Role of contrast-enhanced endoscopic ultrasound in lymph nodes

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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[6] 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]

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

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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 and abdomen closely behind pancreatic diseases.

EUS-FNA cytology has a high accuracy in the diagnosis of enlarged LNs. 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. 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. 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. Unfortunately, those criteria have not been very successful and could only be used in clearly defined clinical settings.

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.

The second important new technique is the contrast-enhanced harmonic-EUS (CH-EUS). After the emergence of the new contrast harmonic imaging techniques for liver diseases, it was inevitable to think about new applications. One of those has been the contrast enhancing the behavior of enlarged LNs. 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. In the following years, this method gained importance. 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.

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.
A few studies have been made using that technique for discriminating benign from malignant LNs. The aim of the studies has been to find differences in the vascular structure of benign to malignant LNs. Demonstration of malignant neovascularization, for example, vessels penetrating the LN capsule has been used as the characteristic feature of LN metastases.

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
- 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).

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

**CONTRAST HARMONIC IMAGING IN ENDOSCOPIC ULTRASOUND**

More studies dealt with contrast harmonic imaging for discrimination of malignant to benign LNs. 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. 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. In the performed studies, this effect has been described and can even been seen in a meta-analysis study. 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. 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. A recommendation could be considered for using the method for tumor cell nest targeting in EUS-FNA cytology and in special clinical settings.

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.
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.\cite{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 \textit{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 \textit{et al.} included ten lymphoma LNs into the malignant group which are known for an equal contrast enhancing effect such as benign LNs.\cite{51}

The next study from Hocke \textit{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,\cite{84} 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.\cite{67}

Recently, there was a study published by Xia \textit{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\%.\cite{68}

Due to pathophysiological reasons, such as mentioned in the section “contrast harmonic imaging” the results should be seen critically.

**CONTRAST-ENHANCED ENDOCOSCOPIC 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. Major advantage can be made using calculating software which can estimate the time and quantity of the contrast enhancer influx. There might be unique contrast enhancing patterns which could be able to overcome the problem of special resolution or focal necrosis.

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. The development of targeting contrast

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**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 lymph node 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.
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.

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There are no conflicts of interest.

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