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● *Review*

CONTRAST-ENHANCED ULTRASOUND FOR MUSCULOSKELETAL APPLICATIONS: A WORLD FEDERATION FOR ULTRASOUND IN MEDICINE AND BIOLOGY POSITION PAPER

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Abstract—This World Federation for Ultrasound in Medicine and Biology position paper reviews the diagnostic potential of ultrasound contrast agents for clinical decision-making and provides general advice for optimal contrast-enhanced ultrasound performance in musculoskeletal issues. In this domain, contrast-enhanced ultrasound performance has increasingly been investigated with promising results, but still lacks everyday clinical application and standardized techniques; therefore, experts summarized current knowledge according to published evidence and best personal experience. The goal was to intensify and standardize the use and administration of ultrasound contrast agents to facilitate correct diagnoses and ultimately to improve the management and outcomes of patients. (E-mail addresses: **mail@medfischer.de**, **Christian.fischer@med.uni-heidelberg.de**)   © 2020 World Federation for Ultrasound in Medicine & Biology. All rights reserved.

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INTRODUCTION

The use of contrast-enhanced ultrasound (CEUS) for musculoskeletal (MSK) indications has increased significantly over the past year. Unlike conventional imaging tools that rely on mere anatomic characteristics including visible changes in bone structure or signs of soft tissue degeneration, the depiction of local microperfusion *via* CEUS sheds light on the (patho)physiologic processes in these tissues. Beyond its utility for basic research, this perspective particularly implies being "up to date" in clinical practice, enabling the early detection of peri-operative conditions that require adjustment of planned or initiated treatment, as well as the comprehensive diagnostic workup, of various MSK pathologies. Therefore, while outlining current research topics, these guidelines intend to facilitate and hence promote the further exploration, as well as the implementation, of the

MSK approach, providing examples of practical application that have proven their practicability in routine use. The recommendations given are primarily intended to serve as *guidance*, based on most recent expert opinions and the referenced studies.

The commonly used ultrasound contrast agent (UCA) for MSK imaging, SonoVue (Bracco, Milan, Italy), is a second-generation UCA containing microbubbles with a mean diameter of $1.5−2.5\ \mu$m that consist of a sulfur hexafluoride core and a stabilizing phospholipid shell (Greis 2004; Committee for Medicinal Products for Human Use [CHMP] 2014). It is the UCA that is the standard used for CEUS examinations in Europe. UCAs have very good safety profiles including SonoVue (Bracco), with a reported rate of serious adverse events of only 0.01%, and there is no need for laboratory tests to assess relevant organ (dys-) functions before examination (Piscaglia and Bolondi 2006; Claudon et al. 2013; CHMP 2014; Sidhu et al. 2018). For further information on SonoVue (Bracco) (*e.g.,* its contraindications) we refer to the manufacturer's recommendations. As the acquisition and analysis of UCA dynamics differ

depending on the information needed, an overview of existing options is introduced first.

UCA DYNAMICS

Qualitative assessment

Outside MSK issues, detection and characterization of lesions and other pathologies are usually realized with qualitative CEUS (Claudon et al. 2013; Sidhu et al. 2018). A single bolus of UCA is injected, and the ultrasound (US) signal is assessed visually. The US transducer may not need to be or even should not be kept at one single position, so that different body regions or views within one examination can be assessed. However, for a semiquantitative evaluation, that is, if scores are to be used to describe the "degree of vascularization" (Klauser et al. 2005a), the examination may be focused on one pre-defined area only.

In MSK application, this is a suitable approach for a general overview of hyperperfused areas, as in rheumatology (Klauser et al. 2005b, 2010) or angiogenic processes after traumatic MSK lesions (Genovese et al. 2007). On the other hand, hypo-perfused areas might be of particular interest (*e.g.,* in muscular transplants). A continuous UCA infusion can prolong the duration of the exam, as in the assessment of inflammatory disease of multiple joints (Klauser et al. 2002).

Because MSK CEUS also addresses questions that are related to critical deviations from physiologic tissue perfusion and is used for monitoring during follow-up, a quantitative analysis of the UCA dynamics providing specific parameters is often required. Thus, objective criteria to assess single measurements (defining terms such as *critical* and *physiologic* for one anatomic region in perfusion-related contexts) can be established and sequential examinations can be compared. Considering the sample sizes of the previous studies, multicentric studies on larger patient cohorts are required for the definition of more representative cutoff values and further standardization.

Quantitative analysis of standard time−intensity curves

The quantitative analysis of standard time−intensity curves (TICs) is one central approach. For this, the transducer needs to be maintained at an identical position, and wash-in as well as wash-out dynamics of the UCA after a single bolus injection within certain regions of interest (ROIs) are calculated. The reduced frame rate of the US machine minimizes bubble destruction, and the derived signals need to be linearized. Suitable post-processing solutions (either on the US platform or external) are a prerequisite for quantification of CEUS data. The entire procedure needs to be standardized to obtain data suitable for intra- or inter-individual comparison. For this purpose, detailed examination protocols should be used.

This standard method of quantitatively analyzing TICs after UCA bolus injection has been applied in various MSK indications such as rheumatology (Schueller-Weidekamm et al. 2007; Stramare et al. 2012; Cai et al. 2015), for assessment of skeletal muscle perfusion (Partovi et al. 2016; Fischer et al. 2017, 2018; Klenze et al. 2017), to assess the microperfusion within long-bone non-unions (Fischer et al. 2016b; Krammer et al. 2018) and to quantify microperfusion of free flaps in reconstruction surgery (Geis et al. 2015).

Various parameters can be calculated with these linearized TICs and when fitting curves are applied (Fig. 1); they are related to the local blood microperfusion and blood volume, respectively. However, an exact calculation of perfusion or blood volume in absolute values is not possible. Parameters such as "area under the curve" (AUC) values may be related to the total blood volume (mL) in a body area, while time-related parameters such as the slope of the enhancement curve basically depend on the local blood flow (mL/s). Perfusion parameters hence describe the measured blood volume or flow or a combination of the two when they refer to the UCA wash-in or wash-out phase or to the entire enhancement. The most often used TIC parameters as depicted in Figure 1 include *peak enhancement* (PE in arbitrary units [a.u.]), which relates to the maximum signal intensity of the enhancement curve; *area under the curve* (AUC [a.u. × s]), the definite integral of the signal intensity against time during wash-in and wash-out; and *wash-in rate* (WiR [a.u.]), the maximum slope of the signal enhancement curve. Time-dependent parameters include *rise time* (RT [s]), the wash-in duration of the UCA; *time to PE* (T_{PE} [s]), the time from UCA application to PE; and *mean transit time* (MTT [s]), the combined wash-in and wash-out duration of the UCA.

These common CEUS parameters are not strictly limited to single perfusion characteristics only; for example, a smaller local blood volume will not only result in a smaller AUC but will also affect the slope. The choice of the most suitable CEUS parameter for perfusion assessment depends on the clinical problem to be addressed.

Quantitative CEUS analysis is an ongoing field of research; further developments have occurred and may further evolve. A pixelwise analysis of TICs has been introduced to characterize arthritis (Rizzo et al. 2015). More advanced technical options, which track individual UCA microbubbles and use super-resolution US, may further improve perfusion assessment with CEUS (Christensen-Jeffries et al. 2015; Lin et al. 2017).

Replenishment kinetics. An alternative technique is based on flash-replenishment kinetics. Although this method is standard for myocardial perfusion analysis in echocardiography (Porter et al. 2018), it is less frequently used elsewhere but has been applied in MSK
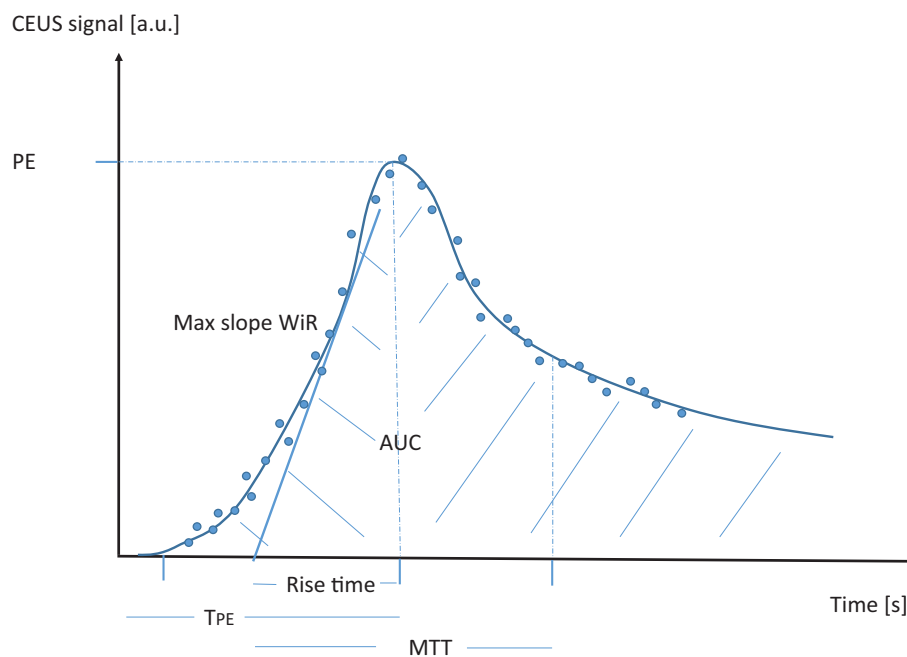
Fig. 1. CEUS standard time−intensity curve after UCA bolus injection. *Blue dots* represent real, linearized values; a fitting curve provides the following CEUS parameters: PE (in a.u.), the maximum signal intensity of the enhancement curve; T_{PE} (s), duration from UCA application to maximum signal; WiR (a.u./s), the maximum slope to peak; RT (s), that is, T_{PE} − time where the maximum slope line originates; AUC (a.u. × s), area under the TIC curve; MTT (s), fitting model dependent. a.u. = arbitrary units; AUC = area under the curve; CEUS = contrast-enhanced ultrasound; MTT = mean transit time; PE = peak enhancement; RT = rise time; T_{PE} = time to peak; UCA = ultrasound contrast agent; WiR = wash-in rate.

CEUS as well, predominantly in a quantitative manner to evaluate skeletal muscle perfusion (Weber et al. 2006c; Davidson et al. 2016, 2017). After the application of US pulses with high acoustic energy (flash), which destroy the UCA microbubbles inside the ROI, the consecutive refilling from outside is monitored. Different perfusion parameters can be derived from such TICs (Fig. 2).

Compared with standard TICs, this method provides perfusion parameters that are more directly related to certain vascularization parameters; that is, the slope parameter β is considered to be strictly linear to local blood flow velocity (m/s), while the plateau value A is proportional to local blood volume (mL). For quantitative analysis, it is usually assumed that the UCA signal is in a steady-state situation. Therefore, a continuous infusion of contrast agent is the first option for this method. In the skeletal muscle, relatively high and long-lasting infusion rates are desired to obtain sufficiently high CEUS signals (*e.g.,* ≥1 mL/min for ≥5 min).

Practical implications of skeletal muscle perfusion characteristics

In a skeletal muscle at rest, the blood perfusion and, thus, the detected CEUS signals are generally very low. Moreover, it must be considered that the muscle has a high physiologic variation in its blood supply. Hence, muscle provocation is often performed before the CEUS examination (Krix et al. 2010, 2011; Amarteifio et al. 2013; Davidson et al. 2017; Fischer et al. 2018, 2019, 2020; Kunz et al. 2019, 2020). Consequently, muscle microperfusion considerably increases, which may also reveal pathologic perfusion patterns (well known from stress myocardial perfusion imaging).

Provocation by muscle exercise can be either dynamic or isometric (Krix et al. 2011; Davidson et al. 2017). Isometric exercise offers the option to measure muscle perfusion with CEUS simultaneously (Krix et al. 2010). In proximal muscle groups like the shoulder girdle, dynamic exercise remains the best option (Fischer et al. 2018). A drug-induced provocation, common in echocardiography, has been reported in MSK CEUS (Davidson et al. 2017). Another option that has been used is transient arterial occlusion using specific cuffs with pressures above arterial occlusion pressure and an automated plethysmograph to simplify the application (Krix et al. 2011; Amarteifio et al. 2013).

After exercise or other provocation methods, the degree of hyperperfusion may vary substantially (Thomas et al. 2015), with intra- and inter-individual variation. Furthermore, other factors influence the detected US signal,
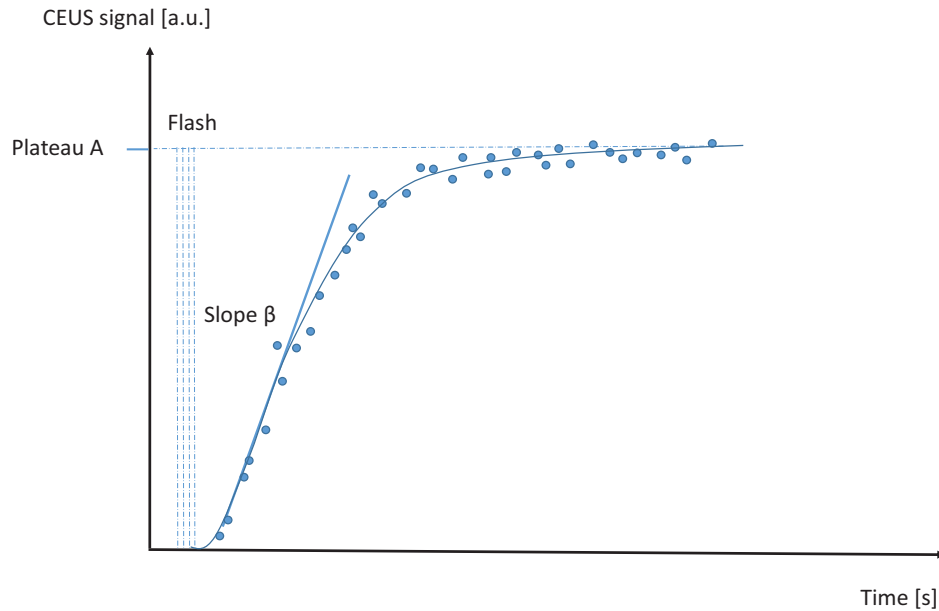
Fig. 2. CEUS time−intensity curve demonstrating flash-replenishment dynamics after the application of a high-energy ultrasonic pulse (flash). *Blue dots* represent real, linearized values. A fitting curve provides the following CEUS parameters: plateau A (a.u.), the signal after complete refilling indicating local blood volume; slope β (a.u./s), the initial slope of the replenishment indicating local blood flow. According to the fitting model, for example, $A \times (1 - \exp[-\beta t])$. a.u. = arbitrary units; CEUS = contrast-enhanced ultrasound.

such as the local acoustic conditions or the general blood circulation, which may differ between patients. For such reasons, the use of relative instead of absolute CEUS values is generally less vulnerable to variations. *Relative* means perfusion parameters of the affected tissue referenced to defined adjacent tissue (Fischer et al. 2017; Kellermann et al. 2017) or in extremities to the contralateral side if the disease is located unilaterally (Fischer et al. 2018). To minimize methodologically-caused variations when measuring muscular microperfusion, a strict examination algorithm, including the provocation method, correct timing of contrast agent application and exact transducer adjustment according to appropriate reference landmarks are paramount (Kunz et al. 2020). With respect to muscular exercise as a provocation method, provocation dynamometers that guide and monitor the degree of force can support standardization. For transducer adjustment, it should be noted that in the case of quantitative CEUS applications, defined landmarks have to be assessed for sufficient sectional plane reproducibility by testing the intra-/inter-observer reliability of measurements in advance (Kunz et al. 2020).

Special types of CEUS methods
In muscle perfusion evaluation, measurement with CEUS is not only feasible *after* a standardized provocation, but also before, during and immediately after. Such assessments may be particularly useful in evaluating therapeutic effects on perfusion reserve or in better understanding training effects in sports medicine.

The UCA signals should be in a steady state before provocation, so a continuous infusion of UCA is needed. In combination with low values of the mechanical index (MI), this enables continuous real-time evaluation of rapid provocation-related changes in signal intensity (Krix et al. 2010). Compared with analogous parameters derived from standard quantitative TICs, the parameters derived from such TICs always have to be considered in terms of these special conditions of acquisition (Fig. 3). Another simplified, special type of quantitative UCA dynamics analysis is to measure arrival times of a contrast bolus. In MSK CEUS this can be used in peripheral muscles for evaluation of peripheral arterial disease or the presence of collaterals in this disease (Duerschmied et al. 2009).

FUNDAMENTALS OF PRACTICAL APPLICATION

Guidelines and recommendations on hepatic and non-hepatic use of CEUS have recently been published, including detailed information on the injection process, the video recording and general technical aspects (Dietrich et al. 2018; Sidhu et al. 2018), and those involved should be familiar with them.

Technical aspects
Technical settings derived for the following MSK applications should cover key features such as "a wide dynamic range," gain "slightly above the noise floor," "a
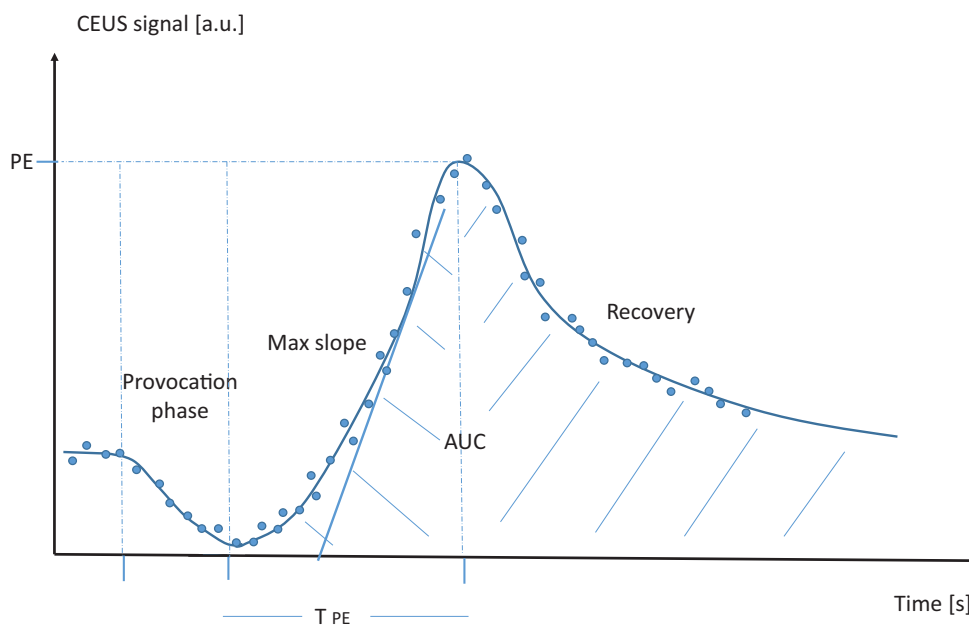
CEUS signal [a.u.]

Fig. 3. CEUS time−intensity curve during ultrasound contrast agent infusion and muscle provocation. CEUS signal during provocation may vary (here continuous decrease in signal), followed by a rapid increase after provocation cessation and subsequent recovery. *Blue dots* represent real, linearized values. A fitting curve provides the following CEUS parameters: PE (a.u.) after cessation of provocation; T_{PE} (s); maximum slope (a.u./s) after provocation; AUC (a.u. × s) after provocation cessation. a.u. = arbitrary units; AUC = area under the curve; CEUS = contrast-enhanced ultrasound; T_{PE} = time to peak; UCA = ultrasound contrast agent.

low mechanical index (MI)" and "the use of a dual-image display format" (Dietrich et al. 2018; Sidhu et al. 2018).

The focus should be positioned below the area of interest to ensure homogeneous imaging of the injected contrast agent (Averkiou et al. 2010; Dietrich et al. 2012). The depth is chosen based on the principle "as little as possible, as much as required," meaning that on the one hand, the target structure (*e.g.,* the fracture gap) should be displayed in large size, but on the other hand, it should be small enough to position the focus adequately (Dietrich et al. 2012).

To obtain the best contrast agent resonance (fundamental stimulating frequency at about 3.5 MHz), broadband linear probes with low frequencies or suitable lower-frequency transducers are preferably used at all relevant resonance frequencies (subharmonic, fundamental, ultraharmonic and second harmonic resonances) for up-to-date second-generation contrast agents are lower than 8 MHz for low-MI imaging. In this context, only applications with highly specific demands on resolution, for example, examination of the finger joints, may justify the use of higher frequencies. For larger or deeper-lying structures, the use of curved probes can be helpful in better depicting the structure at better contrast agent utilization with less resolution.

Preparation and performance of a CEUS procedure

The exact positioning of the patient depends on the region to be examined. In general, we recommend a stable supine or lateral position, which can be easily maintained during the entire measurement to avoid movement artifacts, which impede qualitative and quantitative perfusion assessment. When another setting is more suitable for specific applications, a detailed description is given separately. Afterward, pre-assessment *via* unenhanced US modalities is focused on, which is reasonable before adjusting the primary scan plane for CEUS. To reproduce this scan plane when performing sequential examinations, the exact position and orientation of the transducer have to be documented, particularly in those applications for which no standardized anatomic landmarks are available for orientation. This can be realized with position markers on stored images in B-mode preceding CEUS, supplemented by annotations in the corresponding examination report. The same technical settings should be used to re-examine the patient.

Generally, there are different doses of SonoVue (Bracco) in clinical use, ranging from 1−4.8 mL. For each introduced MSK application, we recommend the most effective UCA dose based on our previous experience in clinical practice. Nevertheless, a final standardization has to be conducted depending on the device used, referred to the anatomic region that is focused by MSK CEUS (Kunz et al. 2020).

If MSK CEUS is performed in a potential multicenter setup requiring the comparison of quantified TIC values, standardization of all described settings is vital and

should also include the interval between UCA application and the start of the video, which is best realized instantaneously. The duration of the recorded video depends on the tissue examined and the capability of local storage capacities for large files. Yet, it will generally be shorter than, for example, in liver diagnostics, as there is no late "portal" phase and the wash-in parameters are usually of most interest. If qualitative instead of quantitative assessment is primary, initial video clips can be recorded during the wash-in phase followed by sequential snapshots at determined intervals.

During measurement, holding the transducer stable with both hands is another key factor in avoiding motion, but at the same time the operator should keep in mind the need to reduce transducer pressure to a negligible level to avoid the risk of small vessel compression.

All examples of the standardized CEUS procedure presented in these guidelines follow the approach to UCA bolus injection and analysis of standard time−intensity curves in case of quantitative evaluation.

PERI-OPERATIVE DIAGNOSTICS

Fractures and non-unions

Background. Osseous perfusion is an essential quality of bone metabolism and regeneration (Giannoudis et al. 2008; Keramaris et al. 2008; Marenzana and Arnett 2013; Bahney et al. 2015). Given a cortical gap permitting US beam penetration, CEUS allows the visualization of osseous microcirculation and, thus, its use as novel diagnostic approach complementing conventional modalities of fracture and non-union diagnostics. Compared with healing tibial fractures, aseptic tibial non-unions have been reported to suffer impaired microcirculation, whereas infected tibial non-unions exhibited excessive perfusion (Fischer et al. 2020), suggesting that the physiologic process of bone healing is reflected by a specific level of osseous perfusion. Moreover, further evidence of the diagnostic value of osseous CEUS can be found in the field of non-union revision surgery, where perfusion measurement is performed at different peri-operative stages.

In a pilot study examining the microperfusion of non-union tissue 12 wk after revision surgery, sufficient perfusion was favorable, as it predicted successful eventual consolidation (Krammer et al. 2018). This finding is the subject of further research, while current clinical interest focuses on osseous CEUS application *before* revision surgery. CEUS hyperperfusion of a non-union pre-operatively indicates infection is an underlying condition (Fig. 4), which is particularly valuable because of the low diagnostic accuracy of clinical, radiologic and laboratory tests (Fischer et al. 2016b).

Derived from the largest patient cohort with lower-extremity non-unions examined to date, CEUS can detect infections with a sensitivity and specificity of 85.1% and 88.7%, respectively, based on P (cutoff value = 81.2 a.u.) (Doll et al. 2019).

Pre-assessment and primary scan plane. Adequate evaluation of osseous perfusion *via* CEUS initially requires identification of the widest visible bone gap in B-mode (Fig. 5), while the transducer basically is oriented in-line with the long bone axis. The primary scan plane for CEUS measurement (Fig. 6) includes an osseous gap that is (i) clearly defined by cortical bone on each side and (ii) well visible without being overlapped by implant material or cortical displacement.

CEUS procedure. After pre-assessment of the bone gap in B-mode, a bolus of 2.4 mL of UCA is administered. To record subsequent contrast agent flow, a 90-s video clip is adequate, beginning with injection. Contrast-specific signals can already be assessed qualitatively during the examination with respect to the visual kinetics of the contrast agent inflow, but the overall evaluation of CEUS sequences is based mainly on the quantitative features of an ROI placed inside the bone gap.

Tissue transplants

Background. Complications rates after free flap transplantation still amount to 10% (Lindau et al. 2013), with free flap loss from vascular compromise remaining a serious complication. It has been reported that large numbers of the failing flaps could be successfully salvaged after early detection of vascular compromise and immediate intervention (Brown et al. 2003; Novakovic et al. 2009; Geis et al. 2013a).

Dynamic CEUS has become an important imaging method yielding information on tissue perfusion of the cutaneous, subcutaneous and deeper layers of free flaps in a hitherto unparalleled feasible and comprehensive way (Geis et al. 2013a, 2013b, 2016; Gao et al. 2016; Kehrer et al. 2017; Mueller et al. 2017; Sidhu et al. 2018).

For surgical planning, knowledge of the flow parameter and the number and exact course of the involved vessels is essential, though challenging to acquire because the small diameters of the vessels (in raised free flaps at most 1−2 mm) (Sidhu et al. 2018). With its high temporal and spatial resolution, CEUS improves such pre-operative assessment. Also in case of perforator mapping, which is important because of the considerable anatomic variations, CEUS had high accuracy (Gao et al. 2016). Thus, CEUS may facilitate the intervention and, in particular, enable a more accurate estimation of the flap size with sufficient blood supply.

The feeding vessels, the optional connection to a bypass, the anastomosis and the transplant's own vessels
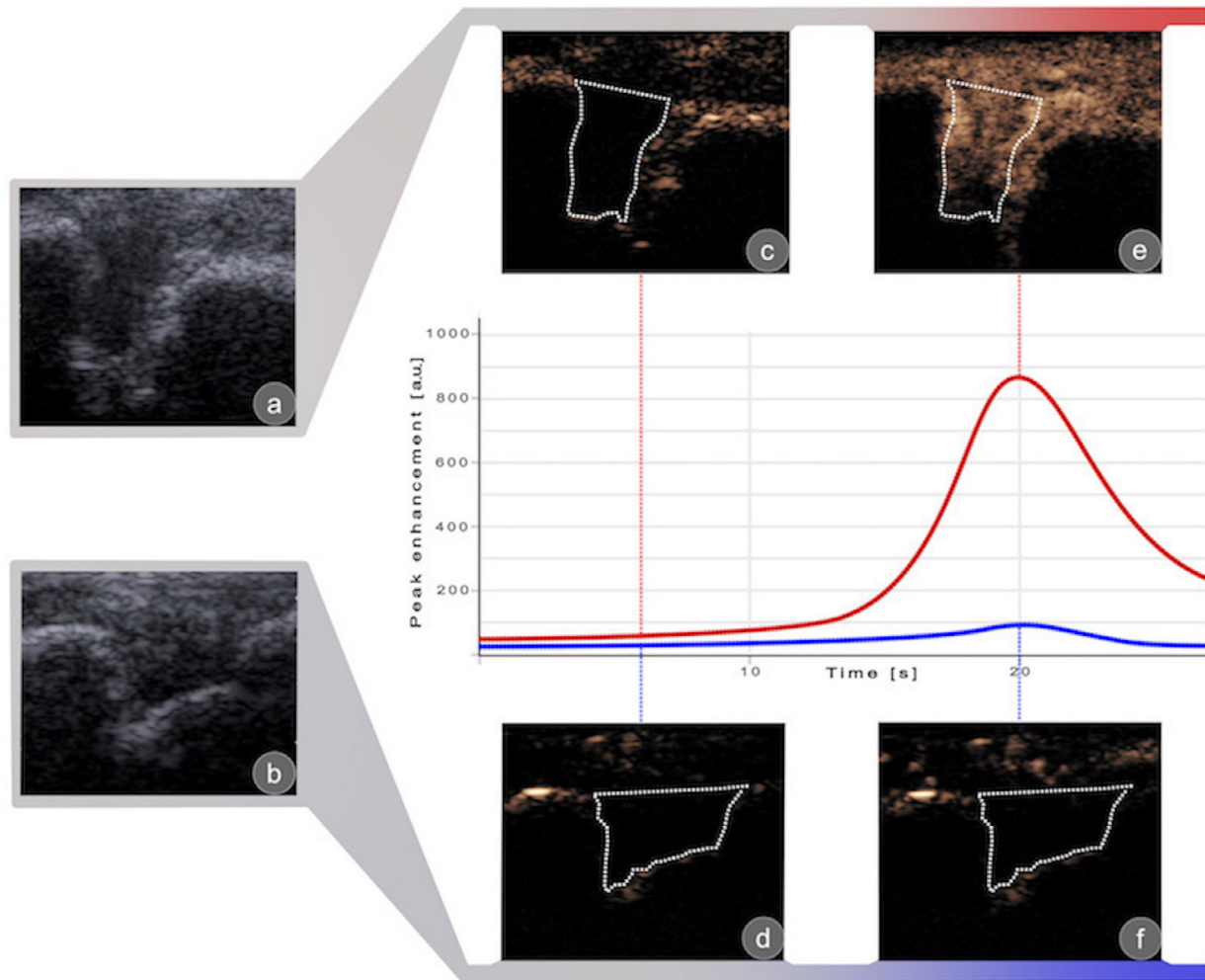
Fig. 4. Time−intensity curves of infected (*red*) and aseptic (*blue*) tibial non-unions before revision surgery. (a, c, e) Corresponding ultrasound images with the non-union gap in B-mode (a) and in contrast mode before (c) and after (e) contrast agent inflow in case of infection. (b, d, f) Corresponding ultrasound images with the non-union gap in B-mode (b) and in contrast mode before (d) and after (f) contrast agent inflow in the case of an aseptic non-union. Informed patient consent for publication available. a.u. = arbitrary units.

can also be examined post-operatively. CEUS provides valuable information on vascular compromises (thrombosis, embolism, twisting, kinking or compression), enabling timely surgical revision. Also, in deeply buried flaps, the entire flap tissue can be evaluated for perfusion abnormalities down to the microcirculatory level (Geis et al. 2013a, 2013b, 2016; Kehrer et al. 2017; Sidhu et al. 2018). *Critical microperfusion of free flaps* means a significant difference between compromised flaps and physiologically vascularized soft tissue. It can well be detected using time to peak (TTP) and regional blood volume (RBV) as CEUS quantification parameters. TTP and RBV appear to be the most precise perfusion parameters available, and first critical thresholds of sufficient flap perfusion could empirically be set for both (Geis et al. 2011, 2013a, 2013b).

Distinct areas with disturbed microperfusion can be detected early with CEUS, independent of the composition of the free flap, so that the identification and characterization (*e.g.,* the exact extent) of hematomas, seromas or partial flap necrosis become possible (Geis et al. 2011, 2015, 2016).

Pre-assessment and primary scan plane. Initially, the whole flap is examined to determine if there are any morphologic abnormalities (*e.g.,* fluid collections). Additionally, the surrounding vessels up to the anastomosis should be delineated for color-coded Doppler sonography (CCDS) and power Doppler-based flow evaluation, which may already identify hemodynamically relevant arterial stenosis or venous thrombosis. To adjust the primary scan plane for CEUS measurement, meaning a representative

Shifting the transducer along with the long bone axis

until the osseous gap occurs in the center of the image

Moving medially and laterally

to display the gap in its maximum width

Perpendicular insonation of the surrounding cortical bone

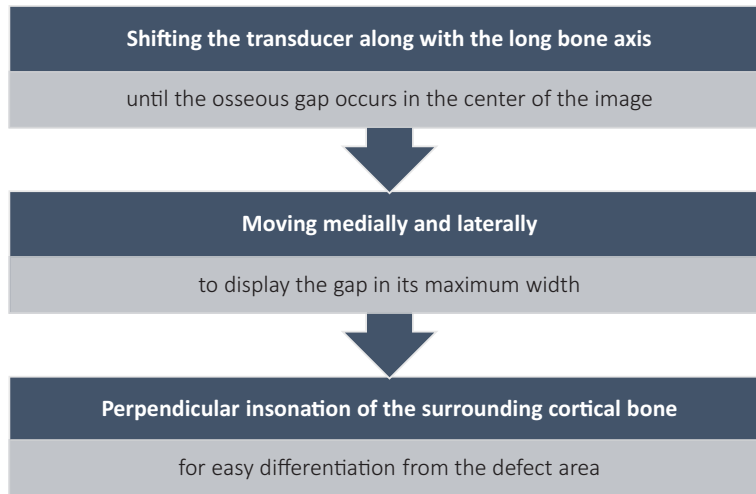for easy differentiation from the defect area

Fig. 5. Adjustment of the primary scan plane preceding CEUS examination of a tibial non-union.
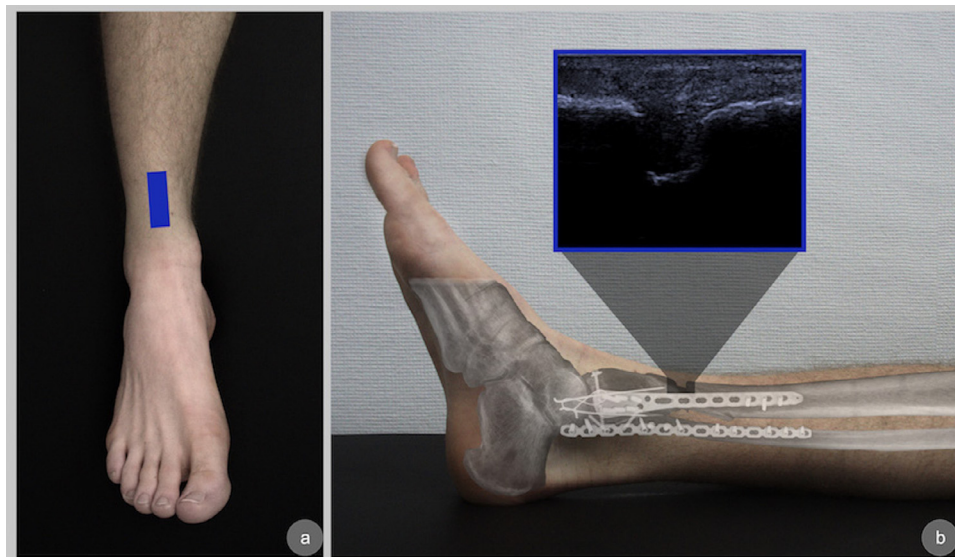

Fig. 6. Primary scan plane for contrast-enhanced ultrasound examination of a tibial non-union. (a) Transducer orientation in-line with the long bone axis. (b) Clearly depicted non-union gap on corresponding B-mode ultrasound without overlapping material, surrounded by intact cortical bone. Informed patient consent for publication available.

US slice of the respective flap tissue, the transducer is positioned in the center of the flap.

CEUS procedure. The standardized step-by-step approach that has been applied in this field starts with a bolus injection of 1−2.4 mL of UCA, followed by continuous recording of contrast-specific signaling for 1 min, with the transducer stabilized in the primary scan plane for subsequent quantitative evaluation of a critical flap perfusion level.

The remainder of the procedure focuses on distinct regions with contrast enhancement indicating abnormal local microcirculation, based on reduced signal intensity and/or altered UCA kinetics. For this purpose, the scanning is continued by sweeping from the primary scan plane over the whole flap and the margins to the normal tissue while CEUS data are digitally stored up to 3 min after the initial injection.

If there are any abnormalities, the respective area is selectively scanned after another bolus injection according to the first part of the examination, but now with the transducer stabilized in the conspicuous plane and for more than 1 min. This separate sequence allows for a detailed qualitative evaluation, the findings of which can additionally be quantified by comparing the TIC analyses of contrast-deficient and surrounding tissue.

MUSCULAR DETERMINANTS OF SHOULDER SURGERY OUTCOME

Supraspinatus muscle tissue

Background. Considering the notable re-tear rate and variability of functional outcomes after rotator cuff repair (McElvany et al. 2015), the issue of identifying a given predisposition to failure appears to be particularly relevant to choosing adequate treatment strategies in this field. Looking beyond common morphologic criteria by revealing muscular microperfusion, which is accessible *via* CEUS as a surrogate marker of supraspinatus muscle vitality and function (Fischer et al. 2018), seems to be promising. In a current prospective study (Kunz et al. 2019b), pre-operative quantification of microperfusion of the supraspinatus muscle tissue could predict the risk of a re-tear within the first 6 mo after repair and correlate it significantly to functional outcome; thus, the latter approach is superior to the use of fatty infiltration and tendon retraction (in magnetic resonance imaging [MRI]) with respect to their predictive potential. In the future, in addition to the intra-individual monitoring of muscle vitality during rehabilitation, CEUS might become useful in routine pre-operative diagnostics when planning rotator cuff surgery. It may optimize the selection of patients who are not likely to benefit from supraspinatus tendon repair but require alternative treatment.

Setting. Seated patients place their arm in neutral position keeping the extended elbow close to the body, while the operator sits behind the shoulder to be examined, with both the patient's and operator's view focused on the US device. The described setting provides easy access to the supraspinatus muscle tissue and enables patients to follow gradual adjustment of different scan planes on the screen as well as to move their arm freely during pre-contrast exercise.

Pre-assessment and primary scan plane. CEUS of the supraspinatus muscle is performed within the area of its maximum cross section, taking advantage of the high muscle/tendon ratio. This requires standardized adjustment of an oblique sagittal plane perpendicular to the muscle's long axis at the suprascapular notch (Fig. 7) (Yanagisawa et al. 2009), which is based on previous attempts to reproduce the Y-view of MRI on US for assessment of supraspinatus muscle atrophy and fatty infiltration (Strobel et al. 2005; Khoury et al. 2008). For CEUS-based evaluation of supraspinatus muscle perfusion, a slightly modified approach is used under consideration of the following criteria (Fig. 8):

1. Apparent delineation of the supraspinatus muscle tissue from the overlying trapezius reference muscle, presenting as hyper-echoic line. This might increase the accuracy of placing an ROI within each muscle during the subsequent quantification process.
2. A distinct osseous boundary (given by the supraspinatus fossa and the scapular spine)

CEUS procedure. To activate the supraspinatus muscle, the specific pre-contrast exercise consists of 120 s of repeated arm raising up to 90° in the plane of the scapula (frequency of direction change = 1 Hz), with the thumb directed downward in full pronation. After exercise, the patient returns to neutral position and CEUS is started immediately with a bolus injection of 4.8 mL UCA. The corresponding 90-s video clip is analyzed quantitatively.

Moving the transducer along the course of the supraspinatus muscle

to identify the suprascapular notch

90° rotation of transducer

to visualize the cross section of the muscle belly inside its fossa

Shifting slightly medially and laterally while basic alignment is maintained
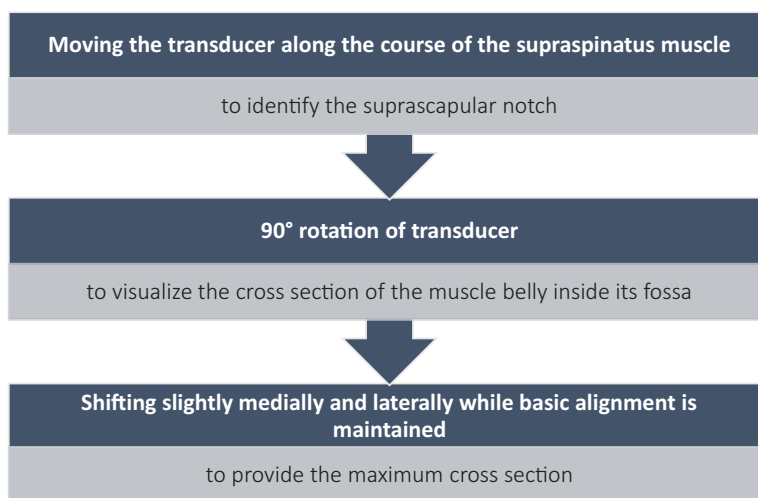
to provide the maximum cross section

Fig. 7. Adjustment of the primary scan plane preceding contrast-enhanced ultrasound examination of the supraspinatus muscle tissue.

Fig. 8. Primary scan plane for contrast-enhanced ultrasound examination of the supraspinatus muscle tissue. (a) Transducer positioning in an oblique sagittal plane at the suprascapular notch. (b) Corresponding B-mode and contrast mode ultrasound images; focus and depth settings optimally adapted. Informed patient consent for publication available.

Deltoid muscle tissue

Background. Muscular CEUS has also shown its diagnostic value when quantifying deltoid muscle microperfusion to peri-operatively assess functional deltoid properties. In an initial study comparing the outcome after open and minimally invasive locked plating of proximal humerus fractures, CEUS was a feasible method to analyze relevant differences in post-operative muscle vitality and soft tissue damage (Fischer et al. 2016a).

Further CEUS-based research focused on the deltoid muscle tissue after reverse shoulder arthroplasty (RSA) (Fischer et al. 2017), the biomechanical concept of which depends on the deltoid muscle's integrity as a substantial component (Boileau et al. 2005; Lam et al. 2007). After RSA, the operated shoulders had reduced perfusion kinetics compared with the contralateral side, which significantly correlated with clinical outcome. Moreover, the measured microperfusion reflected the functional difference between patient groups with above-average and below-average outcomes, thereby revealing its potential as a surrogate marker for deltoid function.

On the basis of these findings, a prospective study on dynamic deltoid perfusion before RSA indicated that pre-operative CEUS assessment was highly predictive of deltoid outcome ($r = 0.84$, $p = 0.0004$) and revealed patient subgroups with a particular need for individually adapted center of rotation positioning (Fischer et al. 2019).

Setting. The basic setting for CEUS of the deltoid muscle corresponds to that described for the peri-operative assessment of supraspinatus muscle tissue, but with the patient's palm on his or her thigh. Thus, optimal exposure of the muscle area to be scanned is achieved while fixing the examined arm, which itself is relaxed, as "counterbearing" for transducer stabilization from (postero-)lateral.

Pre-assessment and primary scan plane. To evaluate deltoid microperfusion, we recommend longitudinal adjustment of the acromial portion at its posterior part (transition zone to the spinal portion) (Fig. 9). This area combines, to a certain extent, the acromial portion's property of contributing significantly to deltoid abductor function (Kuechle et al. 1997; Boileau et al. 2005; Ackland et al. 2008) with the anatomic condition of crossing structures that can be easily used for orientation (teres minor muscle) and quantification (posterior humeral circumflex artery). Finally, the adjusted scan plane visualizes (Fig. 10) the following:

1. The posterior humeral circumflex artery, which as a standardized reference for quantification has been found to be more feasible than the teres minor muscle, which was initially intended for this purpose (Fischer et al. 2016a, 2017).
2. The surgical neck of the humerus, forming a trough with the humeral shaft that comprises the cross section of the teres minor muscle (now serving primarily as a landmark for transducer positioning besides the bony structures)
3. An apparent delineation of the deltoid muscle tissue by its fasciae, complying with the criteria for CEUS assessment of the supraspinatus muscle tissue.

CEUS procedure. Similar to activation of the supraspinatus muscle, the patient is instructed to repeatedly abduct the extended arm up to 90° for a period of 120 s (frequency of direction change = 1 Hz), but in the coronal plane with neutral rotation of the arm. Afterward, the patient rests her or his palm on the thigh and CEUS is started immediately after bolus injection of 2.4 mL of UCA. Finally, quantitative analysis of the resulting 90-s video clip is performed.
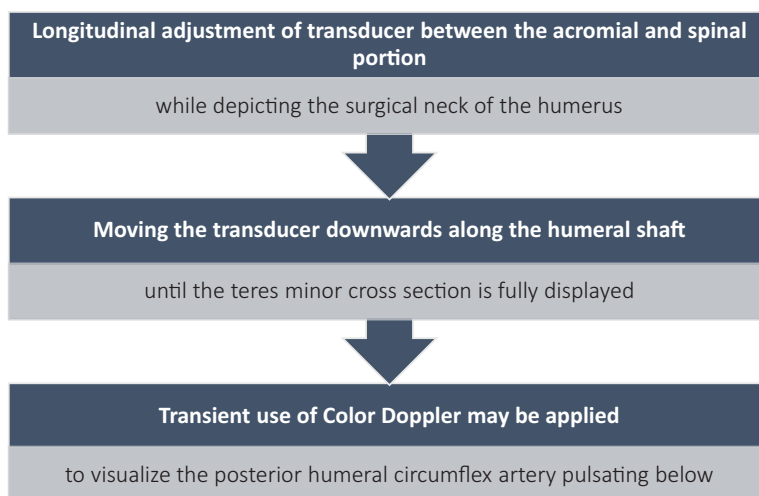
Fig. 9. Adjustment of the primary scan plane preceding contrast-enhanced ultrasound examination of the deltoid muscle tissue.
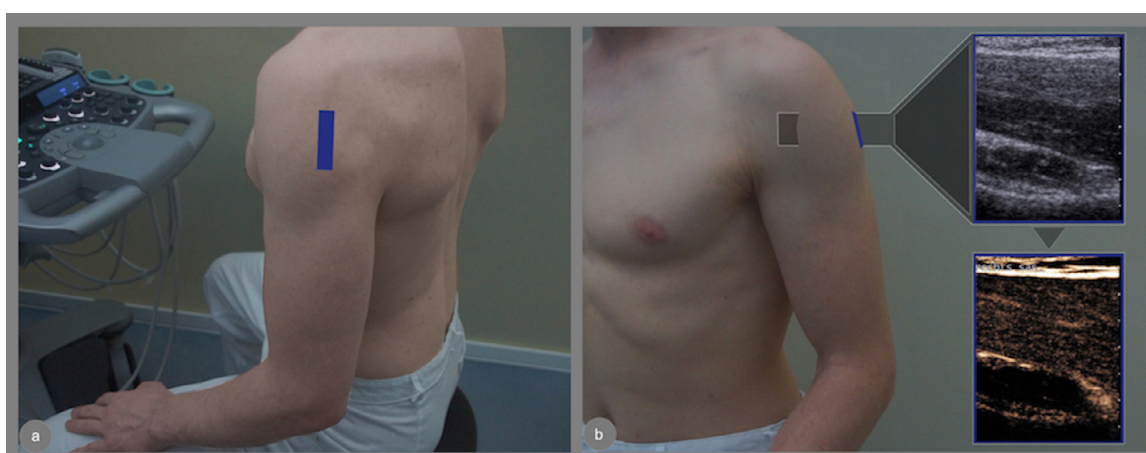

Fig. 10. Primary scan plane for contrast-enhanced ultrasound examination of the deltoid muscle tissue. (a) Longitudinal transducer orientation at the transition between the acromial and spinal portion of the muscle. (b) Corresponding B-mode and contrast mode ultrasound images; focus and depth settings optimally adapted. Informed patient consent for publication available.

Inflammatory, degenerative and neoplastic MSK pathologies

Inflammatory rheumatic diseases. In the case of inflammatory activity within joints and synovia, it is important to differentiate between active and inactive disease. CEUS techniques have been analyzed for these purposes in a number of regions among patients with inflammatory disorders (Wamser et al. 2003; Klauser et al. 2005a, 2009; Rees et al. 2007; Song et al. 2008), predominantly to estimate synovial hypervascularity caused by angiogenesis, which is believed to contribute to articular destruction and erosion (Fig. 11). Some applications focused even further on detectable changes during therapeutic follow-up or the evaluation of soft tissue involvement (Schueller-Weidekamm et al. 2007; Klauser et al. 2010; Mouterde et al. 2014; Tamas et al. 2015).
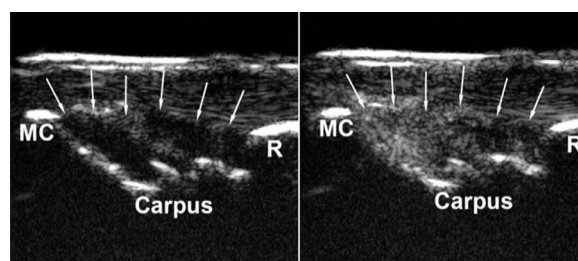

Fig. 11. Contrast-enhanced ultrasound pannus hyperperfusion in a patient with clinically active rheumatoid arthritis. Longitudinal dorsal wrist with high-grade enhancement in the synovial proliferation (*arrows*) after ultrasound contrast agent injection (right side) indicating active joint inflammation. MC = metacarpus; R = radius. Informed patient consent for publication available.

While CEUS revealed poor correlation with clinical symptoms and signs according to Rees et al. (2007), synovial samples of patients with psoriatic arthritis confirmed the association of CEUS perfusion kinetics with histopathological and immunohistochemical signs of microvascular proliferation, suggesting CEUS is a valid method for assessing synovial hypervascularity (Fiocco et al. 2015). However, the exact clinical relevance still needs to be clarified.

Another focus of research is inflammatory myopathies. In this context, CEUS was proposed as an adjuvant method for the detection of acute myositis *via* muscular hyperperfusion (Weber 2009) after patients with histologically confirmed dermatomyositis or polymyositis had manifested significantly higher local muscle perfusion in the affected muscles than patients without these diseases (Weber et al. 2006a, 2006c).

Chronic tendinopathy

In chronic tendinopathy, specific histopathological changes in tendon constituents occur, with localized tendon widening, irregular appearance of fiber structure and hypo-echoic areas as common ultrasonographic correlates (Khan et al. 1999; Alfredson et al. 2003; Xu and Murrell 2008). Moreover, the neovascularization in association with neural ingrowth seen is hypothesized to play a causal role in the presence of pain among affected patients (Alfredson et al. 2003; Ohberg and Alfredson 2004; Xu and Murrell 2008; Li and Hua 2016).

Considering the growing interest in sonographic visualization of such neovascularity, CEUS techniques have been found to be favorable according to previous studies on Achilles tendinopathy. While having higher sensitivity than conventional US modalities, they further contributed to the ongoing debate on the clinical value of neovessel detection and proved promising for tendinopathy research (Genovese et al. 2011; Shen et al. 2012; Pingel et al. 2013a, 2013b; De Marchi et al. 2018; Praet et al. 2018).

Soft tissue masses

Background. Soft tissue masses (STMs) are frequently encountered in everyday practice at a relatively high incidence, but still pose a significant challenge because of their imaging features often overlap between those of benign and malignant lesions. Malignant soft tissue sarcomas (STSs) are rare, with an estimated incidence of 1% of overall STMs (Kransdorf 1995; Toro et al. 2006). The foremost aim in the evaluation of such lesions ought to be a rapid radiologic workup and the differentiation of malignant entities from their benign counterparts, as delay in diagnosis and treatment can worsen the prognosis because of greater local complications, metastases and larger surgical resections (Grimer 2006; Maretty-Nielsen et al. 2014). A manifold approach is usually employed to differentiate malignant STSs from benign or intermediate entities using radiography, US and MRI (Clark et al. 2005; Loizides et al. 2012; Gruber et al. 2017a, 2017b). Despite significant progress in diagnostic imaging, correct classification of malignant soft tissue tumors is rarely achieved based on imaging alone; a guided or open surgical biopsy is usually mandatory.

Various studies based their evaluation of STMs on morphologic features and tumor vascularity using CCDS (Bodner et al. 2002; Datir et al. 2008; Lakkaraju et al. 2009; Widmann et al. 2009). These proved to be helpful in the differentiation between malignant and benign STMs; however, the high degree of uncertainty remaining does not allow for further therapeutic triage.

CEUS has become a helpful adjunct in the radiologic workup and stratification of STMs: vessels with a diameter as small as 40 μm (Lassau et al. 2001), corresponding to the pre- and post-capillary systems, can be visualized. Contrary to other contrast agents used in MRI, the strictly intravascular microbubble UCA with no relevant interstitial phase can depict actual tumor angiogenesis. This enables the definition of four different perfusion patterns as described by Loizides et al. (2012), which allows for a differentiation of STMs beyond the scope of gray-scale and Doppler/Duplex US as follows (Fig. 12): P1 = non-enhancing STM; P2 = peripherally enhancing STM with central non-enhancing region; P3 = diffusely enhancing STM with patchy non-enhancing areas; P4 = completely homogeneously enhancing STM.

The vast majority of STSs exhibit a P2 or P3 perfusion pattern, whereas benign STMs usually exhibit a P1
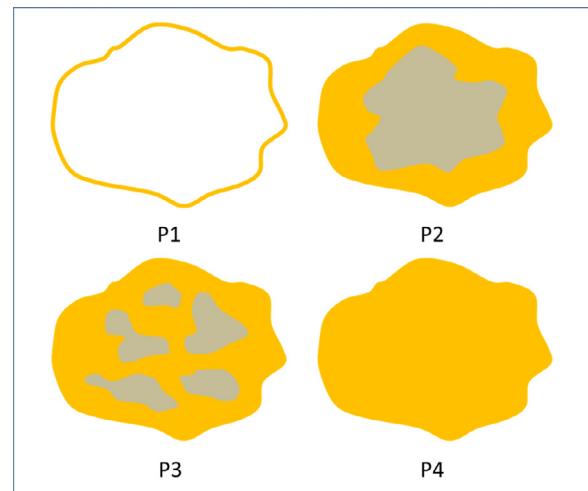

Fig. 12. Contrast-enhanced ultrasound perfusion patterns in the diagnostic workup of soft tissue masses. P1 = no enhancement; P2 = peripheral enhancement with central non-enhancing region; P3 = diffuse enhancement with patchy non-enhancing areas; P4 = completely homogeneous enhancement. It has been reported that the majority of soft tissue sarcomas exhibit a P2 or P3 perfusion pattern in contrast to benign soft tissue masses, which usually exhibit a P1 or P4 perfusion pattern (Loizides et al. 2012).

or P4 pattern (Loizides et al. 2012). This can be explained by analyzing the pathophysiology of STMs: malignant STMs manifest rapid cell proliferation and thus develop a much higher interstitial pressure compared with benign lesions (Jain 1987, 1988). This leads to a central underperfusion, which eventually results, together with pathologic tumor vessels, in areas with necrosis (non-enhancing areas: P2 or P3 pattern). In contrast, benign lesions, which do not outgrow their vital blood supply, are non-enhancing ("pseudotumors") or exhibit a regular, hierarchic and thus stable angiogenesis (Jain 1987, 1988; Bodner et al. 2002).

In evaluation of an STM, radiographic and some known MRI features should be taken into account, in addition to morphologic parameters such as configuration, location, gray-scale and Doppler features (Widmann et al. 2009; Gruber et al. 2017a). All available parameters combined with CEUS can be decisive in a holistic patient workup. Moreover, the definition of vital neoplasm can be depicted *via* CEUS, and if a subsequent

US-guided biopsy is needed, the needle can "target" these areas to minimize the rate of inconclusive histology resulting from biopsy of necrotic tumor areas (Loizides et al. 2011).

Pre-assessment and primary scan plane. An initial gray-scale and Doppler US is performed to assess the dimensions of the STM and to evaluate the surrounding tissue and intended needle path for a subsequent US-guided biopsy. For CEUS, the probe is placed primarily with its orientation in line with the greatest diameter of the STM.

CEUS procedure. Usually, a single injection of 2.4 mL of UCA is used. Because of the often "multicomponent" tissue texture of STMs (and, thus, acoustic signal loss), it might be necessary to escalate the volume administered (as recommended by the vendor) up to 4.8 mL to optimize the visibility of the CEUS texture of the tumor. After contrast injection, continuous digital cine loops as long as 3 min are acquired. During
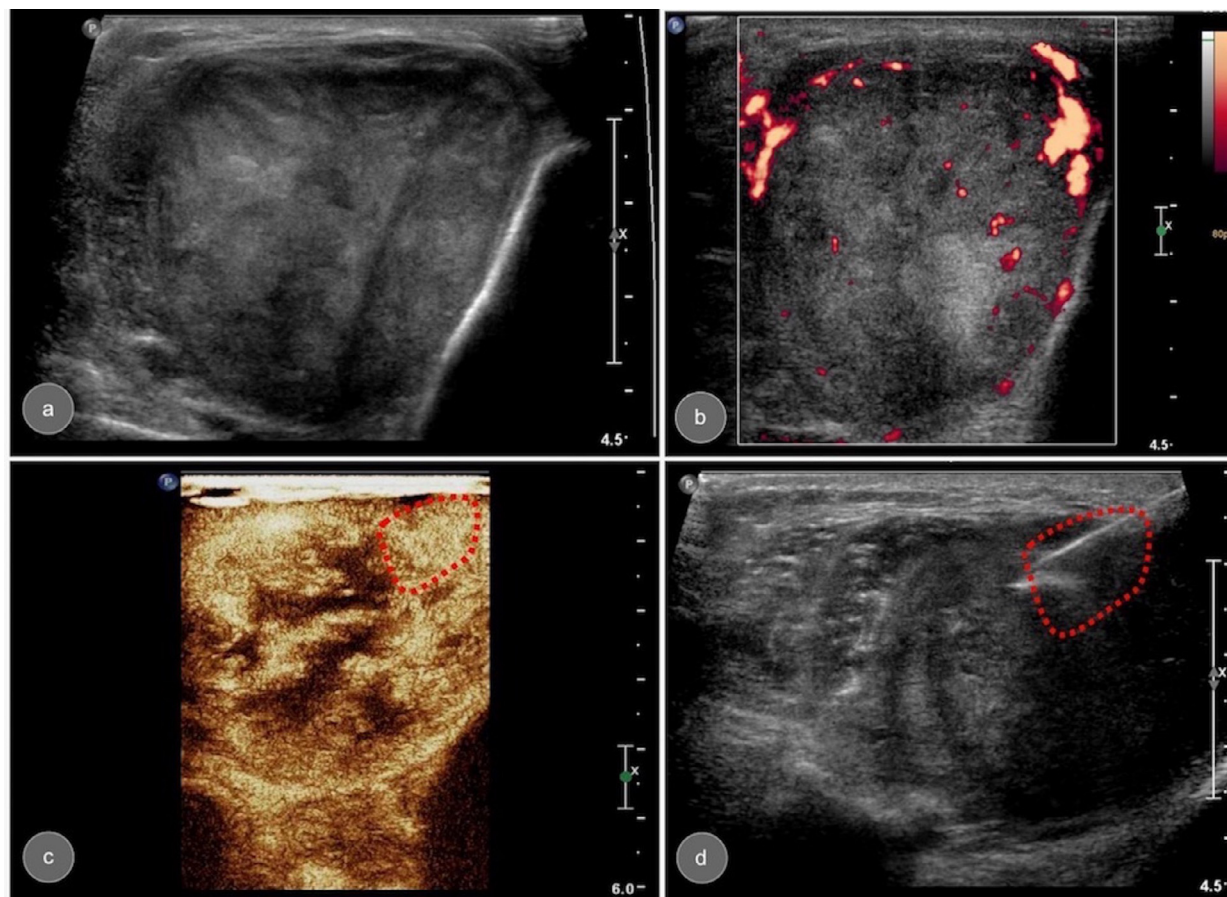

Fig. 13. Ultrasonographic depiction of a histologically verified clear cell sarcoma on the left lower leg of a 47-y-old male patient. (a) B-Mode scan. (b) Power Doppler scan revealing inhomogeneous, mostly peripheral tumour vessels. (c) Contrast-enhanced ultrasound image revealing a P3 perfusion pattern highly suspicious for malignancy. Definition of a target area (*red dotted line*) for the subsequent ultrasound-guided biopsy. (d) Ultrasound-guided biopsy from the predefined region (*red dotted line*). Informed patient consent for publication available.

contrast enhancement, the probe (adjustment unchanged) is moved to both sides along an imaginary axis orthogonal to the primary scan plane so that the whole STM is addressed. Afterward, qualitative assessment of the STM contrast enhancement pattern is performed, enabling the definition of a target area for subsequent US-guided biopsy if requested (Fig. 13).

Through review of the acquired cine loops, the area with the most pronounced enhancement is defined and correlated with the gray-scale images (some US vendors even provide tools for image superimposition). The probe position is marked on the skin, and biopsy is performed using standardized procedures.

BASIC RESEARCH ON MUSCLE PERFUSION PHYSIOLOGY

Apart from the primarily clinical focus on patients who suffer from severe, often chronic conditions, there has been emerging CEUS research on characteristic muscle physiology, which is particularly relevant for healthy and active individuals because of the possible use in sports science/sports medicine.

Early studies on muscular CEUS with high-MI techniques have reported that local blood volume measurements correlated with aerobic capacity (VO_{2max}) and capillary fiber contacts in histology. A great variability in muscle microcirculation at rest was observed, with the highest values for people practicing endurance sports (Weber et al. 2006b, 2007a, 2007b).

Another possibility to investigate muscle perfusion physiology was provided through the application of low-MI CEUS techniques in combination with a continuous infusion of UCA.

During different levels of isometric exercise this method revealed a lower or a higher local blood volume according to Krix et al. (2010), reflecting the effect of the complex relationship between muscle perfusion regulation and local tissue pressure on UCA signals. CEUS detects not only arterial blood flow but also venous blood flow, and the latter can be reduced during muscle exercise, depending on the individual's fitness level.

Current applied research used CEUS to increase accuracy in the ultrasonographic diagnosis of low-grade muscle lesions (Hotfiel et al. 2016, 2018), as well as to examine muscular regeneration processes in professional and recreational athletes (Genovese et al. 2007; Kellermann et al. 2017). Kellermann et al. (2017) reported significantly increased PE and wash-in area under the curve parameters for gastrocnemius muscle perfusion compared with baseline in a delayed-onset muscle soreness model, suggesting CEUS is a feasible diagnostic tool even for ultrastructural muscle lesions, with the potential to evaluate relevant preventative and recovery strategies.

REFERENCES

Ackland DC, Pak P, Richardson M, Pandy MG. Moment arms of the muscles crossing the anatomical shoulder. J Anat 2008;213:383–390.

Alfredson H, Ohberg L, Forsgren S. Is vasculo-neural ingrowth the cause of pain in chronic Achilles tendinosis? An investigation using ultrasonography and colour Doppler, immunohistochemistry, and diagnostic injections. Knee Surg Sports Traumatol Arthrosc 2003;11:334–338.

Amarteifio E, Krix M, Wormsbecher S, Demirel S, Braun S, Delorme S, Kauczor HU, Böckler D, Weber MA. Dynamic contrast-enhanced ultrasound for assessment of therapy effects on skeletal muscle microcirculation in peripheral arterial disease: Pilot study. Eur J Radiol 2013;82:640–646.

Averkiou M, Lampaskis M, Kyriakopoulou K, Skarlos D, Klouvas G, Strouthos C, Leen E. Quantification of tumor microvascularity with respiratory gated contrast enhanced ultrasound for monitoring therapy. Ultrasound Med Biol 2010;36:68–77.

Bahney CS, Hu DP, Miclau T, III, Marcucio RS. The multifaceted role of the vasculature in endochondral fracture repair. Front Endocrinol (Lausanne) 2015;6:4.

Bodner G, Schocke MF, Rachbauer F, Seppi K, Peer S, Fierlinger A, Sununu T, Jaschke WR. Differentiation of malignant and benign musculoskeletal tumors: Combined color and power Doppler US and spectral wave analysis. Radiology 2002;223:410–416.

Boileau P, Watkinson DJ, Hatzidakis AM, Balg F. Grammont reverse prosthesis: Design, rationale, and biomechanics. J Shoulder Elbow Surg 2005;14:147S–161S.

Brown JS, Devine JC, Magennis P, Sillifant P, Rogers SN, Vaughan ED. Factors that influence the outcome of salvage in free tissue transfer. Br J Oral Maxillofac Surg 2003;41:16–20.

Cai XH, Yang SP, Shen HL, Lin LQ, Zhong R, Wu RM, Lv GR. Application of contrast-enhanced ultrasonography and ultrasonography scores in rheumatoid arthritis. Int J Clin Exp Med 2015;8:20056–20064.

Christensen-Jeffries K, Browning RJ, Tang MX, Dunsby C, Eckersley RJ. In vivo acoustic super-resolution and super-resolved velocity mapping using microbubbles. IEEE Trans Med Imaging 2015; 34:433–440.

Clark MA, Fisher C, Judson I, Thomas JM. Soft-tissue sarcomas in adults. N Engl J Med 2005;353:701–711.

Claudon M, Dietrich CF, Choi BI, Cosgrove DO, Kudo M, Nolsoe CP, Piscaglia F, Wilson SR, Barr RG, Chammas MC, Chaubal NG, Chen MH, Clevert DA, Correas JM, Ding H, Forsberg F, Fowlkes JB, Gibson RN, Goldberg BB, Lassau N, Leen EL, Mattrey RF, Moriyasu F, Solbiati L, Weskott HP, Xu HX. 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.

Committee for Medicinal Products for Human Use (CHMP). Assessment report EMA/84084/2014. SonoVue. International non-proprietary name: sulfur hexafluoride, Procedure No. EMEA/H/C/000303/II/0025. 2014.

Datir A, James SLJ, Ali K, Lee J, Ahmad M, Saifuddin A. MRI of soft-tissue masses: The relationship between lesion size, depth, and diagnosis. Clin Radiol 2008;63:373–378.

Davidson BP, Belcik JT, Mott BH, Landry G, Lindner JR. Quantification of residual limb skeletal muscle perfusion with contrast-enhanced ultrasound during application of a focal junctional tourniquet. J Vasc Surg 2016;63:148–153.

Davidson BP, Belcik JT, Landry G, Linden J, Lindner JR. Exercise versus vasodilator stress limb perfusion imaging for the assessment of peripheral artery disease. Echocardiography 2017;34:1187–1194.

De Marchi A, Pozza S, Cenna E, Cavallo F, Gays G, Simbula L, De Petro P, Massè A, Massazza G. In Achilles tendinopathy, the neovascularization, detected by contrast-enhanced ultrasound (CEUS), is abundant but not related to symptoms. Knee Surg Sports Traumatol Arthrosc 2018;26:2051–2058.

Dietrich CF, Averkiou MA, Correas JM, Lassau N, Leen E, Piscaglia F. An EFSUMB introduction into dynamic contrast-enhanced ultrasound (DCE-US) for quantification of tumour perfusion. Ultraschall Med 2012;33:344–351.

Dietrich CF, Averkiou M, Nielsen MB, Barr RG, Burns PN, Calliada F, Cantisani V, Choi B, Chammas MC, Clevert DA, Claudon M, Correas JM, Cui XW, Cosgrove D, D'Onofrio M, Dong Y, Eisenbrey J, Fontanilla T, Gilja OH, Ignee A, Jenssen C, Kono Y, Kudo M, Lassau N, Lyshchik A, Franca Meloni M, Moriyasu F, Nolsoe C, Piscaglia F, Radzina M, Saftoiu A, Sidhu PS, Sporea I, Schreiber-Dietrich D, Sirlin CB, Stanczak M, Weskott HP, Wilson SR, Willmann JK, Kim TK, Jang HJ, Vezeridis A, Westerway S. How to perform contrast-enhanced ultrasound (CEUS). Ultrasound Int Open 2018;4:E2–E15.

Doll J, Gross S, Weber MA, Schmidmaier G, Fischer C. The AMANDUS Project—Advanced Microperfusion Assessed Non-Union Diagnostics with Contrast-Enhanced Ultrasound (CEUS) for the Detection of Infected Lower Extremity Non-Unions. Ultrasound Med Biol 2019;45:2281–2288.

Duerschmied D, Zhou Q, Rink E, Harder D, Freund G, Olschewski M, Bode C, Hehrlein C. Simplified contrast ultrasound accurately reveals muscle perfusion deficits and reflects collateralization in PAD. Atherosclerosis 2009;202:505–512.

Fiocco U, Stramare R, Coran A, Grisan E, Scagliori E, Caso F, Costa L, Lunardi F, Oliviero F, Bianchi FC, Scanu A, Martini V, Boso D, Beltrame V, Vezzu M, Cozzi L, Scarpa R, Sacerdoti D, Punzi L, Doria A, Calabrese F, Rubaltelli L. Vascular perfusion kinetics by contrast-enhanced ultrasound are related to synovial microvascularity in the joints of psoriatic arthritis. Clin Rheumatol 2015;34:1903–1912.

Fischer C, Frank M, Kunz P, Tanner M, Weber MA, Moghaddam A, Schmidmaier G, Hug A. Dynamic contrast-enhanced ultrasound (CEUS) after open and minimally invasive locked plating of proximal humerus fractures. Injury 2016a;47:1725–1731.

Fischer C, Preuss EM, Tanner M, Bruckner T, Krix M, Amarteifio E, Miska M, Moghaddam-Alvandi A, Schmidmaier G, Weber MA. Dynamic contrast-enhanced sonography and dynamic contrast-enhanced magnetic resonance imaging for preoperative diagnosis of infected nonunions. J Ultrasound Med 2016b;35:933–942.

Fischer C, Krammer D, Hug A, Weber MA, Kauczor HU, Krix M, Bruckner T, Kunz P, Schmidmaier G, Zeifang F. Dynamic contrast-enhanced ultrasound and elastography assess deltoid muscle integrity after reverse shoulder arthroplasty. J Shoulder Elbow Surg 2017;26:108–117.

Fischer C, Gross S, Zeifang F, Schmidmaier G, Weber MA, Kunz P. Contrast-enhanced ultrasound determines supraspinatus muscle atrophy after cuff repair and correlates to functional shoulder outcome. Am J Sports Med 2018;46:2735–2742.

Fischer C, Flammer S, Kauczor HU, Zeifang F, Schmidmaier G, Kunz P. Preoperative deltoid assessment by contrast-enhanced ultrasound (CEUS) as predictor for shoulder function after reverse shoulder arthroplasty: a prospective pilot study. Arch Orthop Trauma Surg 2019. doi: 10.1007/s00402-019-03281-w Accessed by 17 Oct 2019. PMID: 31624864. [Epub ahead of print].

Fischer C, Haug T, Weber MA, Kauczor HU, Bruckner T, Schmidmaier G. Contrast-enhanced ultrasound (CEUS) identifies perfusion differences between tibial fracture unions and non-unions. Ultraschall Med 2020;41:44–51.

Gao Y, Yuan Y, Li H, Gu B, Xie F, Herrler T, Li Q, Zan T. Preoperative imaging for thoracic branch of supraclavicular artery flap: A comparative study of contrast-enhanced ultrasound with three-dimensional reconstruction and color duplex ultrasound. Ann Plast Surg 2016;77:201–205.

Geis S, Prantl L, Gehmert S, Lamby P, Nerlich M, Angele P, Egger L, Jung EM. TTP (time to PEAK) and RBV (regional blood volume) as valuable parameters to detect early flap failure. Clin Hemorheol Microcirc 2011;48:81–94.

Geis S, Prantl L, Dolderer J, Lamby P, Mueller S, Jung EM. Postoperative monitoring of local and free flaps with contrast-enhanced ultrasound (CEUS)—Analysis of 112 patients. Ultraschall Med 2013a;34:550–558.

Geis S, Prantl L, Mueller S, Gosau M, Lamby P, Jung EM. Quantitative assessment of bone microvascularization after osteocutaneous flap transplantation using contrast-enhanced ultrasound (CEUS). Ultraschall Med 2013b;34:272–279.

Geis S, Klein S, Prantl L, Dolderer J, Lamby P, Jung EM. Quantitative assessment of free flap viability with CEUS using an integrated perfusion software. Handchir Mikrochir Plast Chir 2015;47:389–395.

Geis S, Prantl L, Schoeneich M, Lamby P, Klein S, Dolderer J, Mueller S, Jung EM. Contrast enhanced ultrasound (CEUS)—An unique monitoring technique to assess microvascularization after buried flap transplantation. Clin Hemorheol Microcirc 2016;62:205–214.

Genovese E, Ronga M, Recaldini C, Fontana F, Callegari L, Maffulli N, Fugazzola C. Analysis of Achilles tendon vascularity with second-generation contrast-enhanced ultrasound. J Clin Ultrasound 2011;39:141–145.

Genovese EA, Callegari L, Combi F, Leonardi A, Angeretti MG, Benazzo F, D'Angelo F, Fugazzola C. Contrast enhanced ultrasound with second generation contrast agent for the follow-up of lower-extremity muscle-strain-repairing processes in professional athletes. Radiol Med 2007;112:740–750.

Giannoudis PV, Einhorn TA, Schmidmaier G, Marsh D. The diamond concept—Open questions. Injury 2008;39(Suppl 2):S5–S8.

Greis C. Technology overview: SonoVue (Bracco, Milan). Eur Radiol 2004;14(Suppl 8):P11–P15.

Grimer RJ. Size matters for sarcomas!. Ann R Coll Surg Engl 2006;88:519–524.

Gruber L, Gruber H, Luger AK, Glodny B, Henninger B, Loizides A. Diagnostic hierarchy of radiological features in soft tissue tumours and proposition of a simple diagnostic algorithm to estimate malignant potential of an unknown mass. Eur J Radiol 2017a;95:102–110.

Gruber L, Loizides A, Luger AK, Glodny B, Moser P, Henninger B, Gruber H. Soft-tissue tumor contrast enhancement patterns: Diagnostic Value and Comparison Between Ultrasound and MRI. AJR Am J Roentgenol 2017b;208:393–401.

Hotfiel T, Carl HD, Swoboda B, Engelhardt M, Heinrich M, Strobel D, Wildner D. [Contrast-enhanced ultrasound in diagnostic imaging of muscle injuries: Perfusion imaging in the early arterial phase]. Sportverletz Sportschaden 2016;30:54–57.

Hotfiel T, Heiss R, Swoboda B, Kellermann M, Gelse K, Grim C, Strobel D, Wildner D. Contrast-enhanced ultrasound as a new investigative tool in diagnostic imaging of muscle injuries—A pilot study evaluating conventional ultrasound, CEUS, and findings in MRI. Clin J Sport Med 2018;28:332–338.

Jain RK. Transport of molecules in the tumor interstitium: A review. Cancer Res 1987;47:3039–3051.

Jain RK. Determinants of tumor blood flow: A review. Cancer Res 1988;48:2641–2658.

Kehrer A, Mandlik V, Taeger C, Geis S, Prantl L, Jung EM. Postoperative control of functional muscle flaps for facial palsy reconstruction: Ultrasound guided tissue monitoring using contrast enhanced ultrasound (CEUS) and ultrasound elastography. Clin Hemorheol Microcirc 2017;67:435–444.

Kellermann M, Heiss R, Swoboda B, Gelse K, Freiwald J, Grim C, Nagel A, Uder M, Wildner D, Hotfiel T. Intramuscular perfusion response in delayed onset muscle soreness (DOMS): A quantitative analysis with contrast-enhanced ultrasound (CEUS). Int J Sports Med 2017;38:833–841.

Keramaris NC, Calori GM, Nikolaou VS, Schemitsch EH, Giannoudis PV. Fracture vascularity and bone healing: A systematic review of the role of VEGF. Injury 2008;39(Suppl 2):S45–S57.

Khan KM, Cook JL, Bonar F, Harcourt P, Astrom M. Histopathology of common tendinopathies: Update and implications for clinical management. Sports Med 1999;27:393–408.

Khoury V, Cardinal E, Brassard P. Atrophy and fatty infiltration of the supraspinatus muscle: Sonography versus MRI. AJR Am J Roentgenol 2008;190:1105–1111.

Klauser A, Frauscher F, Schirmer M, Halpern E, Pallwein L, Herold M, Helweg G, ZurNedden D. The value of contrast-enhanced color Doppler ultrasound in the detection of vascularization of finger joints in patients with rheumatoid arthritis. Arthritis Rheum 2002;46:647–653.

Klauser A, Demharter J, De Marchi A, Sureda D, Barile A, Masciocchi C, Faletti C, Schirmer M, Kleffel T, Bohndorf K. Contrast enhanced gray-scale sonography in assessment of joint vascularity in rheumatoid arthritis: Results from the IACUS study group. Eur Radiol 2005a;15:2404–2410.

Klauser A, Halpern EJ, Frauscher F, Gvozdic D, Duftner C, Springer P, Schirmer M. Inflammatory low back pain: High negative predictive value of contrast-enhanced color Doppler ultrasound in the detection of inflamed sacroiliac joints. Arthritis Rheum 2005b;53:440–444.

Klauser AS, De Zordo T, Bellmann-Weiler R, Feuchtner GM, Sailer-Höck M, Sögner P, Gruber J. Feasibility of second-generation ultrasound contrast media in the detection of active sacroiliitis. Arthritis Rheum 2009;61:909–916.

Klauser AS, Franz M, Arora R, Feuchtner GM, Gruber J, Schirmer M, Jaschke WR, Gabl MF. Detection of vascularity in wrist tenosynovitis: Power Doppler ultrasound compared with contrast-enhanced grey-scale ultrasound. Arthritis Res Ther 2010;12:R209.

Klenze H, Köhler TC, Farquharson F, Walterspacher S, Duerschmied D, Roecker K, Kabitz HJ, Walker DJ. Resting limb muscle perfusion during inspiratory muscle loading in hypoxia and normoxia. Respir Physiol Neurobiol 2017;244:1–9.

Krammer D, Schmidmaier G, Weber MA, Doll J, Rehnitz C, Fischer C. Contrast-enhanced ultrasound quantifies the perfusion within tibial non-unions and predicts the outcome of revision surgery. Ultrasound Med Biol 2018;44:1853–1859.

Kransdorf MJ. Benign soft-tissue tumors in a large referral population: Distribution of specific diagnoses by age, sex, and location. AJR Am J Roentgenol 1995;164:395–402.

Krix M, Weber MA, Kauczor HU, Delorme S, Krakowski-Roosen H. Changes in the micro-circulation of skeletal muscle due to varied isometric exercise assessed by contrast-enhanced ultrasound. Eur J Radiol 2010;76:110–116.

Krix M, Krakowski-Roosen H, Amarteifio E, Furstenberger S, Delorme S, Kauczor HU, Weber MA. Comparison of transient arterial occlusion and muscle exercise provocation for assessment of perfusion reserve in skeletal muscle with real-time contrast-enhanced ultrasound. Eur J Radiol 2011;78:419–424.

Kuechle DK, Newman SR, Itoi E, Morrey BF, An KN. Shoulder muscle moment arms during horizontal flexion and elevation. J Shoulder Elbow Surg 1997;6:429–439.

Kunz P, Mick P, Gross S, Schmidmaier G, Zeifang F, Weber MA, Fischer C. Contrast-enhanced ultrasound (CEUS) as predictor for early retear and functional outcome after supraspinatus tendon repair. J Orthop Res 2019; Available at: https://doi.org/10.1002/jor.24535. Accessed 26 Nov 2019. PMID: 31769543.

Kunz P, Kiesl S, Gro S, Kauczor HU, Schmidmaier G, Fischer C. Intra-observer and device-dependent inter-observer reliability of contrast-enhanced ultrasound for muscle perfusion quantification. Ultrasound Med Biol 2020;46:275–285.

Lakkaraju A, Sinha R, Garikipati R, Edward S, Robinson P. Ultrasound for initial evaluation and triage of clinically suspicious soft-tissue masses. Clin Radiol 2009;64:615–621.

Lam F, Bhatia D, Mostofi S, Van Rooyen K, De Beer J. Biomechanical considerations of the normal and rotator cuff deficient shoulders and the reverse shoulder prosthesis. Curr Orthop 2007;21:40–46.

Lassau N, Koscielny S, Opolon P, De Baere T, Peronneau P, Leclere J, Roche A. Evaluation of contrast-enhanced color Doppler ultrasound for the quantification of angiogenesis in vivo. Invest Radiol 2001;36:50–55.

Li HY, Hua YH. Achilles Tendinopathy: Current concepts about the basic science and clinical treatments. Biomed Res Int 2016;2016 6492597.

Lin F, Tsuruta JK, Rojas JD, Dayton PA. Optimizing sensitivity of ultrasound contrast-enhanced super-resolution imaging by tailoring size distribution of microbubble contrast agent. Ultrasound Med Biol 2017;43:2488–2493.

Lindau RH, Detwiller K, Wax MK. Buried free flaps in head and neck surgery: Outcome analysis. Head Neck 2013;35:1468–1470.

Loizides A, Widmann G, Freuis T, Peer S, Gruber H. Optimizing ultrasound-guided biopsy of musculoskeletal masses by application of an ultrasound contrast agent. Ultraschall Med 2011;32:307–310.

Loizides A, Peer S, Plaikner M, Djurdjevic T, Gruber H. Perfusion pattern of musculoskeletal masses using contrast-enhanced ultrasound: A helpful tool for characterisation? Eur Radiol 2012;22:1803–1811.

Marenzana M, Arnett TR. The key role of the blood supply to bone. Bone Res 2013;1:203–215.

Maretty-Nielsen K, Aggerholm-Pedersen N, Safwat A, Jørgensen PH, Hansen BH, Baerentzen S, Pedersen AB, Keller J. Prognostic factors for local recurrence and mortality in adult soft tissue sarcoma of the extremities and trunk wall. Acta Orthop 2014;85:323–332.

McElvany MD, McGoldrick E, Gee AO, Neradilek MB, Matsen FA, III. Rotator cuff repair: Published evidence on factors associated with repair integrity and clinical outcome. Am J Sports Med 2015;43:491–500.

Mouterde G, Aegerter P, Correas JM, Breban M, D'Agostino MA. Value of contrast-enhanced ultrasonography for the detection and quantification of enthesitis vascularization in patients with spondyloarthritis. Arthritis Care Res (Hoboken) 2014;66:131–138.

Mueller S, Wendl CM, Ettl T, Klingelhöffer C, Geis S, Prantl L, Reichert TE, Jung EM. Contrast-enhanced ultrasonography as a new method for assessing autonomization of pedicled and microvascular free flaps in head and neck reconstructive surgery. Clin Hemorheol Microcirc 2017;65:317–325.

Novakovic D, Patel RS, Goldstein DP, Gullane PJ. Salvage of failed free flaps used in head and neck reconstruction. Head Neck Oncol 2009;1:33.

Ohberg L, Alfredson H. Effects on neovascularisation behind the good results with eccentric training in chronic mid-portion Achilles tendinosis?. Knee Surg Sports Traumatol Arthrosc 2004;12:465–470.

Partovi S, Kaspar M, Aschwanden M, Robbin MR, Bilecen D, Walker UA, Staub D. Quantitative dynamic contrast-enhanced ultrasound for the functional evaluation of the skeletal muscle microcirculation in systemic sclerosis. Clin Hemorheol Microcirc 2016;62:35–44.

Pingel J, Harrison A, Simonsen L, Suetta C, Bülow J, Langberg H. The microvascular volume of the Achilles tendon is increased in patients with tendinopathy at rest and after a 1-hour treadmill run. Am J Sports Med 2013a;41:2400–2408.

Pingel J, Harrison A, Suetta C, Simonsen L, Langberg H, Bülow J. The acute effects of exercise on the microvascular volume of Achilles tendons in healthy young subjects. Clin Physiol Funct Imaging 2013b;33:252–257.

Piscaglia F, Bolondi L. The safety of Sonovue in abdominal applications: Retrospective analysis of 23188 investigations. Ultrasound Med Biol 2006;32:1369–1375.

Porter TR, Mulvagh SL, Abdelmoneim SS, Becher H, Belcik JT, Bierig M, Choy J, Gaibazzi N, Gillam LD, Janardhanan R, Kutty S, Leong-Poi H, Lindner JR, Main ML, Mathias WJ, Park MM, Senior R, Villanueva F. Clinical applications of ultrasonic enhancing agents in echocardiography: 2018 American Society of Echocardiography Guidelines Update. J Am Soc Echocardiogr 2018;31:241–274.

Praet SFE, Ong JH, Purdam C, Welvaert M, Lovell G, Dixon L, Gaida JE, Anglim J, Manzanero S, Vlahovich N, Hughes D, Waddington G. Microvascular volume in symptomatic Achilles tendons is associated with VISA-A score. J Sci Med Sport 2018;21:1185–1191.

Rees JD, Pilcher J, Heron C, Kiely PD. A comparison of clinical vs ultrasound determined synovitis in rheumatoid arthritis utilizing gray-scale, power Doppler and the intravenous microbubble contrast agent 'Sono-Vue'. Rheumatology (Oxford) 2007;46:454–459.

Rizzo G, Raffeiner B, Coran A, Ciprian L, Fiocco U, Botsios C, Stramare R, Grisan E. Pixel-based approach to assess contrast-enhanced ultrasound kinetics parameters for differential diagnosis of rheumatoid arthritis. J Med Imaging (Bellingham) 2015;2 034503.

Schueller-Weidekamm C, Krestan C, Schueller G, Kapral T, Aletaha D, Kainberger F. Power Doppler sonography and pulse-inversion harmonic imaging in evaluation of rheumatoid arthritis synovitis. AJR Am J Roentgenol 2007;188:504–508.

Shen HY, Chen SF, Wu CH, Chen WS, Wang TG, Chang KV. Contrast-enhanced sonography for the evaluation of neovascularization in tendinopathic tissues. J Med Ultrasound 2012;20:109–114.

Sidhu PS, Cantisani V, Dietrich CF, Gilja OH, Saftoiu A, Bartels E, Bertolotto M, Calliada F, Clevert DA, Cosgrove D, Deganello A,

D'Onofrio M, Drudi FM, Freeman S, Harvey C, Jenssen C, Jung EM, Klauser AS, Lassau N, Meloni MF, Leen E, Nicolau C, Nolsoe C, Piscaglia F, Prada F, Prosch H, Radzina M, Savelli L, Weskott HP, Wijkstra H. The EFSUMB Guidelines and Recommendations for the Clinical Practice of Contrast-Enhanced Ultrasound (CEUS) in non-hepatic applications: Update 2017 (long version). Ultraschall Med 2018;39:e2–e44.

Song IH, Althoff CE, Hermann KG, Scheel AK, Knetsch T, Schoenharting M, Werner C, Burmester GR, Backhaus M. Knee osteoarthritis. Efficacy of a new method of contrast-enhanced musculoskeletal ultrasonography in detection of synovitis in patients with knee osteoarthritis in comparison with magnetic resonance imaging. Ann Rheum Dis 2008;67:19–25.

Stramare R, Raffeiner B, Ciprian L, Scagliori E, Coran A, Perissinotto E, Fiocco U, Beltrame V, Rubaltelli L. Evaluation of finger joint synovial vascularity in patients with rheumatoid arthritis using contrast-enhanced ultrasound with water immersion and a stabilized probe. J Clin Ultrasound 2012;40:147–154.

Strobel K, Hodler J, Meyer DC, Pfirrmann CW, Pirkl C, Zanetti M. Fatty atrophy of supraspinatus and infraspinatus muscles: Accuracy of US. Radiology 2005;237:584–589.

Tamas MM, Bondor CI, Rednic N, Ghib LJ, Rednic S. The evolution of time−intensity curves of contrast enhanced ultrasonography in early arthritis patients with wrist involvement. Med Ultrason 2015;17:345–351.

Thomas KN, Cotter JD, Lucas SJ, Hill BG, van Rij AM. Reliability of contrast-enhanced ultrasound for the assessment of muscle perfusion in health and peripheral arterial disease. Ultrasound Med Biol 2015;41:26–34.

Toro JR, Travis LB, Wu HJ, Zhu K, Fletcher CDM, Devesa SS. Incidence patterns of soft tissue sarcomas, regardless of primary site, in the surveillance, epidemiology and end results program, 1978−2001: An analysis of 26,758 cases. Int J Cancer 2006;119:2922–2930.

Wamser G, Bohndorf K, Vollert K, Bücklein W, Schalm J. Power Doppler sonography with and without echo-enhancing contrast agent and contrast-enhanced MRI for the evaluation of rheumatoid arthritis of the shoulder joint: Differentiation between synovitis and joint effusion. Skeletal Radiol 2003;32:351–359.

Weber MA. Ultrasound in the inflammatory myopathies. Ann NY Acad Sci 2009;1154:159–170.

Weber MA, Jappe U, Essig M, Krix M, Ittrich C, Huttner HB, Meyding-Lamade U, Hartmann M, Kauczor HU, Delorme S. Contrast-enhanced ultrasound in dermatomyositis- and polymyositis. J Neurol 2006a; 253:1625–1632.

Weber MA, Krakowski-Roosen H, Delorme S, Renk H, Krix M, Millies J, Kinscherf R, Künkele A, Kauczor HU, Hildebrandt W. Relationship of skeletal muscle perfusion measured by contrast-enhanced ultrasonography to histologic microvascular density. J Ultrasound Med 2006b;25:583–591.

Weber MA, Krix M, Jappe U, Huttner HB, Hartmann M, Meyding-Lamade U, Essig M, Fiehn C, Kauczor HU, Delorme S. Pathologic skeletal muscle perfusion in patients with myositis: Detection with quantitative contrast-enhanced US—Initial results. Radiology 2006c;238:640–649.

Weber MA, Krakowski-Roosen H, Hildebrandt W, Schröder L, Ionescu I, Krix M, Kinscherf R, Bachert P, Kauczor HU, Essig M. Assessment of metabolism and microcirculation of healthy skeletal muscles by magnetic resonance and ultrasound techniques. J Neuroimaging 2007a;17:323–331.

Weber MA, Krix M, Delorme S. Quantitative evaluation of muscle perfusion with CEUS and with MR. Eur Radiol 2007b;17:2663–2674.

Widmann G, Riedl A, Schoepf D, Glodny B, Peer S, Gruber H. State-of-the-art HR-US imaging findings of the most frequent musculoskeletal soft-tissue tumors. Skeletal Radiol 2009;38:637–649.

Xu Y, Murrell GAC. The basic science of tendinopathy. Clin Orthop Relat Res 2008;466:1528–1538.

Yanagisawa O, Dohi M, Okuwaki T, Tawara N, Niitsu M, Takahashi H. Appropriate slice location to assess maximal cross-sectional area of individual rotator cuff muscles in normal adults and athletes. Magn Reson Med Sci 2009;8:65–71.