenchyma; **b** mural nodule from low grade dysplasia (LGD) intraductal papillary mucinous neoplasm (IPMNs); **c** mural nodule from the invasive carcinoma group of IPMNs.

Fig. e7 Comparison of microvessel density in intraductal papillary mucinous neoplasms (IPMNs), between those showing low and intermediate grade dysplasia (LGD/IGD) and those showing high grade dysplasia and invasive carcinoma (HGD/invasive carcinoma). Microvessel density was significantly higher in the HGD/invasive carcinoma group than in the LGD/IGD group ($P = 0.002$)

Fig. e8 Correlation between the echo intensity change and microvessel density of mural nodules in intraductal papillary mucinous neoplasms

(IPMNs). There was a strong, positive linear correlation between echo intensity change and microvessel density ($r = 0.803$, $P < 0.001$).

Table 1 Characteristics of patients, with intraductal papillary mucinous neoplasms (IPMNs) with low grade or intermediate grade dysplasia (LGD/IGD) and high grade dysplasia (HGD) or invasive carcinoma, who underwent contrast-enhanced harmonic endoscopic ultrasonography (CH-EUS).

	LGD/IGD (n = 14)	HGD/invasive carcinoma (n = 16)	<i>P</i> value
Sex, n			n.s.
Male	8	10	
Female	6	6	
Age, mean ± SD, years	69.4 ± 6.2	71.4 ± 7.1	n.s.
Symptoms, n			n.s.
Present	2	3	
Absent	12	13	
Diabetes mellitus, n			n.s.
Present	7	4	
Absent	7	12	
IPMN location, n			n.s.
Head	8	13	
Body–tail	6	3	
IPMN type			n.s.
Main duct	2	4	
Mixed	9	9	
Branch duct	3	3	
Main pancreatic duct diameter, median (IQR)*, mm	6.5 (4.2–8.3)	7.5 (5.3–9.8)	n.s.
Cyst size†, median (IQR)*, mm	31 (15.5–43.5)	33 (25.5–47.8)	n.s.
Mural nodule size, median (IQR)*, mm	7 (5.0–9.3)	7.5 (5.3–11.3)	n.s.
Tumor markers‡, median (IQR)			
CEA, ng/ml	2.6 (1.7–5.0)	2.6 (1.8–3.1)	n.s.
CA19-9, U/ml	10.6 (6.1–17.2)	18.4 (9.7–78)	n.s.
DUPAN-2, U/ml	27 (25–45.3)	27.5 (25–94.3)	n.s.
SPAN-1 level, U/ml	10 (10–10.7)	11.7 (10–39.1)	n.s.

n.s., not significant; SD, standard deviation; IQR, interquartile range; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; DUPAN-2, Duke pancreatic monoclonal antigen type 2; SPAN-1, s-pancrease-1.

*Values of median and IQRs were statistically calculated using the JMP software programs.

†Cyst size was measured only for branch-duct and mixed-type IPMNs.

‡These markers were measured in the serum.

Table 2 Time-intensity curve analysis from patients with intraductal papillary mucinous neoplasms (IPMNs) with low grade or intermediate grade dysplasia (LGD/IGD) or high grade dysplasia or invasive carcinoma (HGD/invasive carcinoma), who underwent contrast-enhanced harmonic endoscopic ultrasonography (CH-EUS).

	LGD/IGD (n = 14 patients)	HGD/invasive carcinoma (n = 16 patients)	<i>P</i> value
Time-intensity curve parameters, median (IQR)			
Echo intensity change, dB	11.22 (8.32–14.07)	15.85 (13.86–17.42)	0.006
Time to contrast enhancement peak, s	11.36 (9.39–15.25)	11.53 (9.04–15.15)	0.84
Velocity of contrast imaging, dB/s	1.03 (0.55–1.29)	1.29 (0.87–1.8)	0.11
Echo intensity reduction rate, %	37 (31.3–41.8)	55 (49–65.5)	<0.001
Nodule/pancreatic parenchyma contrast ratio	0.73 (0.62–0.83)	1.16 (1.02–1.49)	<0.001

IQR, interquartile range

Table 3 Diagnostic performance of time-intensity curve parameters for high grade dysplasia (HGD)/invasive carcinoma in mural nodules of intraductal papillary mucinous neoplasms (IPMNs)

	Sensitivity, % 95%CI No. of patients	Specificity, % 95%CI No. of patients	PPV, % 95%CI No. of patients	NPV, % 95%CI No. of patients	Accuracy, % 95%CI No. of patients
Echo intensity change	75 59.0–83.5 12/16	85.7 64.7–95.4 12/14	85.7 64.7–95.4 12/14	75 59.0–83.5 12/16	80 62.9–89.0 24/30
Echo intensity reduction rate	87.5 72.2–95.0 14/16	85.7 68.2–94.3 12/14	87.5 72.2–95.0 14/16	85.7 68.2–94.3 12/14	86.7 70.3–94.7 26/30
Nodule/pancreatic parenchyma contrast ratio	93.8 80.0–98.3 15/16	92.9 77.2–98.0 13/14	93.8 80.0–98.3 15/16	92.9 77.2–98.0 13/14	93.3 78.7–98.2 28/30

CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value