Review Article

Dynamic contrast-enhanced endoscopic ultrasound: A quantification method

Christoph F. Dietrich, Yi Dong, Eckhart Froehlich[1], Michael Hocke[2]
Department of Internal Medicine 2, Caritas-Krankenhaus Bad Mergentheim, Academic Teaching Hospital of the University of Würzburg, [1]Department of Internal Medicine 1, University of Tuebingen, [2]Department of Internal Medicine 2, Helios Hospital Meiningen GmbH, Academic Teaching Hospital of the University of Jena, Germany

ABSTRACT

Dynamic contrast-enhanced ultrasound (DCE-US) has been recently standardized by guidelines and recommendations. The European Federation of Societies for US in Medicine and Biology position paper describes the use for DCE-US. Comparatively, little is known about the use of contrast-enhanced endoscopic US (CE-EUS). This current paper reviews and discusses the clinical use of CE-EUS and DCE-US. The most important clinical use of DCE-US is the prediction of tumor response to new drugs against vascular angioneogenesis.

Key words: Guidelines, perfusion quantification, therapy monitoring, ultrasound contrast agents

INTRODUCTION

Dynamic contrast-enhanced ultrasound (DCE-US) has been recently standardized by guidelines and recommendations. The European Federation of Societies for US in Medicine and Biology (EFSUMB) position paper describes the use for DCE-US.[1] So far, the technique has been used by the transcutaneous and endoscopic approach. In this publication, the different measurements used for quantification in DCE-US are presented and defined to support the future work in this research field and to facilitate the standardization and recommendations for using the DCE-US technique.[1,2] Updated guidelines for contrast-enhanced US (CEUS) added nonliver applications[3-5] including comments and illustrations.[6-8] EFSUMB guidelines have been published for other related topics as well.[9-13]

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CONTRAST-ENHANCED ENDOSCOPIC ULTRASOUND

Endoscopic US (EUS) has gained importance, and detailed knowledge has been published.[14-17] Comparatively, little is known about the use of contrast-enhanced EUS (CE-EUS).[18-22] The first pilot study performed in 2003 described an experimental technique of CE-EUS based on a linear prototype EUS

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Address for correspondence
Dr. Christoph F. Dietrich, Chefarzt der Med. Klinik 2, Caritas Krankenhaus, Uhlandstr. 7, D-97980 Bad Mergentheim, Germany.
E-mail: christoph.dietrich@ckbm.de
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scope, using a low mechanical index (MI) (0.09–0.25) and a second-generation contrast agent (SonoVue®), which allowed the visualization of early arterial phase and late parenchymal phase enhancement of the pancreas.[20,21] Another pilot study demonstrated both real-time continuous images of finely branching vessels of the pancreas and intermittent homogenous parenchymal perfusion images by using a radial prototype EUS scope, a MI of 0.4, and the same second-generation contrast agent (SonoVue®).[23,24]

This current paper reviews and discusses the clinical use of CE-EUS and DCE-US.[4,5,20,21,24,25]

DYNAMIC CONTRAST-ENHANCED ULTRASOUND

DCE-US is an imaging technique utilizing microbubble contrast agents combined with the accurate quantification of tissue perfusion over time including parametric imaging. Several quantities such as the slope of wash-in or area under the curve (AUC) represent blood flow or blood volume. This means that changes in vascularization can be measured and detected after 1 or 2 weeks of treatment. Software for the application of this technique is commercially available and is already built into several commercial US scanners [Figure 1].[2]

DCE-US is a relatively novel method for the noninvasive quantification of the systemic circulation of different sonographically accessible parenchymatous organs and the gastrointestinal wall.[4,27,28] Reduction in metastatic gastrointestinal stromal tumor (GIST) vascularization after treatment with imatinib was evaluated using DCE-US and computed tomography (CT) scanning with the result of significant advantages for DCE-US due to earlier tumor response evaluation.[29] The CT technique is a volumetric or linear measurement method that does not assess the antivascular effects of the kinase inhibitors, so it is expected to reveal a response later than any DCE technique.[2] One distinct advantage of DCE-EUS as compared with DCE CT or magnetic resonance imaging (MRI) is that second-generation US contrast agents (UCAs) are blood-pool contrast agents and they do not leak into the interstitial space.[24,30,31]

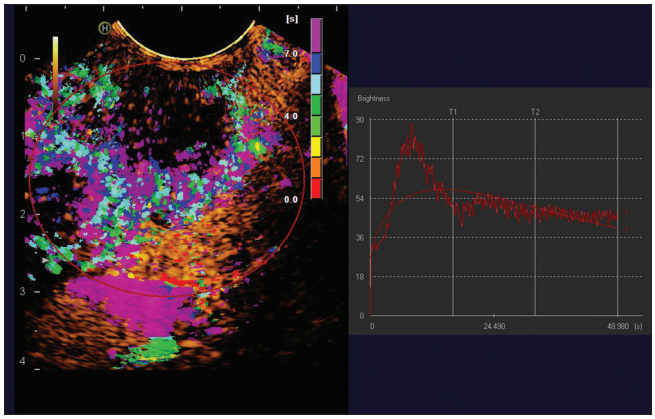

Figure 1. Parametric imaging (left side) and time-intensity curve analysis (right side) using endoscopic ultrasound in a partially cystic tumor of the pancreas.[1,26] Note that time-intensity curve analysis can be displayed by wash-in and wash-out curves, but also as parametric ultrasound by different colors

The different techniques and measurements used for the quantification of DCE-US have been presented and explained in detail in the already mentioned EFSUMB paper.[1] According to these EFSUMB guidelines, DCE-US should ideally be reproducible irrespective of the US equipment, data acquisition, and analysis software used.[2]

Perfusion imaging usually starts with a bolus injection of a second-generation contrast agent, followed by analysis in the early (wash-in) and late (wash-out) phases. Through different postprocessing options, time-intensity curves (TICs) can be reconstructed based on the imaging loops recorded in the US system. TIC analysis can be performed in several regions visualized by US, for example, inside a tumor and/or inside the normal organ parenchyma. The lesions can be defined as hypo-, iso-, or hyper-enhanced, while a certain number of quantitative parameters can be easily defined: Rise time (RT) and mean transit time (MTT), peak intensity and AUC, etc., being correlated with the microvascular blood flow [Table 1].[24]

CE-EUS has also the advantage of allowing liver examination in the late phase of contrast enhancement (after 120 s). Thus, contrast-enhanced examinations can be used for direct guidance of the needle during biopsy procedures, if the lesions are difficult to be visualized during conventional gray-scale US.[9-13,24,30,32]

Time intensity curve reproducibility

The reproducibility of DCE-US in 31 patients was investigated and discussed,[26] showing sources of error and unconvincing results. The following parameters were investigated: AUC, maximum intensity (IMAX), MTT, perfusion index (PI), time to peak (TTP), and RT from 10% to 90% of IMAX (RT) [Table 1].[1,26]

The influence of depth (different tumor positions) and lateral positioning (analysis of a lesion compared to representative liver parenchyma) is of importance to verify the reliability of the method.[26] The parameters describing the inflow curve at the beginning of the enhancement are more stable against positioning of the region of interest (ROI) whereas parameters which describe a longer period of the contrast dynamics are more affected by subtle changes.[26]

TICs should not be analyzed in a depth of more than 4 cm and AUC, PI, and IMAX should not be analyzed in a depth more than 6 cm. The depth penetration for EUS is much less, and data should not be analyzed in a depth more than 3 cm. When comparing more than one ROI, for example, in a tumor *versus* representative parenchyma, they must be compared in the same depth [Figure 2].

Size and shape of a ROI in liver parenchyma do not affect TICs.[26]

Replenishment kinetics that occurs after the destruction of the UCA was first examined by Krix.[36] He found that the median arterial perfusion in the examined liver metastases was more than two and a half times higher than in normal liver tissue, whereas the median perfusion during the portal venous phase was more than five times higher in the liver tissue than in metastases.[2]

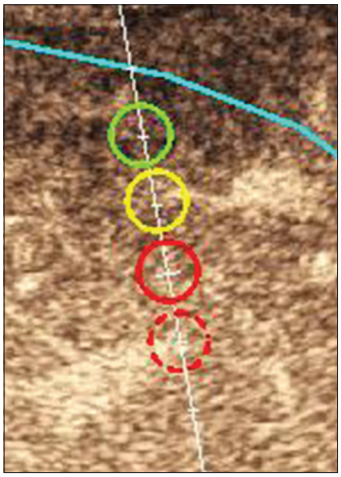

Figure 2. Stability of parameters according to the depth penetration. It could be shown that most parameters show acceptable results between 1.5 and 3.5 cm using endoscopic ultrasound (data not yet published).[1,26] In the near field, bubble destruction and also other artifacts have to be encountered.[33-35]

Dynamic contrast-enhanced endoscopic ultrasound using dynamic vascular pattern

Dynamic vascular pattern (DVP) is a function of quantification software designed for the evaluation of tissue perfusion obtained with real-time CEUS examination, which is true for the transcutaneous and also EUS approach. It combines perfusion quantification tools with perfusion imaging of focal lesions or blood vessels. Variations in tumor vascularity can be represented in sequence processing by color designations. Artifacts can be avoided. DVP software displays a four-quadrant representation of examination results [Figure 3].[37]

Quadrant 1 displays the original clip and quadrant 2 displays the processed DVP sequence. "Warm" colors (yellow to red) within the lesion's ROI

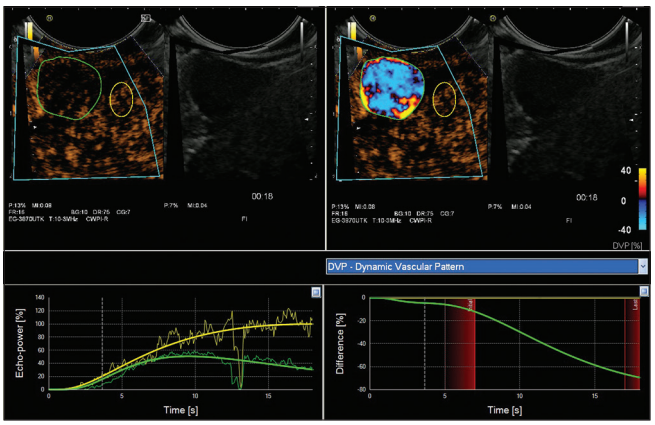

Figure 3. Dynamic vascular pattern. Quadrant 1 displays the original clip and quadrant 2 displays the processed dynamic vascular pattern sequence. "Warm" colors (yellow to red) within the lesion's region of interest indicate hyperenhancement when compared with the surrounding tissue (reference region of interest). "Cold" colors (blue shades) indicate hypoenhancement.[37]

Table 1. Parameters calculated from the time intensity curve and their explanation

Parameter	Abbreviation	Explanation
Area under the curve	AUC	Calculated integral for the time intensity curve
Maximum intensity value	IMAX	Highest value of the curve
Mean transit time	MTT	Time from the rising of the intensity up to decrease to 50% of maximum intensity
Perfusion index	PI	Area under the curve divided by mean transit time
Rise time	RT	Time from 10% to 90% of maximum intensity
Time to peak	TTP	Time from time point zero to maximum intensity

Regarding explanatory figures, we refer to the European Federation of Societies for Ultrasound in Medicine and Biology guidelines on dynamic contrast-enhanced ultrasound

indicate hyperenhancement when compared with the surrounding liver parenchyma (reference ROI). "Cold" colors (blue shades) indicate hypoenhancement. A TIC of the lesion and healthy liver parenchyma is displayed in quadrant 3, and the DVP-processed signals in quadrant 4 are shown as the difference between echo-power signals from the lesion compared with the reference area.[37]

DVP is able to analyze the regional differences in hemodynamics within the lesion because the displayed image's brightness correlates with the intensity of contrast enhancement in each region; areas within the tumor where enhancement is more intense that are brighter with DVP. Subsequently, one or more ROIs can be drawn inside the lesion, and their TIC curve, as well as other quantification parameters vital to characterization, for example, IMAX, TTP, RT, and MTT can be obtained. Thus, DVP allows the comparison of intralesional perfusion patterns, for example, between the center and the periphery, which can aid in the confirmation of either characteristic or atypical lesional perfusion patterns.[37]

The improved characterization of focal liver lesion using DVP software with its unique display of DVPs in all phases and the ability to discriminate between regions of differential hemodynamic patterns inside the lesion simplifies the diagnosis process/procedure and amplifies diagnostic accuracy, thus benefiting many researchers and medical workers.[37]

DVP affords the following features to the clinicians:[37]

1. Increased accuracy in the characterization of suspicious lesions visualized during a contrast EUS examination after a bolus injection of contrast medium
2. Better differentiation between benign and malignant lesions
3. A method of training clinicians who are less experienced in CEUS and to establish an integrated measuring system for correct diagnosis.

CLINICAL APPLICATIONS

The role of DCE-US in the liver has been described in detail.[30,32,38-40] AUC and Area under Wash Out are the most reliable TIC measurements for assessing the perfusion of the liver and kidneys.[26,41] The potential of DCE-EUS as an instrument to differentiate benign from malignant nonliver neoplasia is less promising since the nonliver organs do not display two different vascularities and renal cell carcinoma that are not in the scope of EUS. The literature was recently summarized in patients with pancreatic disease, lymph nodes, and other organs, which is important to know also for EUS.[2,5] The data are summarized below.

Pancreas

After the initial description of the CE-EUS technique,[20,21,23,24] several other groups reported the use of second-generation contrast agents with low MI techniques thereafter.[42-44] Quantitative analysis based on histograms and index of the contrast uptake ratio was employed to differentiate focal pancreatic masses and yielded an accuracy of 86%. Most of the cases with pancreatic adenocarcinoma were hypovascular on contrast-enhanced harmonic EUS (CEH-EUS) as compared to the surrounding parenchyma,[44] thus supporting the previous reports.[24,25,45]

Thirty patients with suspected pancreatic solid lesions were studied prospectively by DCE-US using SonoVue® by Seicean *et al.*[44] DCE-US was performed using SonoVue® and a low MI (0.3–0.4), followed by EUS-guided fine-needle aspiration (FNA). The quantitative analysis was based on histograms obtained from each video recording. DCE-US showed a hypoenhanced pattern in 14 cases of adenocarcinoma and in 10 cases of chronic pancreatitis (CP). The index of the contrast uptake ratio was significantly lower in adenocarcinoma than in mass-forming CP. A cutoff uptake ratio index value of 0.17 for diagnosing adenocarcinoma corresponded to an AUC of 0.86 with a sensitivity of 80%, a specificity of 91.7%, a positive predictive value of 92.8%, and a negative predictive value of 78%. The size of the pancreatic mass was assessed effectively by DCE-US.[2]

Differentiation between an inflammatory focal lesion of the pancreas and a pancreatic carcinoma using DCE-US in sixty patients was studied by Kersting *et al.*[46] TICs were obtained for all examinations in two ROIs; one within the lesion and the other within the normal pancreatic tissue. The following measurements were obtained: IMAX, arrival time (AT), time-to-peak intensity (TPI), and AUC. Absolute values and differences between the lesion and the normal tissue were evaluated. Histology analysis revealed 45 pancreatic ductal adenocarcinomas (PDACs) and 15 inflammatory masses in patients with CP. Although markedly lower than in a healthy pancreas, the IMAX and AUC data were not significantly different between PDACs and

focal lesions in patients with CP. They compared the enhancement in the lesions to the representative parenchyma and found significantly longer values for AT and TPI in adenocarcinoma than in inflammatory masses. There were no significant differences for IMAX and AUC. They concluded that cases of CP, PDAC, and focal masses have different perfusion patterns at a capillary level and that DCE-US offers a new instrument to facilitate the differential diagnosis of focal lesions in pancreatic cancer and CP.[2]

Lymph nodes

TIC analysis is a promising tool for lymph node characterization, with two studies identified that examined the enhancement in malignant infiltrated lymph nodes. Ouyang *et al.*[47] examined whether DCE-US is able to discriminate between metastatic and nonmetastatic lymph nodes in 51 patients with breast carcinoma. They correlated the CEUS-characteristics of metastatic lymph nodes with the tumor aggressiveness. Lymph nodes with metastasis were characterized by centripetal progress and a heterogeneous pattern, and no, or scarce, perfusion. Lymph nodes with nonmetastasis were characterized by centrifugal enhancement and a homogeneous pattern. The difference between the hyperintense and hypointense regions was higher in metastatic lymph nodes than nonmetastatic ones. A histopathologic diagnosis was predicted with sensitivity, specificity, and accuracy of 92.6%, 76.0%, and 84.6%, respectively. They concluded that noninvasive CEUS can discriminate metastatic from nonmetastatic lymph nodes, and it is able to predict the aggressiveness in patients with breast cancer. There was no difference in AT, TPI, or PI. The study relies on the subjective detection of hypoperfused areas, and the addition of TIC analysis does not lead to further information.[2]

TIC analysis of general lymph node contrast uptake was able to discriminate benign from malignant lymph nodes and lymphomas as studied by Yu *et al.*[48] They investigated 94 superficially enlarged lymph nodes in 94 patients with CEUS using SonoVue®. Of the 94 enlarged lymph nodes, 50 (53%) were malignant (location: 63% neck, 25% axilla, 13% inguinal region) and 44 (47%) were benign. Of the 50 malignant nodes, 33 (66%) were metastatic and 17 (33%) were lymphomas. In addition to the conventional characteristics, the authors found a significantly higher AUC as well as TPI for the benign lymph nodes in comparison to both the metastatic lymph nodes and the lymphomas.[2] Additional information will be given in a separate paper in this special issue of EUS.

Adrenal masses

DCE-US was used in the diagnostic work-up of adrenal mass to differentiate benign from malignant tumors by Friedrich-Rust *et al.*[49] They evaluated 116 adrenal masses using the contrast agent SonoVue®. The dynamic contrast enhancement was analyzed using TICs. In addition, all patients received CT/MRI and hormonal testing. In suspicious cases, a biopsy or adrenalectomy was performed. The sensitivity and specificity of DCE-US for the diagnosis of malignant adrenal mass were 100% and 67%, respectively. Overall, histology was available as a reference method for forty adrenal masses. In 68% of the histologically diagnosed adrenal masses, CT/MRI and DCE-US were congruent concerning the characterization of malignant *versus* benign adrenal mass. They concluded that DCE-US is a useful method in the diagnostic work-up of adrenal mass with excellent sensitivity for the diagnosis of malignancy.[2] According to our own results, the differentiation of benign and malignant neoplasia of the adrenal glands is much less promising.[50,51]

Fluid collections

DCE-US was used to visualize the complications of drained fluid collections in the abdomen by applying UCA via drainage catheters.[52,53] Complications such as fistulae to the intestine or to the peritoneal cavity are precisely displayed. Orally applied, the contrast agent was longtime visible, and thus, insufficient anastomoses or spontaneous perforations unto the colon became detectable. Furthermore, applying the agents in the biliary tract via endoscopic retrograde cholangiopancreatography, the biliary system is shown in a detailed description.[54,55]

Additional information

DCE-EUS was first reported by Kato *et al.*, who used fundamental EUS with carbon dioxide gas.[56] Compared to other imaging modalities, EUS has limitations in terms of image enhancement. With the development of second-generation UCAs, EUS has been evolved. Contrast-enhanced Doppler EUS (CD-EUS) can be used to characterize lesions detected by EUS with enhancement of Doppler signals from vessels. This was particularly useful for avoiding vessels during EUS-guided FNA. However, CD-EUS suffers from artifacts such as blooming and flash.[57,58]

Compared to CD-EUS imaging, which depicts vessel flow, DCE-EUS depicts the microbubble perfusions. DCE-EUS is useful for characterizing pancreatic adenocarcinomas as hypoenhancement lesions, with a sensitivity of 94% and a specificity of 89%.[5,59] Particularly, DCE-EUS was significantly more accurate than CT in diagnosing small ductal carcinomas of ≤2 cm.[60] A recent prospective multicenter trial indicated that in diagnosing pancreatic adenocarcinoma, DCE-EUS and EUS-FNA had respective accuracy, sensitivity, specificity, positive predictive value, and negative predictive value of 95%, 96%, 94%, 97%, and 91%, and of 95%, 93%, 100%, 100%, and 86%, without significant difference. The performance of DCE-EUS for the diagnosis of pancreatic adenocarcinoma was excellent. The good intra- and inter-observer concordances suggest an excellent reproducibility. DCE-EUS could help to guide the choice between surgery and follow-up when EUS-FNA is inconclusive.[61]

DCE-US with a broadband transducer allows the display of parenchymal perfusion and quantitative analysis of microvascular with TIC curve.[45,62,63] Recent articles on DCE-EUS with TIC quantitative analyses revealed that some quantitative indexes, such as IMAX, accumulated intensity during observation, intensity reduction rate, the ratio between the uptake inside the mass, and the uptake of the surrounding parenchyma, are useful for the discrimination of pancreatic carcinomas from autoimmune pancreatitis, pseudo tumors, and neuroendocrine tumors.[44,63-65] Although the most reliable variable of TIC should be identified by further large multicenter studies, this quantitative perfusion analysis may be complementary for classification according to enhancement patterns to characterize pancreatic solid lesions.[66] In subepithelial tumors, the DCE-EUS with TIC revealed that echo intensity in GISTs was significantly higher than that in benign tumors such as lipomas.[67]

During the guidance of EUS-FNA, DCE-EUS can be used to identify the target of FNA or to identify a specific site which would be more suitable for FNA.[60] In addition, it was helpful for identification and for biopsy avoidance of internal avascular necrotic areas.[68]

Accurate preoperative T staging, particularly regarding vascular involvement, is crucial in guiding the appropriate management for pancreatobiliary malignancies.[58] Compared with EUS, DCE-EUS was significantly better than EUS alone for preoperative T staging, with an overall accuracy of 92% and 69%, respectively.[69] In addition, DCE-EUS is useful for N-staging of pancreatobiliary tumors, with a sensitivity of 96.3% and a specificity of 100%.[70]

During the EUS-guided local ablation for pancreatic tumor, the assessment of vascularization and altered perfusion is crucial to differentiate necrotic area from residual viable tumor. However, conventional EUS does not provide any reliable information about the efficacy of ablative treatment. DCE-EUS can provide valuable information both of pre- and post-treatment assessment of tumor vascularity. The typical imaging that indicates complete ablation is the disappearance of any previously visualized internal enhancement on DCE-EUS. The size of the post-treatment nonenhanced volume of necrosis area could be compared with that of the pretreatment volume. DCE-EUS facilitates post-treatment follow-up by improving the visualization of pancreatic perfusion.[71,72]

Only few articles had been published that describe the characterization of gallbladder diseases with DCE-EUS. Imazu *et al.* compared conventional EUS and DCE-EUS for the differentiation of gallbladder wall thickening. They concluded that the inhomogeneous enhanced pattern in DCE-EUS was a strong predictive factor of malignant gallbladder wall thickening. The overall accuracy of DCE-EUS (94.4%) was significantly higher than conventional EUS (73.1%).[73] In differentiation between benign and malignant polyps of the gallbladder, an irregular vessel pattern on DCE-EUS had a sensitivity of 90.3% and a specificity of 96.6% in the diagnosis of malignant polyps.[74]

Functional assessment of tumor response

Due to recent advances in angiogenesis and its use for therapeutic indications, for example, tyrosine kinase inhibitory therapy in GISTs, it is obvious that for these purposes, new imaging modalities are needed. The commonly used Response Evaluation Criteria in Solid Tumors (RECIST) criteria based on the diameter of lesions do not fulfill the requirements for functional assessment of tumor response to the targeted therapies mentioned. The use of wash-in and wash-out curves of contrast agents is proposed as a more functional and distinct analysis of this issue. Hereby, the change of brightness over time after injection of a contrast agent monitors the inflow and outflow of a contrast agent representing the vitality of a tumor. Hence, in a

second step, the changes of the vascularization can be interpreted as a response to a certain therapy.[26]

Despite studies published in the literature up to now, there is no consensus concerning the following.[26]

- The validity of the parameters used
- The different principles transforming the raw US signals into the resulting curve to be analyzed (raw data – smoothing)
- The comparability of different software sources used. Mainly, the latter are offered by the manufacturer of the US machine only.

"Linear data" are uncompressed US data that are used to produce TICs, which are then analyzed for amplitude and temporal components. Linear data processing seems to be the only mathematically valid method for determining tumor response to antivascular drugs analyzing TIC.[2,75]

A correlation was shown between intratumor microvessel density, different angiogenic factors (including vascular endothelial growth factor), and the microvascularity of tumors, thus paving a way for early assessment and monitoring of the efficacy of anti-angiogenic agents based on tumor perfusion quantification, before morphological changes become apparent. The increased resolution of CEH-EUS will potentially improve quantification and monitoring of the results of chemotherapy and anti-angiogenic treatments in unresectable patients with digestive cancers, which are difficult to be examined by conventional cross-sectional imaging techniques (US, CT, or MR). The literature has been recently summarized.

SUMMARY AND CONCLUSIONS

This review concludes that DCE-US is, at the moment, the most sensitive cross-sectional real-time method for measuring the perfusion of organs noninvasively.[2]

- The most important routine clinical role of DCE-US is the early prediction of tumor response to treatment within a very short time, earlier than with RECIST criteria
- Tumor differentiation between benign and malignant is possible in some parenchymatous organs as demonstrated in both animals and humans. Future studies are needed
- One current limitation of DCE-US was found to be the lack of volume data that are available for both DCE-CT and DCE-MR.

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Conflicts of interest
There are no conflicts of interest.

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