Original Research

Contrast-enhanced harmonic endoscopic ultrasound using time-intensity curve analysis predicts pathological grade of pancreatic neuroendocrine neoplasm

CH-EUS WITH TIC ANALYSIS PREDICTS GRADE OF PNEN

Saimon Takada, MD; Hironari Kato, MD*; Yosuke Saragai, MD; Shin-Ichiro Muro, MD; Daisuke Uchida, MD; Takeshi Tomoda, MD; Kazuyuki Matsumoto, MD; Shigeru Horiguchi, MD; Noriyuki Tanaka, MD; Hiroyuki Okada, MD

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

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

*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

E-mail: katou-h@cc.okayama-u.ac.jp

ABSTRACT

Purpose

Histological grading is important to treat algorism in pancreatic neuroendocrine neoplasms (PNEN). The present study examined the efficacy of contrast-enhanced harmonic endoscopic ultrasound (CH-EUS) and time-intensity curve (TIC) analysis of PNEN diagnosis and grading.

Methods

TIC analysis was performed in 30 patients using data obtained from CH-EUS, and histopathological diagnosis was performed via EUS-guided fine needle aspiration or surgical resection. The TIC parameters were analyzed by dividing them into G1/G2 and G3/NEC groups. Then, patients were classified into non-aggressive and aggressive groups and evaluated.

Results

Twenty-six patients were classified as G1/G2, and 4 as G3/NEC. From the TIC analysis, five parameters of I; echo intensity change, II; time for peak enhancement, III; speed of contrast, IV; decrease rate for enhancement, and V; enhancement ratio for node / pancreatic parenchyma were obtained. Three of these parameters, I, IV and V, showed high diagnostic performance. Using the cutoff value obtained from the receiver operating characteristic (ROC) analysis, the correct diagnostic rates of parameter I, IV and V were 96.7%, 100%, and 100%, respectively, between G1/G2 and G3/NEC. A total of 21 patients were classified into the non-aggressive group, and 9 into the aggressive group. Using the cutoff value obtained from the ROC analysis, the accurate diagnostic rates of I, IV and V were 86.7%, 86.7%, and 88.5%, respectively, between the non-aggressive and aggressive groups.

Conclusion

CH-EUS and TIC analysis showed high diagnostic accuracy for grade diagnosis of PNEN. Quantitative perfusion analysis is useful to predict PNEN grade diagnosis preoperatively.

Keywords

Contrast-enhanced harmonic endoscopic ultrasound (CH-EUS); Pancreatic neuroendocrine neoplasm (PNEN); time-intensity curve (TIC) analysis

INTRODUCTION

Recently, there has been an increasing number of incidentally discovered pancreatic neuroendocrine neoplasms (PNEN) because of high-quality image inspection [1,2]. Especially, endoscopic ultrasound (EUS) is a useful technique in detecting small PNEN [3,4]. PNEN include various histological malignancies, and the WHO defined the pathological classifications as G1, G2, G3, and neuroendocrine carcinoma (NEC) according to the mitotic and Ki-67 index in 2017. The prognosis varies greatly depending on the grade, and treatment strategies are different among G1, G2, G3, and NEC. Though surgical resection is the main treatment for PNEN, limited resection, such as enucleation or resection without lymph node dissection, is acceptable in cases of low-grade PNEN [5], and grade diagnosis is also necessary for the selection of chemotherapy in unresectable cases. Thus, grade diagnosis of PNEN is important to determine the appropriate treatment. On the other hand, patients with G1/G2 PNEN sometimes have distant metastasis, however, unfortunately, it is currently difficult for the grade diagnosis to distinguish these patients from other patients without metastasis.

There are several reports describing the effectiveness of grade diagnosis before treatment with EUS-guided fine needle aspiration (EUS-FNA) [6-9]. However, there was a report in which the Ki-67 index varies within the tumor and does not necessarily mean that the hotspot is punctured [10]. Grade diagnosis should not be determined when tumor cells are not sufficiently contained in biopsy specimens. Therefore, another evaluation method is necessary in case pathological evidence for grading diagnosis cannot be acquired with EUS-FNA.

Contrast-enhanced harmonic EUS (CH-EUS) allows us to depict micro-vasculatures in real time. In addition, temporal change of the echo enhancement intensity can be measured, and a time-intensity curve (TIC) can be achieved. With percutaneous contrast-enhanced ultrasound, the usefulness of TIC analysis has been reported in breast tumors, renal masses, and liver tumors [11-13]. For pancreatic diseases, EUS is effective because it can approach and perform detailed observation. It has been reported that CH-EUS using TIC analysis is effective in differentiating various pancreatic tumors [14,15]. In addition, in PNEN, several papers reported the effectiveness of CH-EUS for evaluation of malignant potential. [16, 17]. However, there are no extensive reports about the relationship between PNEN and the accuracy of CH-EUS with TIC analysis.

We retrospectively analyzed a series of PNENs to determine whether CH-EUS with TIC analysis predicted the pathological grade and the malignant potential in G1/G2 with distant metastasis.

METHODS

Patients and study design

Eligibility criteria was the following: (i) PNEN for which CH-EUS and TIC analysis had been performed and (ii) a pathologically proven PNEN grade with evaluation specimens acquired via surgical resection or EUS-FNA. Endoscopic, radiological, and clinical data were retrospectively extracted from clinical records of all patients.

Patients pathologically diagnosed as PNEN G1/G2 via surgical resection or as PNEN G3/NEC via EUS-FNA at our institution from November 2009 and March 2018 were included in the analysis. These pathological samples were carefully examined by a pathologist according to the WHO 2017 classification. The tumor staging of all patients was evaluated with computed tomography (CT), based on the European Neuroendocrine Tumor Society (ENET) guidelines. In addition to the WHO 2017 classification, the subjects were analyzed by dividing them into the non-aggressive and aggressive group more clinically. Tumors were thought to be aggressive when there were morphological findings of an advanced disease (close organ involvement, lymph node involvement, and distant organ metastasis) or histological findings of a G3/NEC.

The study was approved by the review board of our institution, and informed consent regarding CH-EUS with TIC analysis was obtained from all patients.

EUS-FNA and pathological evaluation

EUS-FNA was performed using convex echoendoscopes (GF-UCT260; Olympus Optical Co. Ltd., Tokyo, Japan) with 19-, 22-, or 25-G needles. Immediately after tissue collection, a part of aspirated material was examined by a cell pathologist through a Diff-Quick staining method to ensure that the specimen was adequate as rapid on-site evaluation. As a surgical specimen, the remaining material was fixed in 10% formalin in a specimen bottle. If the specimens were too small for histopathological diagnosis, the specimens were centrifuged and then seated in paraffin for cell-block analysis.

The formalin-fixed FNA and surgical specimens were processed into paraffin, and staining of 5-μm sections with hematoxylin and eosin was performed for conventional histology and evaluated according to the WHO 2017 guidelines. Immunohistochemistry was performed using the primary antibodies against CD31, chromogranin A, synaptophysin, and Ki-67. The proliferation index for Ki-67 was evluated by the WHO 2017 guidelines.

CH-EUS protocol

EUS was performed using electronic radial echoendoscopes (GF-UE260-AL5; Olympus Optical Co. Ltd., Tokyo, Japan) or convex echoendoscopes (GF-UCT260). The apparatus used was ProSound α10 or F75 (Hitachi Aloka Co., Tokyo, Japan).

In case of suspected PNEN by B-mode EUS, CH-EUS was employed. An extended harmonic detection mode in which the filtered fundamental frequency and second harmonic component frequency were combined with a transmission frequency of 4.7 MHz was used. After intravenous administration of the contrast agent Sonazoid (Daiichi Sankyo, Tokyo, Japan), bloodstream of micro vessels in the tumor was evaluated. Sonazoid was a second-generation contrast agent for ultrasound containing perfluorobutane microbubbles with a median diameter of 2–3 μm. One vial of Sonazoid had 16 μL of perfluorobutane in 2 mL of distilled water, which was administered with transvenous injection at 0.015 mL/kg. After injection, the lesion suspected of PNEN was continuously observed for 120 seconds, and its enhancement was compared with that of the surrounding pancreatic parenchyma.

TIC analysis

The digital CH-EUS data were recorded continuously for 120 seconds following administration of the contrast medium. Subsequently, those data stored on the hard drive were retrieved and analyzed. Two circular regions of interest (ROIs) were placed in the tumor and surrounding parenchyma of the pancreas. To prevent incorrect settings, the position of the ROI was determined by two endoscopists with experience in CH-EUS. No knowledge of the final diagnosis was provided to these two endoscopists. The position of the ROI was calibrated to the respiratory movement of the patient. The size of ROI was decided according to the tumor size, and ROI was settled as widely as possible to cover the entire tumor. If the tumor contained cystic change, the ROI was placed avoiding cystic area. Especially in case with a large tumor, the content was sometimes heterogeneous with solid and cystic part. In such a case, ROI was placed at the largest solid part of several solid parts. The echo intensity of the ROI was quantified, and a TIC was computed with a software program embedded in the ultrasound imaging system.

The following parameters were measured from the TIC (Figure 1):

I. Echo intensity change

II. Time for peak enhancement

III. Speed of contrast

IV. Decrease rate for enhancement

V. Enhancement ratio for node / pancreatic parenchyma

These parameters were compared among the groups G1/G2 and G3/NEC. Histopathological examination findings from excised specimens provided the reference criteria. In addition to the comparison of parameters among histopathological grading, differences between the non-aggressive and aggressive groups and that between cystic PNEN and solid PNEN were analyzed.

Statistical data was analyzed using the JMP software program version 13.0 (SAS Institute, Cary, North Carolina, USA). Categorical values were compared using Fisher's exact test. Continuous values were presented as median and interquartile range and compared using the Mann-Whitney U test. The Youden index calculation was used, and the cutoff values were determined by a receiver operating characteristic (ROC) analysis for the diagnosis based on the TIC. P-values <0.05 were considered to be statistically significant.

RESULTS

Patients

EUS was performed on 77 patients suspected of PNEN. Fifty-one patients underwent EUS-FNA or surgical resection, and were diagnosed with PNEN pathologically. Grade diagnosis was possible in 40 patients. Of the 40 patients, 30 in whom both grade diagnosis and TIC analysis were applicable were enrolled in this study (Figure 2).

The clinical characteristics of the 30 patients are shown in Table 1. All 30 patients underwent surgical resection or EUS-FNA, and the final diagnosis was determined via the pathological findings. A total of 19 patients were classified as G1, 7 patients as G2, and 4 patients as G3/NEC. There were no significant differences with regard to the clinical and morphological findings observed among the three groups. There was a significant difference in tumor size and stage according to the ENET guideline.

Of all 30 patients, 24 patients underwent surgical resection after EUS-FNA. Ki-67 could not be measured in FNA specimens in 13 patients, and the correct diagnostic rate for PNEN grade diagnosis via EUS-FNA was 37.5% (9/24). Therefore, the grade diagnosis was obtained via both EUS-FNA and surgical resection in 9 patients and via only surgical resection in 17 patients. Of these 26 patients, 19 was G1, and 7 was G2. Remaining four of G3/NEC patients were diagnosed via only EUS-FNA.

The subjects were analyzed by dividing them into aggressive and non-aggressive groups as shown in Table 2. A total of 21 patients were classified in the non-aggressive group and 9 patients were classified in the aggressive group. No significant differences were noted regarding the clinical and morphological factors observed between the two groups. There was a significant difference in tumor size and stage ENET.

Cystic degeneration was observed in 11 of 30 patients. The rate of cystic degeneration was 38.5% in G1/G2 (10/26) and 25% in G3/NEC (1/4) (P=0.59). The rate of cystic degeneration was 33.3% (7/21) in the non-aggressive group and 44.4% (4/9) in aggressive group (P=0.56).

TIC parameters of PNEN

Typical findings and TICs of G1 and G3/NEC cases are shown in Figures 3 and 4.

All tumors were contrasted at the early stage, and then the contrast weakened over time. G1 was intensely contrasted at an early stage, and the contrast lasted interminably. G3/NEC was contrasted weakly at an early stage, and its level declined quickly. G2 had roughly a middle contrast effect. Non-significant adverse events were occurred associated with CH-EUS.

TIC analysis quantified the contrast effect as a level and was evaluated. Considering the G1/G2 and G3/NEC groups, the three parameters, I; echo intensity change, IV; decrease rate for enhancement, and V; enhancement ratio for node / pancreatic parenchyma, showed high diagnostic performance (Table 3). The cutoff values determined by ROC analysis between G1/G2 and G3/NEC were 92.5, 0.76, and 0.92 for parameter I, IV, and V, respectively. Using the cutoff value obtained by ROC analysis, the correct diagnostic rates differentiating G1/G2 and G3/NEC of parameter I, IV, and V were 96.7%, 100%, and 100%, respectively (Table 4).

Considering the non-aggressive and aggressive groups, the three parameters, I, IV, and V, showed high diagnostic performance (Table 5). The cutoff values determined by ROC analysis between the non-aggressive and aggressive groups were 95.2, 0.645, and 0.928 for parameter I, IV, and V, respectively. Using the cutoff value obtained by ROC analysis, the accurate diagnostic rates differentiating the non-aggressive and aggressive groups of parameter I, IV, and V were 86.7%, 86.7%, and 88.5%, respectively (Table 6).

In addition, the G1/G2 group included 21 patients from the non-aggressive group and 5 patients from the aggressive group. The cut-off values obtained by ROC analysis between the non-aggressive and aggressive groups within the G1/G2 group were 138.8, 0.428, and 1.362 for parameter I, IV, and V, respectively. Using the cutoff values obtained by ROC analysis, the accurate diagnostic rates differentiating the non-aggressive and aggressive groups for parameter I, IV, and V were 46.2%, 73.1%, and 45.8%, respectively, in the G1/ G2 group.

The median values of the parameter I, IV, and V in cystic PNEN were 136, 0.37, and 1.79. Those in solid PNEN were 120, 0.48, and 1.36, respectively. There was no significant difference between cystic and solid PNEN on each TIC parameter.

Comparison of grade diagnostic ability between EUS-FNA and TIC analysis

Of 24 patients undergoing both EUS-FNA and surgical resection, 17 patients were G1 and seven patients were G2. The cut-off values obtained by ROC analysis between the 17 patients of G1 and the 7 patients of G2 were 103, 0.37, and 2.03 for parameter I, IV, and V. Using the cutoff values obtained by ROC analysis, the accurate diagnostic rates differentiating between the two groups was 76.9%, 65.4%, and 75.0%. The accurate diagnostic rate for PNEN grade diagnosis via EUS-FNA was 37.5% (9/24).

DISCUSSION

The prognosis of PNEN differs depending on the pathological grade. In several guidelines of PNEN, a pathological grade diagnosis is recommended to determine treatment strategies, especially in the differential diagnosis between G1/G2 and G3/NEC [5,17]. Several reports described the effectiveness of pathological grading via EUS-FNA in preoperative and unresectable cases. The accuracy of pathological grading was reported to be 69.2–87.5% [8,9,18-20]. However, the controversies on the diagnostic ability of EUS-FNA for pathological grading of PNEN remain, because the samples obtained through EUS-FNA or liver biopsy are sometimes so small that pathological grading is difficult, and underestimation might occur.

Several studies have reported the effectiveness of CH-EUS in patients with PNEN. Kitano et al. reported that CH-EUS depicted hypervascular enhancement diagnosed as PNEN with a sensitivity and specificity of 78.9% and 98.7%, respectively [3]. Ishikawa et al. reported the heterogeneous ultrasonographic texture as malignant PNEN and that the sensitivity, specificity, and accuracy of conventional EUS for malignancy were 90.5%, 85.0%, and 87.8%, respectively [16]. Palazzo et al. reported that CH-EUS is accurate for predicting aggression by evaluating heterogeneous patterns within the PNEN and the overall accuracy, sensitivity, specificity, positive predictive value, and negative predictive value of CH-EUS for the diagnosis of tumor aggressiveness were 86%, 96%, 82%, 71%, and 98%, respectively [17]. However, no study evaluated the usefulness of CH-EUS with TIC analysis for pathological grading of PNEN. This is the first study using CH-EUS with TIC analysis for malignancy prediction of PNEN.

In most studies in which the effectiveness of CH-EUS was estimated, evaluation of the contrast pattern depends on the intuition of the operator, and a large difference among operators or facilities is sometimes observed. In TIC analysis, quantitative blood flow dynamics can be evaluated by setting the ROI and measuring the contrast intensity. We found a correlation between pathological grade and CH-EUS with TIC analysis. Especially, the echo intensity reduction rate was extremely different between G1/G2 and G3/NEC. High diagnostic accuracy could be obtained using the cutoff value determined from the ROC analysis, and the grade could be predicted from the echo intensity reduction rate. CH-EUS with TIC analysis is useful as one of the diagnostic modalities for the pathological grading of PNEN. In addition, the grade diagnostic ability was compared between EUS-FNA and TIC analysis in this study. Although it was limited in analysis between G1 and G2, the grade diagnostic ability of TIC analysis superior to that of EUS-FNA.

However, it is sometimes difficult to differentiate PNEN and pancreatic cancer only via imaging modalities such as contrast enhanced computed tomography and magnetic resonance imaging. We think that the differentiation using every diagnostic imaging modality including CH-EUS is difficult in cases of no findings with hyper vascular tumor which is a typical finding of PNEN. Fortunately, there were no cases who were misdiagnosed with pancreatic cancer preoperatively in this study. However, EUS-FNA is necessary for differentiation between pancreatic neuroendocrine tumor and pancreatic cancer in such cases.

In contrast, the issue on the choice of treatment strategy according to pathological grading is that the prediction of G1 and G2 cases with distant metastasis due to high biological malignancy is impossible. In our study, two of seven (28.5%) patients with G2 (one, liver metastasis; one, liver and bone metastasis) had distant metastasis of the liver (Table 1). Therefore, the presence or absence of distant metastasis is not always associated with pathological grade. We think that the results are influenced by microvessel density (MVD) within the tumor. MVD has been suggested a prognostic parameter in many malignant tumors. In PNEN, it has been reported that MVD was significantly higher in well-differentiated benign endocrine tumors than in tumors of uncertain behavior and carcinomas [21-23]. Palazzo et al. compared MVD between PNEN with or without distant metastasis, and there were significant differences between them, irrespective of the pathological grade [17]. Therefore, we think that TIC analysis based on CH-EUS findings may be able to evaluate biological malignancy of PNEN in the patients with G1/G2. Actually, we could differentiate G1 and G2 with high biological malignancy from those with low biological malignancy in this study by parameter IV; decrease rate for enhancement (73.1%).

Recently, several papers reported that cystic PNEN tend to be biologically less aggressive compared with their solid counterparts [24-26]. 11 of 30 cases had cystic degeneration in this study. However, there was no significant relationship between the presence of cystic degeneration and tumor aggressiveness. Although we also evaluated the differentiation of TIC parameters between cystic PNEN and solid PNEN, significant difference was not found in each TIC parameter.

This study had several limitations. First, our study was retrospective with results from a single institution. Owing to the small sample size, especially for G3/NEC, only 4 cases could be analyzed. Second, the diagnosis of the patients with G3/NEC were determined via only EUS-FNA. However, the misdiagnosis of pathological grading by EUS-FNA is mainly due to underestimation, and G3/NEC had less possibility of underestimation. Third, there is a technical problem. There are several cases in which it is difficult to continuously visualize the target tumor or pancreatic parenchyma with EUS and the TIC could not be measured. Fourth, this study mixed G3 and NEC as one group. The WHO 2017 grading system distinguishes G3 and NEC pathologically. However, recent papers written under pathological classification before WHO 2017 mixed G3 and NEC. These papers showed poor prognosis in G3, including NEC, compared with G1/G2. Several papers reported the relationship between micro vessel density (MVD) and PNEN [17,22,27,28]. In those papers, MVD has a significant relationship with the Ki-67 index, which is significantly higher in G3/NEC than in G1/G2. In addition, the results of the TIC study are reported to be affected by MVD [15,29,30]. Therefore, we think that differentiation between G1/G2 and G3/NEC by TIC analysis is reasonable and feasible.

In conclusion, CH-EUS can be employed for the small PNEN, in which enough sample for pathological grading is difficult to obtain, and TIC facilitates accurate quantitative analysis of the CH-EUS results. The combination of CH-EUS with TIC analysis and EUS-FNA and/or surgery has the potential of establishing a new treatment strategy for PNEN. For accurate conclusions, a larger sample size is warranted.

Acknowledgment

We gratefully acknowledge the work of past and present members of our laboratory.

Conflict of Interest

The authors have no conflicts of interest directly relevant to the content of this article.

Ethical statements

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. Informed consent was obtained from all patients for being included in the study.

References

1. Jae Gu Jung, Kyu Taek Lee, Young Sik Woo, et al. Behavior of small, asymptomatic, nonfunctioning pancreatic neuroendocrine tumors (NF-PNETs). Medicine. 2015; 94: e983

2. Ito T, Sasano H, Tanaka M, et al. Epidemiological study of gastroenteropancreatic neuroendocrine tumors in Japan. J Gastroenterol. 2010; 45: 234–43

3. 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: 303–10

4. 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: 1073–9

5. Falconia M, Erikssonb B, Kaltsasc G, et al. Consensus guidelines update for the management of functional p-NETs (F-p-NETs) and non-functional p-NETs (NF-p-NETs). Neuroendocrinology. 2016; 103: 153–71

6. Alexiev BA, Darwin PE, Goloubeva O, et al. Proliferative rate in endoscopic ultrasound fine-needle aspiration of pancreatic endocrine tumors. Cancer Cytopathol. 2009; 117: 40–5

7. Fujimori N, Osoegawa T, Lee L, et al. Efficacy of endoscopic ultrasonography and endoscopic ultrasonography-guided fine-needle aspiration for the diagnosis and grading of pancreatic neuroendocrine tumors. Scand J Gastroenterol. 2016; 51: 245–52

8. Hasegawa T, Yamao K, Hijioka S, et al. Evaluation of Ki-67 index in EUS–FNA specimens for the assessment of malignancy risk in pancreatic neuroendocrine tumors. Endoscopy. 2014; 46: 32–8

9. Sugimoto M, Takagi T, Hikichi T, et al. Efficacy of endoscopic ultrasonography-guided fine needle aspiration for pancreatic neuroendocrine tumor grading. World J Gastroenterol. 2015; 21: 8118–24

10. Yang Z, Tang LH, Klimstra DS, et al. Effect of tumor heterogeneity on the assessment of Ki67 labeling index in well-differentiated neuroendocrine tumors metastatic to the liver: implications for prognostic stratification. Am J Surg Pathol. 2011; 35: 853–60

11. Zhang Q, Yuan C, Dai W, et al. Evaluating pathologic response of breast cancer to neoadjuvant chemotherapy with computer-extracted features from contrast-enhanced ultrasound videos. Phys Med. 2017; 39: 156–63

12. King KG, Gulati M, Malhi H, et al. Quantitative assessment of solid renal masses by contrast-enhanced ultrasound with time-intensity curves: how we do it. Abdom Imaging. 2015; 40: 2461–71

13. EM Jung, DA Clevert, AG Schreyer, et al. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: a prospective controlled two-center study. World J Gastroenterol. 2007; 13: 6356–64

14. Omoto S, Takenaka M, Kitano M, et al. Characterization of pancreatic tumors with quantitative perfusion analysis in contrast-enhanced harmonic endoscopic ultrasonography. Oncology. 2017; 93: 55–60

15. Yamamoto N, Kato H, Tomoda T, et al. Contrast-enhanced harmonic endoscopic ultrasonography with time-intensity curve analysis for intraductal papillary mucinous neoplasms of the pancreas. Endoscopy. 2016; 48: 1–10

16. Ishikawa T, Itoh A, Kawashima H, et al. Usefulness of EUS combined with contrast-enhancement in the differential diagnosis of malignant versus benign and preoperative localization of pancreatic endocrine tumors. Gastrointest Endosc. 2010; 71: 951–9

17. Palazzo M, Napoléon B, Gincul R, et al. Contrast harmonic EUS for the prediction of pancreatic neuroendocrine tumor aggressiveness (with videos). Gastrointest Endosc. 2018; 87: 1481–8

18. Capelli P, Fassan M, Scarpa A, et al. Pathology – grading and staging of GEP-NETs. Best Pract Res Clin Gastroenterol. 2012; 26: 705–17

19. Larghi A, Capurso G, Carnuccio A, et al. Ki-67 grading of nonfunctioning pancreatic neuroendocrine tumors on histologic samples obtained by EUS-guided fine-needle tissue acquisition: a prospective study. Gastrointest Endosc. 2012; 76: 570–7

20. Sugimoto M, Takagi T, Hikichi T, et al. Efficacy of endoscopic ultrasonography-guided fine needle aspiration for pancreatic neuroendocrine tumor grading. World J Gastroenterol. 2015; 21: 8118–24

21. Farrell JM, Pang JC, Kim GE, et al. Pancreatic neuroendocrine tumors: accurate grading with Ki-67 index on fine-needle aspiration specimens using the WHO 2010/ENETS criteria. Cancer Cytopathol. 2014; 770–8

22. Marion-Audibert AM, Barel C, Gouysse G, et al. Low microvessel density is an unfavorable histoprognostic factor in pancreatic endocrine tumors. Gastroenterology. 2003; 125: 1094–104

23. Couvelard A, O'Toole D, Turley H, et al. Microvascular density and hypoxia-inducible factor pathway in pancreatic endocrine tumours: negative correlation of microvascular density and VEGF expression with tumour progression. Br J Cancer. 2005; 92: 94–101

24. Singhi AD, Chu LC, Tatsas AD, et al. Cystic pancreatic neuroendocrine tumors: a clinicopathologic study. Am J Surg Pathol 2012; 36: 1666-73

25. Koh YX, Chok AY, Zheng HL, Tan CS, Goh BK. A systematic review and meta-analysis of the clinicopathologic characteristics of cystic versus solid pancreatic neuroendocrine neoplasms. Surgery 2014; 156: 83-96 e2.

26. Cloyd JM, Kopecky KE, Norton JA, et al. Neuroendocrine tumors of the pancreas: Degree of cystic component predicts prognosis. Surgery 2016; 160: 708-13

27. Kuiper P, Hawinkels LJAC, de Jonge-Muller ESM, et al. Angiogenic markers endoglin and vascular endothelial growth factor in gastroenteropancreatic neuroendocrine tumors. World J Gastroenterol. 2011; 17: 219–25

28. Horiguchi S, Kato H, Shiraha H, et al. Dynamic computed tomography is useful for prediction of pathological grade in pancreatic neuroendocrine neoplasm. J Gastroenterol Hepatol. 2017; 32: 925-31

29. Jiang J, Shang X, Zhang H, et al. Correlation between maximum intensity and microvessel density for differentiation of malignant from benign thyroid nodules on contrast-enhanced sonography. J Ultrasound Med. 2014; 33: 1257-63.

30. Wang Y, Li L, Wang YX, et al. Time-intensity curve parameters in rectal cancer measured using endorectal ultrasonography with sterile coupling gels filling the rectum: correlations with tumor angiogenesis and clinicopathological features. Biomed Res Int. 2014; 2014: 587806.

Table 1 Characteristics of patients with PNEN, who underwent contrast-enhanced harmonic endoscopic ultrasonography (CH-EUS).

	G1/G2 n=26	G3/NEC n=4	P value
Gender			0.7041
Male	9	1	
Female	17	3	
Mean age median (IQR)[1]	65.5 (45-69.5)	71.5 (50.25-83.75)	0.2712
Functioning tumor			0.8961
Insulinoma	3	1	
Gastrinoma	1	-	
Nonfunctioning tumor	19	3	
MEN type 1	3	-	
Size[2], median (mm)(IQR)[1]	15 (12.75-34.25)	33.5 (23-41.75)	0.1113
Stage ENET			0.0010
I	15	-	
IIa/IIb	7	-	
IIIa/IIIb	2	-	
IV	2	4	

NEC, Neuroendocrine carcinoma; IQR, interquartile range; MEM, Multiple Endocrine Neoplasia

ENET, European Neuroendocrine Tumor Society

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

2 Tumor size was determined on pathologic report or on imaging report and the largest value was used.

Table 2 Characteristics of patients, with PNEN with non aggressive and aggresive, who underwent contrast-enhanced harmonic endoscopic ultrasonography (CH-EUS).

	non aggressive n=21	aggressive n=9	P value
Gender			0.3980
Male	8	2	
Female	13	7	
Mean age median (IQR)[1]	66(46-70)	66(33.5-76)	1.0000
Functioning tumor			0.4479
Insulinoma	3	1	
Gastrinoma	1	-	
Nonfunctioning tumor	15	8	
MEN type 1	2	-	
Size[2], median (mm)(IQR)[1]	15(11.5-26)	35(33-57)	0.0003
Stage ENET			<0.0001
I	15	-	
IIa/IIb	6	1	
IIIa/IIIb	-	2	
IV	-	6	

IQR, interquartile range; MEM, Multiple Endocrine Neoplasia

ENET, European Neuroendocrine Tumor Society

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

2 Tumor size was determined on pathologic report or on imaging report and the largest value was used.

Table 3 Time-intensity curve analysis from patients with PNEN, who underwent contrast-enhanced harmonic endoscopic ultrasonography (CH-EUS) .

	G1/G2 n=26	G3/NEC n=4	P value
Time-intensity curve parameters, median (IQR)			
Echo intensity change, level	131.88 (109.24-141.83)	75.92 (53.79-92.15)	0.0031
Time for peak enhancement, s	10.11 (7.37-13.57)	10.61 (7.37-20.29)	0.6473
Speed of contrast, level/s	13.33 (9.56-20.16)	7.39 (3.40-11.35)	0.0546
Decrease rate for enhancement, %	0.37 (0.28-0.48)	0.82 (0.77-0.87)	0.0017
Enhancement ratio for node / pancreatic parenchyma	1.51 (1.28-1.85)	0.77 (0.62-0.92)	0.0237

NEC, Neuroendocrine carcinoma; IQR, interquartile range

Table 4 Diagnostic performance of time-intensity curve parameters between PNEN G1/G2 and G3/NEC

	Sensitivity, % 95%Cl No.of patients	Specificity, % 95%Cl No.of patients	PPV,% 95%Cl No.of patients	NPV,% 95%Cl No.of patients	Accuracy,% 95%Cl No.of patients
Echo intensity change	100	96.2	80	100	96.7
	59.5-100	89.9-96.2	47.6-80	93.5-100	85.9-96.7
	4/4	25/26	4/5	25/25	29/30
Decrease rate for enhancement	100	100	100	100	100
	63.5-100	94.4-100	63.5-100	94.4-100	90.3-100
	4/4	26/26	4/4	26/26	30/30
Enhancement ratio for node / pancreatic parenchyma	100	100	100	100	100
	43.1-100	95.3-100	43.1-100	95.3-100	91.2-100
	2/2	24/24	2/2	24/24	26/26

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

Table 5 Time-intensity curve analysis from patients with PNEN non-aggressive group or aggressive group, who underwent contrast-enhanced harmonic endoscopic ultrasonography (CH-EUS) .

	non aggressive n=21	aggressive n=9	P value
Time-intensity curve parameters, median (IQR)			
Echo intensity change, level	134.97 (113.85-147.69)	92.5 (59.72-129.97)	0.0099
Time for peak enhancement, s	9.31 (6.73-12.67)	13.43 (8.4-14.5)	0.1606
Speed of contrast, level/s	13.68 (11.31-21.62)	8.02 (5.29-12.73)	0.0113
Decrease rate for enhancement, %	0.35 (0.28-0.46)	0.64 (0.40-0.82)	0.0087
Enhancement ratio for node / pancreatic parenchyma	1.46 (1.26-1.83)	1.36 (0.77-1.80)	0.2979

IQR, interquartile range

Table 6 Diagnostic performance of time-intensity curve parameters for PNEN non-aggressive group or aggressive group

	Sensitivity,% 95%Cl No.of patients	Specificity,% 95%Cl No.of patients	PPV,% 95%Cl No.of patients	NPV,% 95%Cl No.of patients	Accuracy,% 95%Cl No.of patients
Echo intensity change	55.6	100	100	84.0	86.7
	34.5-55.6	91.0-100	62.1-100	76.4-84.0	74.0-86.7
	5/9	21/21	5/5	21/25	26/30
Decrease rate for enhancement	55.6	100	100	84	86.7
	34.5-55.6	91.0-100	62.1-100	76.4-84.0	74.0-86.7
	5/9	21/21	5/5	21/25	26/30
Enhancement ratio for node / pancreatic parenchyma	40.0	100	100	87.5	88.5
	14.8-40.0	94.0-100	37.1-100	82.3-87.5	78.8-88.5
	2/5	21/21	2/2	21/24	23/26

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

Figure and Figure Legends

Figure 1. Schematic of a time-intensity curve showing the measured parameters

Ipeak − Ibase, echo intensity change

tpeak, time to contrast enhancement

(Ipeak − Ibase)/tpeak, speed of contrast

(Ipeak − I120)/Ipeak, decrease rate for enhancement

(Ipeak − Ibase, for the nodule)/ (Ipeak − Ibase, for the parenchyma), enhancement ratio for node / pancreatic parenchyma

Figure 2. Flowchart of the study

Figure 3. Pancreatic endocrine tumor in the G1/G2 group within the pancreatic tail a. Contrast-enhanced computed tomography image b. Fundamental B-mode endoscopic ultrasonography (EUS) shows a hypoechoic lesion c. A pre-enhancement image d. EUS image at the peak of contrast enhancement. The yellow and purple circles show the regions of interest (ROIs) in the tumor and pancreatic parenchyma, respectively, both of which were enhanced e. EUS image obtained 120 s after Sonazoid injection, revealing continued enhancement in both the tumor (yellow circle) and pancreatic parenchyma (purple circle) f. Time-intensity characteristics of the tumor (yellow line) and pancreatic parenchyma (purple line). At 120 s after injection, the echo intensity from the peak decreased in the pancreatic tumor rather than the pancreatic parenchyma. When analyzing the TIC, the echo intensity change was 128.75, decrease rate for enhancement was 23.5, and enhancement ratio for node / pancreatic parenchyma was 2.85.

Figure 4. A pancreatic endocrine tumor in the G3/NEC group within the pancreatic body. a. Contrast-enhanced computed tomography image b. Fundamental B-mode endoscopic ultrasonography (EUS) showed a hypoechoic lesion c. A pre-enhancement image d. EUS image at the peak of contrast enhancement. The yellow and purple circles show the regions of interest (ROIs) in the tumor and pancreatic parenchyma, respectively, both of which were enhanced. e. EUS image obtained 120 s after Sonazoid injection, revealing continued enhancement in both the tumor (yellow circle) and pancreatic parenchyma (purple circle) f. Time-intensity characteristics of the tumor (yellow line) and pancreatic parenchyma (purple line). At 120 s after injection, the enhanced echo intensity of the pancreatic parenchyma was slightly reduced, whereas that of the tumor was markedly decreased from the peak intensity. When analyzing TIC, the echo intensity change was 92.5, decrease rate for enhancement was 85.5, and enhancement ratio for node / pancreatic parenchyma was 0.92.