Review Article

Role of contrast-enhanced endoscopic ultrasound in lymph nodes

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ABSTRACT

Diagnosing unclear lymph node (LN) enlargements in the mediastinum and abdomen is the most important indication of endoscopic ultrasound (EUS)-fine needle aspiration (FNA) after the diagnosis and treatment of pancreatic diseases. Investigating LNs in these areas can happen in different clinical settings. Mostly, it is the first modality in general LN diseases without any peripheral LN enlargements. On the other hand, it can be the question of LN involvement in a known or suspected primary tumor. Due to EUS-FNA cytology, those questions can be answered highly, accurately. However, a primary discrimination of LNs might be helpful to increase the diagnostic value of the FNA cytology, especially in cases with multiple LN enlargements and hard to reach enlarged LNs for example by vessel interposition. Because of the unreliability of B-mode criteria, further diagnostic improvements such as elastography and contrast-enhanced EUS are investigated to increase the accuracy of the initial diagnosis.

Key words: Benign and malignant, contrast-enhanced endoscopic ultrasound, discrimination, elastography, lymph node

INTRODUCTION

Endoscopic ultrasound (EUS) is currently the modality with the highest innovative potential in gastroenterology.[1-3] Massive progress has been made over the last few years in the diagnostic as well as in the therapeutic abilities of the method. Even the indication for performing EUS has changed over the years. It was initially used mainly for the diagnostic of luminal changes of the upper gastrointestinal tract such as cancer staging of esophageal and gastric cancer[4] as well as submucosal tumors,[5,6] it has been used more and more for diagnosing and treating of extraluminal processes such as the pancreas, bile duct, or lymph nodes (LNs). The use of ultrasound (US) and EUS-guided interventions has been recently published in the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) guidelines.[1,2,7-14] We also refer to the EFSUMB guidelines on the use of contrast-enhanced ultrasound (CEUS) in other organs.[15-18]

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According to a German survey, the most important EUS targets for EUS-fine needle aspiration (FNA) cytology are unclear LN enlargements of the mediastinum[19-21] and abdomen closely behind pancreatic diseases.

EUS-FNA cytology has a high accuracy in the diagnosis of enlarged LNs.[22,23] However, in cases were the LNs cannot be reached by the needle, in patients with contraindications against fine needle puncture and in patients with multiple LN enlargement, a noninvasive diagnostic procedure for initial estimation of the nature of the enlargement would be preferable.

The differentiation of malignant from benign LNs by the US, computed tomography, and magnetic resonance imaging traditionally relies mainly on size measurements and topographic distribution.[24] However, sensitivity and specificity in the differentiation of benign and malignant LNs are disappointing using only size parameters. Reasons for the low accuracy includes that malignant infiltration occurs in up to 30% in LNs of <5 mm which has been shown for lung, esophageal, gastric, pancreatic, and rectal carcinoma.[24] Studies from high-resolution percutaneous US tried to find reliable morphological B-mode and color Doppler criteria for the discrimination of benign and malignant LNs.[25-27] Unfortunately, those criteria have not been very successful and could only be used in clearly defined clinical settings.[28-31]

Two major new techniques have targeted the same question. Elastography is able to differentiate between different stiffness values of the tissue. Under the impression that cancerous tissue is harder than benign LN tissue multiple studies investigated the impact of this interesting method.[32-37]

The second important new technique is the contrast-enhanced harmonic-EUS (CH-EUS).[15,24,38-41] After the emergence of the new contrast harmonic imaging techniques for liver diseases, it was inevitable to think about new applications.[33,42,43] One of those has been the contrast enhancing the behavior of enlarged LNs.[44,45] The major problem, in the beginning, was the incompatibility of the high-resolution US with contrast harmonic techniques. Neither the contrast enhancer bubbles nor the software for high-frequency US probes was capable of producing reliable pictures. One of the main disadvantages is the presence of artifacts, including flash and blooming.

Whereas the problem was targeted relatively, quickly in the percutaneous technique, systems for EUS are only available since 2005.[39] In the following years, this method gained importance.[40] This was the reason some contrast-enhanced EUS (CE-EUS) studies, with the aim of discriminating malignant from benign LNs, have been performed using contrast-enhanced Doppler techniques and not contrast harmonic imagine methods.[27,32,34,46-48]

PRINCIPLES OF CONTRAST-ENHANCED TECHNIQUES IN ENDOSCOPIC ULTRASOUND

Contrast enhancers can be used even without contrast harmonic imaging as color Doppler enhancers. The bubbles are able to increase the color Doppler signal of vessels with a diameter of approximately 0.1–0.4 mm. Those vessels are normally called arterioles and venoules. No signal can be detected from the capillary bed. The effect of the commonly used US contrast enhancer SonoVue® (Bracco, Milano, Italy) can be visualized for approximately 3–4 min.

Using contrast harmonic techniques, the result of the investigation is quite different. With the technique, the resolution of the method is so good that a single bubble can be identified as a bright spot. Taken into account that a bubble is roughly the size of an erythrocyte; it is easy to understand that the enhancing effect shows the capillary bed of the LN. The bubbles are not able to leave the capillary bed that means no parenchymal enhancement is possible using Sonovue®.

DOPPLER ENHANCING USE OF ULTRASOUND CONTRAST ENHANCERS IN ENDOSCOPIC ULTRASOUND

Color Doppler ultrasound (color Doppler imaging) adds value for the differentiation of malignant from normal or reactive nodes by displaying the macrovessel architecture. Normal LNs generally show hilar predominant normal vascularity. Inflammatory LNs are typically more vascularized without changes of the predominant hilar vessel architecture. In contrast metastatic LNs present peripheral or mixed vascularity and loss of the hilar type of vascularization.[24]

A few studies have been made using that technique for discriminating benign from malignant LNs.[49,50] The aim of the studies has been to find differences in the vascular structure of benign to malignant LNs.[51] The assumption was that a difference in the neovascularization of malignant to benign LNs exists in the level of arterioles and venoules.[32,34,52] Demonstration of malignant neovascularization, for example, vessels penetrating the LN capsule has been used as the characteristic feature of LN metastases.[24]

To read those studies in the right light, it has to be taken into account that there is a morphological difference in between malignant LNs involved in a lymphoma and malignant LNs involved into a solid carcinoma. The vessel structure of lymphomas does not differ very much from benign LNs due to the fact that the LN itself stays intact in its original structure. Further on the pathophysiology of malignant LNs regarding solid tumor involvement should be borne in mind. It is rarely the case that the whole LN is involved in the process. The LN rather serves as a metastatic organ. In pathohistology, a LN counts as malignant if a small area of malignant cells is detectable. This area can be as small as a few cells, which is out of any resolution abilities of the modern diagnostic methods. These areas can definitely not be visualized using Doppler enhancing CE-EUS. Possible differences in the neovascularization of the arterioles and venoules can only be detected if the whole LN is involved in the process. Theoretically, the principle of neovascularization of solid tumors such as adenocarcinoma of the pancreas should stay the same in the metastasis of the LNs. However, the size of the metastasis could be the major limitation in the detection of those principles. It could be demonstrated in patients with pancreatic carcinoma that certain criteria can describe the typical neovascularization of adenocarcinoma, which are:

- diminished vessel system with irregular vessels
- only arterial vessels with high-resistant index visible[53]
- no venous vessels visible because of the invasive behavior of the cancer cells and the higher tissue pressure (venous vessels are present but not detectable using this method).[53-55]

In one study those criteria have been used; however, it could only improve the specificity of the method, but not the sensitivity.[56]

CONTRAST HARMONIC IMAGING IN ENDOSCOPIC ULTRASOUND

More studies dealt with contrast harmonic imaging for discrimination of malignant to benign LNs.[24,34,57,58] Contrast harmonic imaging is combining a method with a very high-resolution, with the display of contrast enhancing the behavior of the capillary bed of the LN.[59] Assuming that the capillary bed of the solid malignant metastasis is destroyed, the estimated behavior should be of a less enhancing effect within the whole LNs or within certain areas of the LN.[60,61] In the performed studies, this effect has been described and can even been seen in a meta-analysis study.[62] However, the same pathophysiological problems mentioned above have to be taken into account. Lymphoma LNs are known to have a good vascularization in the capillary bed and cannot be identified using the method. The second problem regards the zones of necrosis within benign LNs. These zones can be easily misinterpreted into metastatic involvement of the LN and this makes the discrimination even harder (e.g., LN tuberculosis).[63-65] The third problem is that even in this method with a very high-resolution, cancer cell nests within the LN can be easily overlooked simply because of the size of the nests. This is the reason that the EFSUMB guidelines do not recommend the CH-EUS for discrimination of malignant to benign LNs.[15,41] A recommendation could be considered for using the method for tumor cell nest targeting in EUS-FNA cytology and in special clinical settings.[1,2,5,56,66]

Carcinoma infiltration causes the development of pathological vessels (neoangiogenesis) and therefore, a change of the perfusion pattern with heterogeneous enhancement due to the presence of caliber changes of the neoplastic vessels and arteriovenous shunts. Focal hypoenhancement may result from the partial insufficiency of blood supply due to overpressure in the LN caused by the neoplastic infiltration. Malignant LNs not only have a greater number of peripheral vessels but also longer contrast enhancement duration than benign LNs. Destructive avascular necroses are an important imaging sign for malignant infiltration. Criteria for carcinomatous LN infiltration on CEUS are centripetal inhomogeneous enhancement and perfusion defects.[24]

The very few studies published so far showed that in lymphoma contrast enhancement patterns are highly variable. The most often observed pattern is intense homogeneous enhancement, which is not different from reactive inflammatory LNs.[24]

Most inflammatory processes do not change the hilum predominant vessel architecture of LNs. According to the majority of published papers, normal and inflammatory LNs are characterized by a centrifugal and homogeneous enhancement pattern. Therefore, inflammation changes the enhancement pattern only by the amount (peak) enhancement but not by changes of distribution. It is worth mentioning that nondestructive necrosis, which is reflected in avascular areas on CEUS, can be also found in granulomatous lymphadenitis, for example, cat-scratch disease (bartonellosis), tuberculosis, and sarcoidosis.

OVERVIEW ABOUT THE LITERATURE

Currently, there are only three studies published. The first from Kanamori *et al.* the investigators used Sonozaid as contrast agent and performed a color Doppler study. They described a sensitivity from 100% and specificity of 81.8% for discrimination of malignant and benign LNs in 46 patients. The benign LNs have been characterized by homogenous Doppler enhancing the effect over the whole LN, whereas malignant LNs showed areas of no enhancing effect. The major inconsistency of the study however is that Kanamori *et al.* included ten lymphoma LNs into the malignant group which are known for an equal contrast enhancing effect such as benign LNs.[51]

The next study from Hocke *et al.* tried to confirm the Kanamori results, however, failed to do so. The Doppler enhancing effect, achieved with the contrast enhancer Sonovue, did not perform much better than the B-mode criteria in 122 patients. The study did not just use the Kanamori effect; it tried to install a new criterium as well. According to the promising Doppler enhancing results for discrimination of pancreatic carcinoma and chronic pancreatitis,[54] the study tried to discriminate LNs by the vessel pattern. Although the specificity of discriminating malignant from benign LNs improved to 91.9%, the sensitivity decreased to 60.4% and therefore could not be used for clinical practice.[67]

Recently, there was a study published by Xia *et al.* about CH-EUS for discriminating unclear abdominal lesions. In the study, 43 patients have been included. The discrimination was made by the contrast enhancing the effect of Sonozaid. The differential diagnosis was made by the discrimination of a homogenous contrast enhancer pattern in benign lesions and inhomogenous enhancer pattern in malignant lesions. Although the majority of lesions have been LNs, other lesions such as GIST tumors have been included as well. The authors describe a high sensitivity of 96.3% and specificity of 100%.[68] Due to pathophysiological reasons, such as mentioned in the section "contrast harmonic imaging" the results should be seen critically.

CONTRAST-ENHANCED ENDOSCOPIC ULTRASOUND FOR LYMPH NODES – CURRENT STATUS

In our personal experience, CE-EUS is not very helpful in clinical practice today. The possibility of performing EUS-FNA cytology with a high diagnostic accuracy makes the method unnecessary for daily routine investigations. Like Elastograophy, it could be used for better needle targeting such as to avoid necrotic areas in large LNs or chosing the most promising LN in a LN chain. However, the effect seems to be minor. If performed, the most interesting setting currently is the CH-EUS because of the discrimination of vital and avital areas within the LN. The currently best dosage seems to be a full dose of contrast enhancer (e.g., 4.8 mL of Sonovue) in the contrast harmonic mode. It has to be taken into account that due to probe pressure in the near areas of the LNs capillary bed, the probe near areas often show no contrast enhancing effect and cannot be voted as malignant or avital. After investigation in the CH-EUS mode, the CE-EUS mode can still be performed with enough Doppler enhancing effect to display the small LNs vessels and vessel discrimination. For CH-EUS, the installed program values should be used, although mostly the very low-mechanical index has to be slightly increased to get good results. For CE-EUS, the pulse repetition frequency should be as low as possible (e.g., 5 cm/s) and the color gain should be as high as possible just to avoid artifacts. If possible it is recommendable to lower the mechanical index of the B-mode and color mode to approximately 50% to avoid high bubble destruction.

Examples of benign and malignant lymph nodes, lymph nodes of a lymphoma and a patient with lymph node

tuberculosis are given in Figures 1-4. Please note the typical patterns of the different techniques. A short overview of the different patterns are also given in Table 1.

FUTURE PERSPECTIVES OF CONTRAST HARMONIC IMAGING IN ENDOSCOPIC ULTRASOUND

This recommendation might have to be revised when time progresses. New developments and discoveries will further improve the method.[58] Major advantage can be made using calculating software which can estimate the time and quantity of the contrast enhancer influx.[48,69] There might be unique contrast enhancing patterns which could be able to overcome the problem of special resolution or focal necrosis.[70-74]

In addition, contrast enhancers might be possible, which are loaded with specific antibodies on the surface, which could serve as a kind of red flag technique.[41] The development of targeting contrast

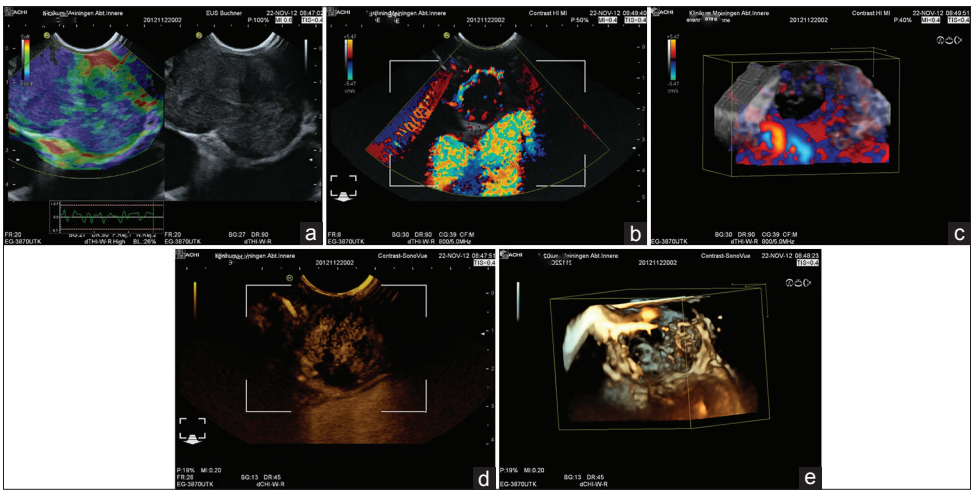

Figure 1. Typical malignant lymph node in the mediastinum of a patient with squamous cell carcinoma of the lung; (a) elastography shows stiff areas within the lymph node which are suspect of malignant infiltration; (b) high-mechanical index contrast-enhanced endosonography after injection of 4.8 mL Sonovue shows a diminished and destroyed vessel system without hilus vessels; (c) three-dimensional reconstruction of the same contrast-enhanced mode shows the diminished vessel system more pronounced; (d) low-mechanical index contrast-enhanced endosonography after injection of 4.8 mL Sonovue shows nonperfused lymphnode areas which are suspect of malignant infiltration; (e) three-dimensional reconstruction of the same contrast mode shows these areas more pronounced

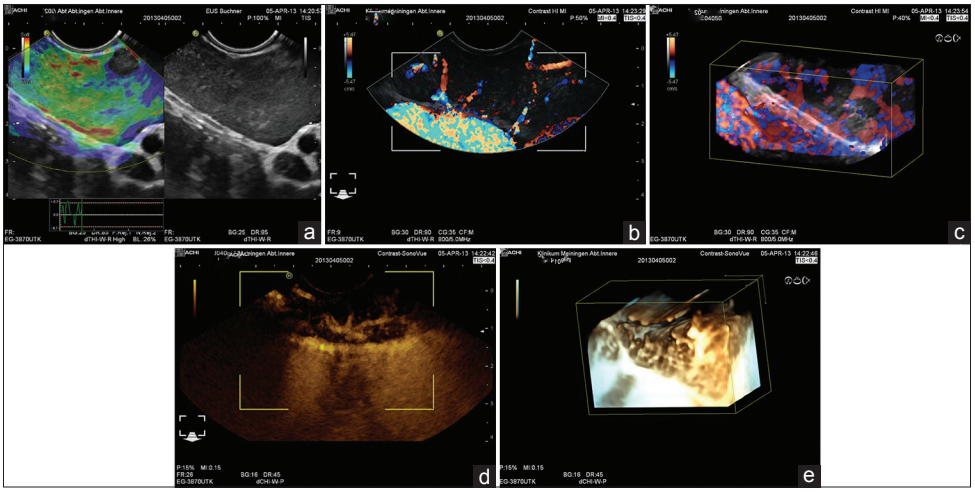

Figure 2. Typical benign lymph node in the mediastinum of a patient with sarcoidosis; (a) elastography shows a homogenous soft tissue of the lymph node; (b) high-mechanical index contrast-enhanced endosonography after injection of 4.8 mL Sonovue shows a regular vessel system; (c) three-dimensional reconstruction of the same contrast-enhanced mode shows the healthy vessel more system pronounced; (d) low-mechanical index contrast-enhanced endosonography after injection of 4.8 mL Sonovue shows homogenous lymph node perfusion; (e) three-dimensional reconstruction of the same contrast mode shows the perfusion more pronounced

Table 1: Typical contrast enhancement patterns of lymphnodes

	Contrast-enhanced-endoscopic ultrasound	Contrast harmonic-endoscopic ultrasonography
Benign LN	Hilus vessels, arterial, and venous vessels visible	Homogenous enhancement of contrast enhancer
Malignant LN	Rarefication of vessels, incoming vessels from the peripherie, only arterial vessels visible	Patchy or missing contrast enhancing effect
Lymphoma	Rich vessels system with venous and arterial vessels visible	Homogenous contrast enhancing effect
Tuberculosis	Arterial and venous vessels detectable but rarefication of the vessel system	Patchy or missing contrast enhancing effect

LN: Lymph node

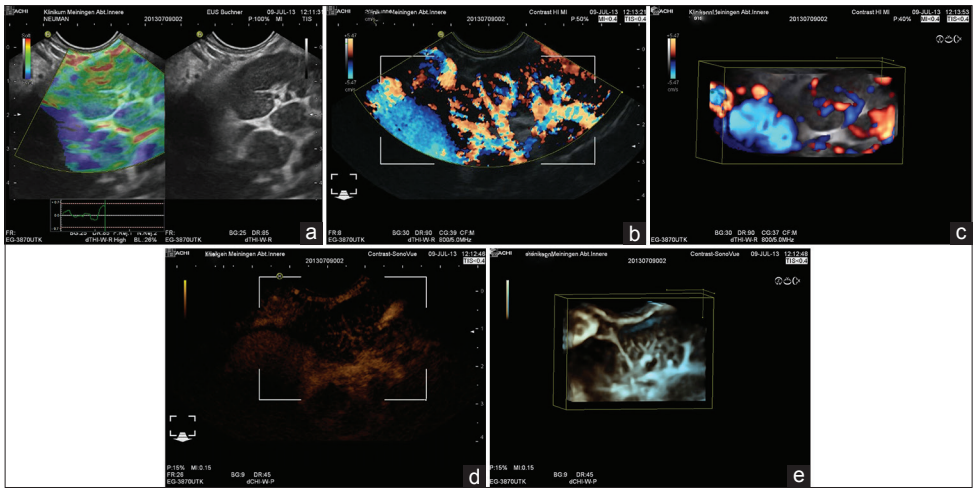

Figure 3. Typical lymph node in the mediastinum of a patient with non-Hodgkin lymphoma; (a) elastography shows a homogenous soft tissue of the lymph node; (b) high-mechanical index contrast-enhanced endosonography after injection of 4.8 mL Sonovue shows a regular vessel system; (c) three-dimensional reconstruction of the same contrast-enhanced mode shows the healthy vessel more system pronounced; (d) low-mechanical index contrast-enhanced endosonography after injection of 4.8 mL Sonovue shows homogenous lymph node perfusion; (e) three-dimensional reconstruction of the same contrast mode shows the perfusion more pronounced

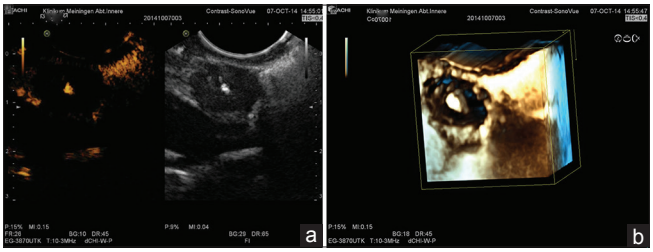

Figure 4. Typical lymph node in the mediastinum of a patient with tuberculosis; (a) low-mechanical index contrast-enhanced endosonography after injection of 4.8 mL Sonovue shows no lymph node perfusion but a central calcification; (b) three-dimensional reconstruction of the same contrast mode shows the lack of perfusion and the calcification more pronounced

enhancer bubbles would even overcome the problem of different contrast enhancing behavior of tumors different to squamous cell carcinoma or adenocarcinomas.

The use of CH-EUS in pediatric patients will be challenged as well.[75,76]

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Fusaroli P, Jenssen C, Hocke M, *et al.* EFSUMB guidelines on interventional ultrasound (INVUS), part V. *Ultraschall Med* 2015. Epub ahead of print.
2. Jenssen C, Hocke M, Fusaroli P, *et al.* EFSUMB guidelines on interventional ultrasound (INVUS), part IV – EUS-guided interventions: General aspects and EUS-guided sampling (Long Version). *Ultraschall Med* 2016;37:E33-76.
3. Jenssen C, Lauer NJ, Burmester E, *et al.* Art meets science - Thoughts about an overlap of art and sience in connection with information and fascination of endoscopic picture documetation, Endoskopie heute, 2013;26:2-12.
4. Moehler M, Al-Batran SE, Andus T, *et al.* German S3-guideline "Diagnosis and treatment of esophagogastric cancer". *Z Gastroenterol* 2011;49:461-531.
5. Dietrich CF, Hocke M, Jenssen C. Interventional endosonography. *Ultraschall Med* 2011;32:8-22.
6. Dietrich CF, Jenssen C. Evidence based endoscopic ultrasound. *Z Gastroenterol* 2011;49:599-621.
7. Sidhu PS, Brabrand K, Cantisani V, *et al.* EFSUMB guidelines on interventional ultrasound (INVUS), part II. *Ultraschall Med* 2015;36:E15-35.
8. Lorentzen T, Nolsøe CP, Ewertsen C, *et al.* EFSUMB guidelines on interventional ultrasound (INVUS), part I. General aspects (long Version). *Ultraschall Med* 2015;36:E1-14.
9. Lorentzen T, Nolsøe CP, Ewertsen C, *et al.* EFSUMB guidelines on interventional ultrasound (INVUS), part I. General aspects (Short Version).

Ultraschall Med 2015;36:464-72.
10. Jenssen C, Brkljacic B, Hocke M, *et al*. EFSUMB guidelines on interventional ultrasound (INVUS), part VI. *Ultraschall Med* 2015. [Epub ahead of print].
11. Jenssen C, Brkljacic B, Hocke M, *et al*. EFSUMB guidelines on interventional ultrasound (INVUS), part VI – Ultrasound-guided vascular interventions. *Ultraschall Med* 2015. [Epub ahead of print].
12. Dietrich CF, Lorentzen T, Sidhu PS, *et al*. An introduction to the EFSUMB guidelines on interventional ultrasound (INVUS). *Ultraschall Med* 2015;36:460-3.
13. Dietrich CF, Lorentzen T, Appelbaum L, *et al*. EFSUMB guidelines on interventional ultrasound (INVUS), part III – Abdominal treatment procedures (Long Version). *Ultraschall Med* 2016;37:E1-32.
14. Dietrich CF. EFSUMB guidelines 2015 on interventional ultrasound. *Med Ultrason* 2015;17:521-7.
15. Piscaglia F, Nolsøe C, Dietrich CF, *et al*. The EFSUMB guidelines and recommendations on the clinical practice of contrast enhanced ultrasound (CEUS): Update 2011 on non-hepatic applications. *Ultraschall Med* 2012;33:33-59.
16. Claudon M, Dietrich CF, Choi BI, *et al*. Guidelines and good clinical practice recommendations for contrast enhanced ultrasound (CEUS) in the liver – Update 2012: A WFUMB-EFSUMB initiative in cooperation with representatives of AFSUMB, AIUM, ASUM, FLAUS and ICUS. *Ultrasound Med Biol* 2013;39:187-210.
17. Claudon M, Dietrich CF, Choi BI, *et al*. Guidelines and good clinical practice recommendations for contrast enhanced ultrasound (CEUS) in the liver – Update 2012: A WFUMB-EFSUMB initiative in cooperation with representatives of AFSUMB, AIUM, ASUM, FLAUS and ICUS. *Ultraschall Med* 2013;34:11-29.
18. Dietrich CF, Rudd L. The EFSUMB website, a guide for better understanding. *Med Ultrason* 2013;15:215-23.
19. Jenssen C, Annema JT, Clementsen P, *et al*. Ultrasound techniques in the evaluation of the mediastinum, part 2: Mediastinal lymph node anatomy and diagnostic reach of ultrasound techniques, clinical work up of neoplastic and inflammatory mediastinal lymphadenopathy using ultrasound techniques and how to learn mediastinal endosonography. *J Thorac Dis* 2015;7:E439-58.
20. Dietrich CF, Annema JT, Clementsen P, *et al*. Ultrasound techniques in the evaluation of the mediastinum, part I: Endoscopic ultrasound (EUS), endobronchial ultrasound (EBUS) and transcutaneous mediastinal ultrasound (TMUS), introduction into ultrasound techniques. *J Thorac Dis* 2015;7:E311-25.
21. Dietrich CF, Mathis G, Cui XW, *et al*. Ultrasound of the pleurae and lungs. *Ultrasound Med Biol* 2015;41:351-65.
22. Cameron SE, Andrade RS, Pambuccian SE. Endobronchial ultrasound-guided transbronchial needle aspiration cytology: A state of the art review. *Cytopathology* 2010;21:6-26.
23. Zhang R, Ying K, Shi L, *et al*. Combined endobronchial and endoscopic ultrasound-guided fine needle aspiration for mediastinal lymph node staging of lung cancer: A meta-analysis. *Eur J Cancer* 2013;49:1860-7.
24. Cui XW, Jenssen C, Saftoiu A, *et al*. New ultrasound techniques for lymph node evaluation. *World J Gastroenterol* 2013;19:4850-60.
25. Jenssen C. Clinical value of lymph node sonography. *Praxis (Bern 1994)* 2009;98:581-8.
26. Dietrich CF, Hocke M, Jenssen C. Ultrasound for abdominal lymphadenopathy. *Dtsch Med Wochenschr* 2013;138:1001-18.
27. Cui XW, Hocke M, Jenssen C, *et al*. Conventional ultrasound for lymph node evaluation, update 2013. *Z Gastroenterol* 2014;52:212-21.
28. Moritz JD, Ludwig A, Oestmann JW. Contrast-enhanced color Doppler sonography for evaluation of enlarged cervical lymph nodes in head and neck tumors. *AJR Am J Roentgenol* 2000;174:1279-84.
29. Dietrich CF, Lee JH, Herrmann G, *et al*. Enlargement of perihepatic lymph nodes in relation to liver histology and viremia in patients with chronic hepatitis C. *Hepatology* 1997;26:467-72.
30. Hirche TO, Russler J, Braden B, *et al*. Sonographic detection of perihepatic lymphadenopathy is an indicator for primary sclerosing cholangitis in patients with inflammatory bowel disease. *Int J Colorectal Dis* 2004;19:586-94.
31. Dietrich CF, Viel K, Braden B, *et al*. Mediastinal lymphadenopathy: An extrahepatic manifestation of chronic hepatitis C? *Z Gastroenterol* 2000;38:143-52.
32. Chiorean L, Barr RG, Braden B, *et al*. Transcutaneous ultrasound: Elastographic lymph node evaluation. Current clinical applications and literature review. *Ultrasound Med Biol* 2016;42:16-30.
33. Guang Y, Xie L, Ding H, *et al*. Diagnosis value of focal liver lesions with SonoVue® – Enhanced ultrasound compared with contrast-enhanced computed tomography and contrast-enhanced MRI: A meta-analysis. *J Cancer Res Clin Oncol* 2011;137:1595-605.
34. Dietrich CF, Jenssen C, Arcidiacono PG, *et al*. Endoscopic ultrasound: Elastographic lymph node evaluation. *Endosc Ultrasound* 2015;4:176-90.
35. Cosgrove D, Piscaglia F, Bamber J, *et al*. EFSUMB guidelines and recommendations on the clinical use of ultrasound elastography. Part 2: Clinical applications. *Ultraschall Med* 2013;34:238-53.
36. Bamber J, Cosgrove D, Dietrich CF, *et al*. EFSUMB guidelines and recommendations on the clinical use of ultrasound elastography. Part 1: Basic principles and technology. *Ultraschall Med* 2013;34:169-84.
37. Shiina T, Nightingale KR, Palmeri ML, *et al*. WFUMB guidelines and recommendations for clinical use of ultrasound elastography: Part 1: Basic principles and terminology. *Ultrasound Med Biol* 2015;41:1126-47.
38. Ying M, Bhatia KS, Lee YP, *et al*. Review of ultrasonography of malignant neck nodes: Greyscale, Doppler, contrast enhancement and elastography. *Cancer Imaging* 2014;13:658-69.
39. Dietrich CF, Ignee A, Frey H. Contrast-enhanced endoscopic ultrasound with low mechanical index: A new technique. *Z Gastroenterol* 2005;43:1219-23.
40. Dietrich CF, Sharma M, Hocke M. Contrast-enhanced endoscopic ultrasound. *Endosc Ultrasound* 2012;1:130-6.
41. Dietrich CF, Cui XW, Barreiros AP, *et al*. EFSUMB guidelines 2011: Comment on emergent indications and visions. *Ultraschall Med* 2012;33 Suppl 1:S39-47.
42. Xie L, Guang Y, Ding H, *et al*. Diagnostic value of contrast-enhanced ultrasound, computed tomography and magnetic resonance imaging for focal liver lesions: A meta-analysis. *Ultrasound Med Biol* 2011;37:854-61.
43. Friedrich-Rust M, Klopffleisch T, Nierhoff J, *et al*. Contrast-enhanced ultrasound for the differentiation of benign and malignant focal liver lesions: A meta-analysis. *Liver Int* 2013;33:739-55.
44. Rubaltelli L, Khadivi Y, Tregnaghi A, *et al*. Evaluation of lymph node perfusion using continuous mode harmonic ultrasonography with a second-generation contrast agent. *J Ultrasound Med* 2004;23:829-36.
45. Schmid-Wendtner MH, Partscht K, Korting HC, *et al*. Improved differentiation of benign and malignant lymphadenopathy in patients with cutaneous melanoma by contrast-enhanced color Doppler sonography. *Arch Dermatol* 2002;138:491-7.
46. Dietrich CF, Ignee A, Hocke M, *et al*. Pitfalls and artefacts using contrast enhanced ultrasound. *Z Gastroenterol* 2011;49:350-6.
47. Hocke M, Ignee A, Dietrich CF. Advanced endosonographic diagnostic tools for discrimination of focal chronic pancreatitis and pancreatic carcinoma – Elastography, contrast enhanced high mechanical index (CEHMI) and low mechanical index (CELMI) endosonography in direct comparison. *Z Gastroenterol* 2012;50:199-203.
48. Dietrich CF, Averkiou MA, Correas JM, *et al*. An EFSUMB introduction into dynamic contrast-enhanced ultrasound (DCE-US) for quantification of tumour perfusion. *Ultraschall Med* 2012;33:344-51.
49. Schulte-Altedorneburg G, Demharter J, Linné R, *et al*. Does ultrasound contrast agent improve the diagnostic value of colour and power Doppler sonography in superficial lymph node enlargement? *Eur J Radiol* 2003;48:252-7.
50. Schmid-Wendtner MH, Dill-Müller D, Baumert J, *et al*. Lymph node metastases in patients with cutaneous melanoma: Improvements in diagnosis by signal-enhanced color Doppler sonography. *Melanoma Res* 2004;14:269-76.
51. Kanamori A, Hirooka Y, Itoh A, *et al*. Usefulness of contrast-enhanced endoscopic ultrasonography in the differentiation between malignant and benign lymphadenopathy. *Am J Gastroenterol* 2006;101:45-51.

52. Zenk J, Bozzato A, Steinhart H, *et al*. Metastatic and inflammatory cervical lymph nodes as analyzed by contrast-enhanced color-coded Doppler ultrasonography: Quantitative dynamic perfusion patterns and histopathologic correlation. *Ann Otol Rhinol Laryngol* 2005;114 (1 Pt 1):43-7.
53. Hocke M, Ignee A, Topalidis T, *et al*. Contrast-enhanced endosonographic Doppler spectrum analysis is helpful in discrimination between focal chronic pancreatitis and pancreatic cancer. *Pancreas* 2007;35:286-8.
54. Hocke M, Schulze E, Gottschalk P, *et al*. Contrast-enhanced endoscopic ultrasound in discrimination between focal pancreatitis and pancreatic cancer. *World J Gastroenterol* 2006;12:246-50.
55. Hocke M, Dietrich CF. Vascularisation pattern of chronic pancreatitis compared with pancreatic carcinoma: Results from contrast-enhanced endoscopic ultrasound. *Int J Inflam* 2012;2012:420787.
56. Rubaltelli L, Beltrame V, Scagliori E, *et al.* Potential use of contrast-enhanced ultrasound (CEUS) in the detection of metastatic superficial lymph nodes in melanoma patients. *Ultraschall Med* 2014;35:67-71.
57. Cui XW, Ignee A, Jedrzejczyk M, *et al*. Dynamic vascular pattern (DVP), a quantification tool for contrast enhanced ultrasound. *Z Gastroenterol* 2013;51:427-31.
58. Cui X, Ignee A, Nielsen MB, *et al*. Contrast enhanced ultrasound of sentinel lymph nodes. *J Ultrason* 2013;13:73-81.
59. Dudau C, Hameed S, Gibson D, *et al.* Can contrast-enhanced ultrasound distinguish malignant from reactive lymph nodes in patients with head and neck cancers? *Ultrasound Med Biol* 2014;40:747-54.
60. Aoki T, Moriyasu F, Yamamoto K, *et al*. Image of tumor metastasis and inflammatory lymph node enlargement by contrast-enhanced ultrasonography. *World J Radiol* 2011;3:298-305.
61. Poanta L, Serban O, Pascu I, *et al*. The place of CEUS in distinguishing benign from malignant cervical lymph nodes: A prospective study. *Med Ultrason* 2014;16:7-14.
62. Jin Y, He YS, Zhang MM, *et al.* Value of contrast-enhanced ultrasonography in the differential diagnosis of enlarged lymph nodes: A meta-analysis of diagnostic accuracy studies. *Asian Pac J Cancer Prev* 2015;16:2361-8.
63. Dietrich CF, Lembcke B, Jenssen C, *et al*. Intestinal ultrasound in rare gastrointestinal diseases, update, part 1. *Ultraschall Med* 2014;35:400-21.
64. Dietrich CF, Lembcke B, Jenssen C, *et al*. Intestinal ultrasound in rare gastrointestinal diseases, update, part 2. *Ultraschall Med* 2015;36:428-56.
65. Barreiros AP, Braden B, Schieferstein-Knauer C, *et al*. Characteristics of intestinal tuberculosis in ultrasonographic techniques. *Scand J Gastroenterol* 2008;43:1224-31.
66. Jenssen C, Dietrich CF. Endoscopic ultrasound-guided fine-needle aspiration biopsy and trucut biopsy in gastroenterology – An overview. *Best Pract Res Clin Gastroenterol* 2009;23:743-59.
67. Hocke M, Menges M, Topalidis T, *et al*. Contrast-enhanced endoscopic ultrasound in discrimination between benign and malignant mediastinal and abdominal lymph nodes. *J Cancer Res Clin Oncol* 2008;134:473-80.
68. Xia Y, Kitano M, Kudo M, *et al.* Characterization of intra-abdominal lesions of undetermined origin by contrast-enhanced harmonic EUS (with videos). *Gastrointest Endosc* 2010;72:637-42.
69. Rubaltelli L, Corradin S, Dorigo A, *et al*. Automated quantitative evaluation of lymph node perfusion on contrast-enhanced sonography. *AJR Am J Roentgenol* 2007;188:977-83.
70. Li L, Mori S, Kodama M, *et al*. Enhanced sonographic imaging to diagnose lymph node metastasis: Importance of blood vessel volume and density. *Cancer Res* 2013;73:2082-92.
71. Jin Y, Peng YL, Qiu L, *et al.* The perfusion pattern of lymphadenopathy in contrast-enhanced ultrasonograph. Under different reference conditions. *Sichuan Da Xue Xue Bao Yi Xue Ban* 2014;45:1005-9.
72. Xiang D, Hong Y, Zhang B, *et al.* Contrast-enhanced ultrasound (CEUS) facilitated US in detecting lateral neck lymph node metastasis of thyroid cancer patients: Diagnosis value and enhancement patterns of malignant lymph nodes. *Eur Radiol* 2014;24:2513-9.
73. Matsuzawa F, Einama T, Abe H, *et al.* Accurate diagnosis of axillary lymph node metastasis using contrast-enhanced ultrasonography with Sonazoid. *Mol Clin Oncol* 2015;3:299-302.
74. Matsuzawa F, Omoto K, Einama T, *et al.* Accurate evaluation of axillary sentinel lymph node metastasis using contrast-enhanced ultrasonography with Sonazoid in breast cancer: A preliminary clinical trial. *Springerplus* 2015;4:509.
75. Schreiber-Dietrich DG, Cui XW, Piscaglia F, *et al*. Contrast enhanced ultrasound in pediatric patients: A real challenge. *Z Gastroenterol* 2014;52:1178-84.
76. Schreiber-Dietrich D, Pohl M, Cui XW, *et al*. Perihepatic lymphadenopathy in children with chronic viral hepatitis. *J Ultrason* 2015;15:137-50.