Review

Contrast-Enhanced Harmonic Endoscopic Ultrasonography of Solid Pancreatic Lesions

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Abstract

Endoscopic ultrasonography is the best modality for pancreatic lesion evaluation as its superior spatial resolution allows small lesions to be identified and fine needle aspiration (FNA) cytology performed under ultrasound-guidance. Despite this, differentiating benign from malignant lesions remains a challenge as conventional ultrasound imaging is unable to differentiate lesions accurately and tissue yield is poorly diagnostic or limited in patients with the chronic inflammation. Contrast-harmonic technology uses a wide-band transducer capable of inducing sufficient acoustic energy to create harmonic microbubble oscillations of the newer second-generation ultrasound contrast agents (UCAs). These microbubbles are more stable, remaining within the intravascular component longer and emit significantly more harmonic content than surrounding tissue, thus allowing pancreatic parenchymal differentiation and microvascular architecture visualization. The use of UCAs is generally safe, but should be especially avoided in patients with unstable ischemic heart disease. During CH endosonography, pancreatic adenocarcinoma is commonly seen as an inhomogenous hypoenhancing lesion, focal pancreatitis as a hypo- or iso-enhancing lesion and neuroendocrine tumor as a hyperenhancing lesion. The presence of hyperenhancement is a strong predictor of non-adenocarcinoma etiology. Furthermore, in patients with the chronic pancreatitis or biliary stents that may obscure pancreatic inspection, the addition of contrast-harmonic endosonography to guide FNA cytology improves its diagnostic yield and accuracy. Quantitative analysis of perfusion through the time intensity curve is promising as an objective and accurate method to differentiate pancreatic lesions. Furthermore, studies are required to fully determine the role of contrast harmonic endosonography in the differential diagnosis of solid pancreatic lesions.

Keywords: Contrast-harmonic; pancreas; endoscopic ultrasonography

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INTRODUCTION

Ever since endoscopic ultrasonography (EUS) was first introduced in 1980,[1-3] its superior spatial resolution cemented its popularity as an important tool to localize and characterize pancreatic lesions.[4-6] Distinguishing malignant lesions from benign ones allows appropriate and early curative surgery for resectable adenocarcinoma and avoids surgery for non-neoplastic lesions such as inflammatory masses associated with chronic pancreatitis. However, despite the advances in technology of multi-detector row computed tomography (CT), magnetic resonance imaging (MRI) and conventional EUS, none of these modalities is able to differentiate the types of solid pancreatic lesions reliably.[7-9] Also, the addition of EUS-guided fine-needle aspiration (FNA) cytology did not improve diagnostic accuracy reliably as the negative predictive value for diagnosing cancer remains low at about 70%.[10]

Contrast-enhanced (CE) EUS evolved from simple power Doppler EUS evaluation with contrast injection to a wideband dedicated transducer, which is now widely known as contrast-enhanced harmonic-EUS (CH-EUS).[11] Although power Doppler sonography is able to detect vascular flow patterns, it has low sensitivity for detecting microvessels and is limited by multiple tissue artifacts such as blooming (overpainting) and tissue motion (flash).[10,12] Harmonic technology allows detection of microbubbles within slow-flow microvessels without Doppler-related artifacts. CH-EUS studies have described specific enhancement patterns for pancreatic lesions. This technology was made possible with the combined advances of having a EUS

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transducer strong enough to produce sufficient acoustic power and the availability of contrast agents that remain in the microcirculation while producing harmonic signals at lower power requirements.

This review describes the technology of CH-EUS and its clinical application in patients with solid pancreatic lesions.

ULTRASOUND CONTRAST AGENTS (UCAs)

In comparison with contrast agents for CT or MRI, UCAs remain within the intravascular space and do not diffuse into the interstitial space.[13] The second generation UCAs contain inert gases with lower water-solubility and diffusion coefficient than the first generation agents and thus improved stability and contrast duration.[14] These high molecular weight gases include perfluoropentane and sulfurhexafluoride, whereas the microbubble shells consist of phospholipids, polymers, surfactant or albumin.

Commonly used agents include sonovue (Bracco Imaging, Milan, Italy), sonazoid (Daiichi-Sankyo, Tokyo, Japan), definity (Lantheus Medical Imaging, Mass, USA) and imagent (IMCOR Pharmaceutical Co.). Among these, the most experience is with sonovue. It is microbubbles contain sulfurhexafluoride, an inert and poorly soluble gas. It is provided as a lyophilisate powder in a phospoholipid-stabilized sulfurhexafluoride microbubble, sealed within a glass vial. Reconstitution with the addition of saline and gentle shaking lead to a white milky solution. The recommended dosage is 2-2.4 ml, which is injected intravenously into a large peripheral vein, where it remains within the intravascular compartment till the gas dissolves and is expired in the lungs.[15] Sonazoid has microbubbles of perfluorobutane with a lipid membrane encapsulation. Definity consists of perfluopenthane within a phospholipid shell and is commonly used in contrast echocardiography.[16] UCAs differ in their signal intensity and duration following infusion. It has been described that sonovue's intensity declines within 60 s,[11] whereas sonazoid allows parenchymal perfusion observation for at least 90 s.[17] However, in clinical practice, the duration of contrast observation may vary as contrast destruction depends not only on the type of contrast used, but also on the target lesion's distance from the transducer as well as imaging parameters such as the mechanical index (MI), pulse repetition frequency and pulse length.

HARMONIC TECHNOLOGY

The basic principle of UCAs is that while gases are compressible, tissue is almost incompressible.[14] During an ultrasound wave, volumetric oscillation of microbubbles occurs with compression during pressure peaks and expansion during pressure nadirs. Besides the type of gas and property of the bubble shell, the acoustic power of the ultrasound influences the vibration of the microbubbles. To quantify the insonation energy from the transmitted ultrasound wave transducer, the MI is calculated from the maximum negative sound pressure divided by the square root of the sound frequency. It reflects the probability of cavitation resulting from an acoustic beam. Varying acoustic powers produces varying effects. Very low acoustic power (MI <0.1) produces symmetrical oscillations and the scattered signal frequency is similar to that of the emitted sound wave. At low acoustic powers (MI: 0.1-0.6), a non-linear response occurs due to the microbubbles resisting compression more strongly than expansion. This leads to multiples of insonating frequency being present within the returning signals. These higher frequency components are known as harmonics. Because microbubbles produce harmonic content that are significantly higher than tissue, CH imaging highlights areas with higher harmonic content of microbubbles and filter the lower signals that arise from tissue.[18] This strong acoustic signal is detected and visualized as an opacification on an ultrasound image. When the acoustic power is further increased (MI >0.6), disruption of the microbubble shell occurs and transient resonance of free gas bubbles result in a very strong echo signal.

There are various imaging modalities to detect UCA and the chosen technique is based on the contrast agent used and on the machine platform. Contrast-specific imaging procedures include single-pulse and multi-pulse procedures, of which pulse-inversion mode is the simplest modality.

In 2005, Dietrich *et al.*, first reported the use of CH-EUS in six patients.[19] They used sonovue injection with an adapted dynamic contrast harmonic wide-band pulse-inversion software with low MI, demonstrating that arterial, portal venous and parenchymal contrast-enhancement were possible. Since then, numerous studies showed the usefulness of CH-EUS in differentiating benign from malignant lesions. Kitano *et al.*, in particular, described the optimal settings and clinical role of CH-EUS in evaluating the parenchymal perfusion and microvessel architecture in pancreaticobiliary and other gastrointestinal (GI) diseases.[11] In his study, sonovue infusion with intermittent imaging at MI of 0.4 and interval delay-scanning time of more than 2 s produced homogenous enhancement of the pancreas. With MI of 0.4 and real-time continuous imaging, microvascular architecture could be visualized.

HOW TO PERFORM CH ENDOSONOGRAPHY

At our center, we use the ALOKA ProSound SSD α-10 image processor (ALOKA, Tokyo, Japan) with a dedicated echoendoscope (GF-UE160-AL5, Olympus Medical Systems, Tokyo, Japan). With the patient in the left lateral decubitus position and sedation with intravenous midazolam and fentanyl, the echoendoscope is inserted into the upper GI tract and the whole pancreas is surveyed by conventional B mode EUS. Subsequently, we switch to the extended pure

harmonic detection (ExPHD) imaging mode. ExPHD for tissue harmonic echo (THE) and for contrast harmonic echo (CHE) are used. THE mode is better for imaging the entire anatomical structures of the pancreaticobiliary system, whereas CHE is more specific in accentuating the vascular pattern following contrast injection. At our center in Singapore, the UCA definity and sonovue are available. In Japan, sonazoid and sonovue are utilized whereas in Korea, sonovue is used. Definity is used at a dose of 10 ul/kg body weight, injected into an 18-gauge intravenous cannula situated in the antecubital fossa, followed by 5 ml of saline flush. The MI is set at 0.3 as it provided the optimal balance between contrast enhancement and effect duration of contrast.[20] At a frequency of 5 MHz, we used intermittent mode for parenchymal evaluation and continuous mode for microvascular assessment. For Sonovue, we inject 2.4 ml of the reconstituted solution, followed by 5 ml of saline. MI is set at 0.4.

CH-EUS FEATURES OF SOLID PANCREATIC LESIONS

Adenocarcinoma

Ductal adenocarcinoma (Fig. 1A-C) is characteristically poorly vascularized. On CH-EUS, the presence of irregular network-like vessels in a relatively hypovascular lesion were highly suggestive of pancreatic adenocarcinoma.[11,20] In 49 of 51 patients with pancreatic adenocarcinoma, Fusaroli *et al.*, reported presence of inhomogeneous hypoenhancement with fast washout. However, rarely, some pancreatic adenocarcinoma may appear iso- or hyperenhancing.[21]

Focal pancreatitis/autoimmune pancreatitis

The differentiation of focal pancreatitis from pancreatic adenocarcinoma is challenging as cytological evaluation in the setting of chronic pancreatitis is limited by inflammatory infiltrates that could obscure or simulate malignancy.[22] Fusaroli *et al.*,[21] reported hypoenhancement in nine patients and isoenhancement in four patients with focal pancreatitis. In this regard, hypoenhancement alone has poor specificity and may not reliably diagnose malignancy.

To improve on its accuracy to differentiate autoimmune pancreatitis (Fig. 2A-C) from pancreatic adenocarcinoma, Imazu *et al.*, used CH-EUS with quantitative perfusion analysis via a "time intensity curve" software.[23] Using maximum intensity gain (MIG), which was the difference between peak intensity and base intensity before contrast injection, they showed that a MIG cut-off value of 12.5 dB had excellent sensitivity and specificity to differentiate autoimmune pancreatitis from pancreatic carcinoma. Though promising, further studies are required to determine its utility for differentiating various types of pancreatic lesions.

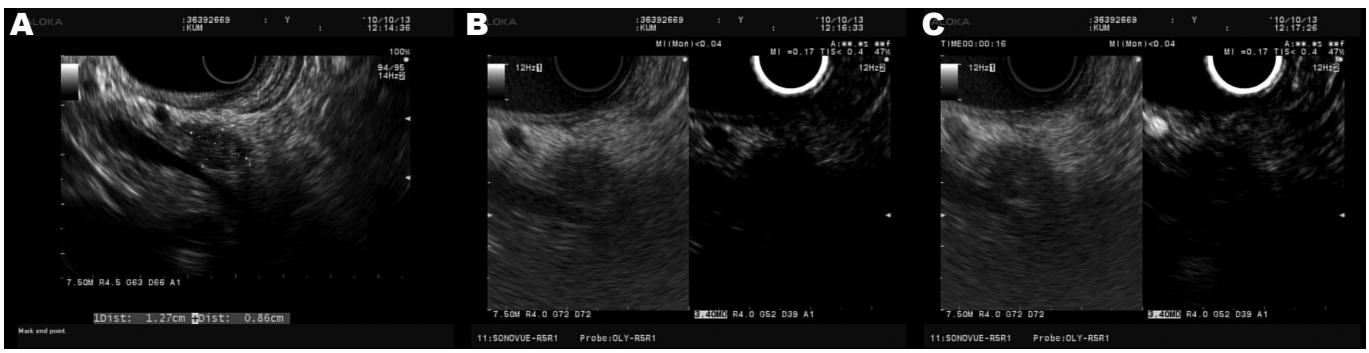

Figure 1. Pancreatic adenocarcinoma: Hypoechoic pancreatic mass. (A) On conventional endoscopic ultrasonography (EUS); (B) on contrast-enhanced harmonic (CH)-EUS (pre-contrast); (C) on CH-EUS (post-contrast)

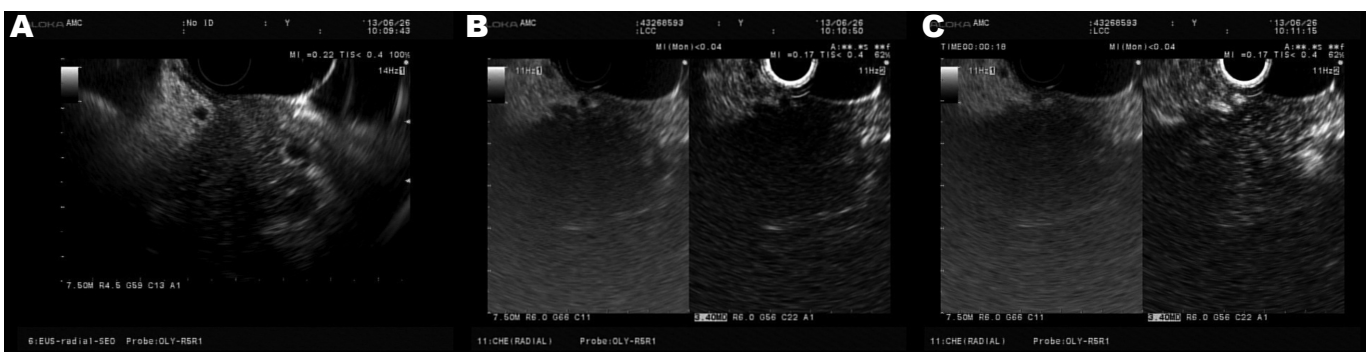

Figure 2. Focal autoimmune pancreatitis. (A) Hypoechoic pancreatic mass on conventional endoscopic ultrasonography; (B) hypoechoic pancreatic mass on contrast-enhanced harmonic endoscopic ultrasonography (CH-EUS) (pre-contrast); (C) iso-enhancing pancreatic mass on CH-EUS (post-contrast)

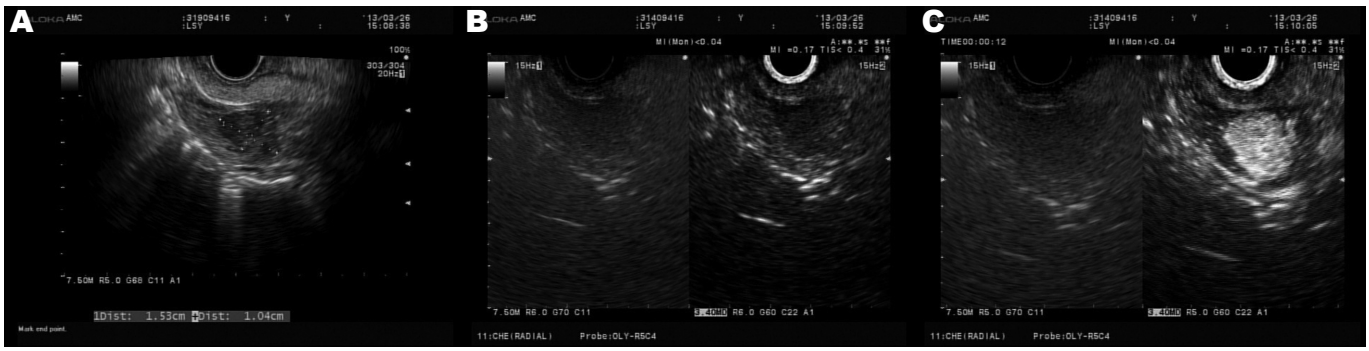

Figure 3. Neuroendocrine tumor. A: Hypoechoic pancreatic mass on conventional endoscopic ultrasonography; B: hypoechoic pancreatic mass on contrast-enhanced harmonic endoscopic ultrasonography (CH)-EUS (pre-contrast); C: homogenously-enhancing pancreatic mass on CH-EUS (post-contrast)

Neuroendocrine tumor

Although, it appears hypoechoic on conventional EUS, neuroendocrine tumors (Fig. 3A-C) are classically homogenously enhancing on CH-EUS.[11] This is consistent with previous reports on CE transabdominal US that described hypervascular enhancement in 80% to 87% of endocrine tumors.[24,25] Although hyperenhancement is not specific for neuroendocrine tumor, this feature is a strong predictor for histology other than adenocarcinoma (94% positive predictive value), with neuroendocrine tumor being the most common (56%).[21]

SAFETY AND COMPLICATIONS

UCAs approved for clinical use are generally well-tolerated and safe. The risks associated with UCAs are related to biologic effects from ultrasound-induced cavitation, toxicity, embolic risk and anaphylactic risk. Implosion of microbubbles or the cavitation phenomenon occurs due to the low-pressure and high-pressure wave phases of the ultrasound. While the gas bubble is formed during the low-pressure phase, this gas bubble collapses during the high-pressure phase, leading to a large amount of energy being transmitted causing significant changes in pressure and temperature within the close vicinity. This can result in formation of free radicals, electromagnetic radiation and lysis of adjacent cells. Although hemolysis and platelet aggregation effects have been seen in animal studies, these side-effects have not been seen in clinical practice.[26] The toxicity and embolic potential of UCAs were also deemed to be of no clinical significance by the European committee for medical ultrasound safety.[14] However, as the component shell and excipients of the UCAs are derived from macromolecular substances, there is a risk of allergic reactions. These are mostly mild such as transient rash or sensation of heat but rarely, anaphylactic hypersensitivity reactions may occur. In addition, UCAs should not be used in patients with unstable ischemic heart disease.[27] The European Medicine Agency has limited the use of the agent sulfurhexafluoride in patients with cardiac disease after a number of serious allergic reactions with cardiovascular complications and deaths were reported. Other listed contraindications include the presence of right-to-left shunts, severe pulmonary hypertension, uncontrolled systemic hypertension and adult respiratory distress syndrome.

PERFORMANCE CHARACTERISTICS

Prior to CH-EUS studies, Sakamoto *et al.*, compared the sensitivity of non-harmonic CE EUS, power-Doppler EUS and CE multidetector computed tomography (MDCT) for differentiating pancreatic tumors 2 cm or smaller. Despite its limitation in depicting fine vessels and parenchymal perfusion, CE-EUS had the best sensitivity for differentiating ductal adenocarcinoma from other tumors; 83.3%, 11.0% and 50.0%, respectively.[28]

The value of CH-EUS was demonstrated by Kitano *et al.*, in a study of 277 patients with solid pancreatic lesions.[17] In his study, besides diagnosing ductal adenocarcinoma with high sensitivity and specificity (95.1% and 89.0%, respectively) based on a hypoenhancement pattern on CH-EUS, there was also an excellent inter-observer agreement with a kappa coefficient of 0.94. In addition, neuroendocrine tumors were diagnosed by the presence of hypervascular enhancement, with sensitivity and specificity of 78.9% and 98.7%, respectively. Table 1 summarizes the performance characteristics of recent studies using CH-EUS in patients with solid pancreatic lesions.

Similarly, a study by Napolean *et al.*, reported sensitivity, specificity, negative predictive value, positive predictive value and accuracy of hypointensity for diagnosing pancreatic adenocarcinoma of 89%, 88%, 88%, 89% and 88.5%.[10] The value of CH-EUS was highlighted when compared with EUS-guided EUS-FNA, which had corresponding performance characteristics of 72%, 100%, 77%, 100% and 86%. Although the study of Kitano *et al.*[17] did not show CH-EUS to be more superior to EUS-FNA, five patients with false-negative EUS-FNA findings had hypo-enhancement suggestive of adenocarcinoma on CH-EUS. By combining CH-EUS with EUS-FNA, the sensitivity for cancer diagnosis was increased from 92.2% to 100%.

Table 1. Performance characteristics of CH-EUS in patients with pancreatic lesions

Author	Year	*n*	Contrast agent	Diagnosis	CH-EUS feature	Sensitivity (%)	Specificity (%)	Complication
Kitano[17]	2012	277	Sonazoid	Adenocarcinoma	Hypoenhancement	95.1	89.0	Nil
				Small carcinoma (≤2 cm)	Hypoenhancement	91.2	94.4	
				Neuroendocrine tumor	Hyperenhancement	78.9	98.0	
Imazu[23]	2012	30	Sonazoid	Autoimmune pancreatitis *vs* adenocarcinoma	MIG>12.5	100	100	Nil
				Adenocarcinoma	Hypoenhancement (heterogenous)	81.8	100	
				Autoimmune pancreatitis	Isoenhancement (homogenous)	100	95.5	
Fusaroli[21]	2010	90	Sonovue	Adenocarcinoma	Hypoenhancement	96	64	Nil
				Neuroendocrine tumor	Hyperenhancement	69	90	
Napoleon[10]	2010	35	Sonovue	Adenocarcinoma	Hypoenhancement	89	88	Nil
				Non-adenocarcinoma	Hyperenhancement	88	89	

CH-EUS: Contrast-enhanced harmonic-endoscopic ultrasonography; MIG: Maximum intensity gain (decibel)

In comparison with CH-EUS, MDCT did not differ significantly in its ability to diagnose ductal adenocarcinoma; 95.1% *vs* 91.7%, respectively.[17] However, due to EUS's better spatial resolution, it was not surprising that CH-EUS was significantly more sensitive than MDCT in detecting small cancers <2 cm; 91.2% *vs* 70.6%, respectively.

FUTURE DIRECTIONS

In seven patients where presence of biliary stent or diffuse chronic pancreatitis led to poor pancreatic visualization on conventional EUS, CH-EUS was able to detect small hypoenhancing lesions. This guided FNA and pancreatic adenocarcinoma was subsequently diagnosed.[21] The value of CH-EUS as a guide for not only detection, but improving histological yield will require larger studies for further confirmation.

Imazu *et al*., described an improvement in T-staging for patients with pancreaticobiliary malignancy when CH-EUS was used instead of harmonic EUS alone; 92.4% *vs* 69.2%, respectively.[29] Larger studies with multimodality comparison including that with current high resolution CE MDCT may further distinguish its role in tumor staging.

A limitation of CH-EUS is the subjectivity of an operator's impression of lesion enhancement, which is dependent on one's experience. To overcome this limitation, quantitative methods of recording perfusion based on contrast intensity was created and studied by various authors.[23,30] This objective technique may further improve the accuracy of CH-EUS to differentiate various pancreatic etiologies.

Another promising feature of contrast-harmonic technology is its potential to selectively deliver therapy and reduce undesired side-effects in other non-targeted organs using contrast microbubbles as drug/gene carriers. This concept relies on the high acoustic pressure ultrasound-induced microbubble destruction to release the carrier drug or plasmid deoxyribonucleic acid at the targeted site.[31]

CONCLUSION

CH-EUS allows detailed parenchymal and microvascular evaluation of focal solid pancreatic lesions. It overcomes the limitation of artifacts associated with contrast-enhancement with Doppler EUS. The presence of hyperenhancement has a strong predictive value of non-adenocarcinoma diagnosis. Objective methods to quantify perfusion analyses may improve its diagnostic accuracy in the differential diagnosis of pancreatic lesions.

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