

EFSUMB Technical Review – Update 2023: Dynamic Contrast-Enhanced Ultrasound (DCE-CEUS) for the Quantification of Tumor Perfusion

Technisches Review der EFSUMB – Update 2023: Dynamischer Kontrastverstärkter Ultraschall (DCE-US) zur Quantifizierung der Tumorperfusion

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ABSTRACT

Dynamic contrast-enhanced ultrasound (DCE-US) is a technique to quantify tissue perfusion based on phase-specific enhancement after the injection of microbubble contrast agents for diagnostic ultrasound. The guidelines of the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) published in 2004 and updated in 2008, 2011, and 2020 focused on the use of contrast-enhanced ultrasound (CEUS), including essential technical requirements, training, investigational procedures and steps, guidance regarding image interpretation, established and recommended clinical indications, and safety considerations. However, the quantifica-

tion of phase-specific enhancement patterns acquired with ultrasound contrast agents (UCAs) is not discussed here. The purpose of this EFSUMB Technical Review is to further establish a basis for the standardization of DCE-US focusing on treatment monitoring in oncology. It provides some recommendations and descriptions as to how to quantify dynamic ultrasound contrast enhancement, and technical explanations for the analysis of time-intensity curves (TICs). This update of the 2012 EFSUMB introduction to DCE-US includes clinical aspects for data collection, analysis, and interpretation that have emerged from recent studies. The current study not only aims to support future work in this research field but also to facilitate a transition to clinical routine use of DCE-US.

ZUSAMMENFASSUNG

Der DCE-US (Dynamic contrast-enhanced ultrasound) ist eine Quantifizierungstechnik des kontrastverstärkten Ultraschalls. Die EFSUMB-Leitlinien von 2004, mit Updates aus den Jahren

2008, 2011, 2013 und 2020, erläutern die Grundlagen der Ultraschall-Kontrastmitteltechniken, geben aber keine detaillierten Informationen zu den Anwendungsmöglichkeiten, der Vorgehensweise und den Besonderheiten des DCE-US. Ziel dieses EFSUMB-Dokuments ist es nun, auf der Basis einer aktuellen Literaturrecherche Standardisierungsgrundlagen zur Methodik des DCE-US – insbesondere für das Therapiemonitoring bei onkologischen Erkrankungen – weiter zu vertiefen. Die notwendigen Grundlagen und technischen Voraussetzungen für die Analyse von Zeit-Intensitätskurven werden vorgestellt. Das vorliegende Update eines EFSUMB-Statements aus dem Jahr 2012 berücksichtigt klinische Aspekte aufgrund jüngster Studien für einen standardisierten Ablauf der Daten-Akquise und -Analyse sowie Empfehlungen zur Interpretation. Die aktuelle Arbeit zielt nicht nur darauf ab, künftige Arbeiten auf diesem Forschungsgebiet zu unterstützen, sondern auch den Übergang zur klinischen Routineanwendung des DCE-US zu erleichtern.

Introduction

Dynamic contrast-enhanced ultrasound (DCE-US) is a technique to quantify tissue perfusion down to the capillary level based on phase-specific enhancement after injection of microbubble contrast agents for diagnostic ultrasound. In addition, the quantitative analysis of the dynamics of contrast enhancement overcomes its subjective comparison between normal and abnormal parenchyma, or between a focal lesion and the surrounding tissue.

The guidelines of the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) published in 2004 [1] and updated in 2008 [2], 2012 [3], 2013 [4, 5], and 2020 [6, 7] focused on the use of contrast-enhanced ultrasound (CEUS), including essential technical requirements, training, investigational procedures and steps, guidance regarding image interpretation, established and recommended clinical indications, and safety considerations. However, the quantification of phase-specific tissue enhancement acquired with ultrasound contrast agents (UCAs) is not discussed. The basis for the standardization of DCE-US has been established and published by the EFSUMB introductory paper in 2012 [3]. It provided some recommendations and descriptions of the quantification of DCE-US images, and technical explanations for the analysis of time-intensity curves (TICs).

As part of the development of professional standards for diagnostic ultrasound techniques [8] and in accordance with the regulations for EFSUMB policy documents published in 2019 [9], the current update was prepared on the basis of an up-to-date literature search. It includes clinical aspects for data collection, analysis, and interpretation in the quantification of tumor perfusion, which are derived from recent studies. This study focuses on the clinical assessment in oncology, but the basic considerations are generally transferable to other DCE-US indications such as treatment monitoring in inflammatory bowel disease or chronic kidney disease. The current study not only aims to support future work in this research field but also to facilitate a transition to clinical routine use of DCE-US.

Why do we need quantification?

Quantification of CEUS is needed to evaluate data objectively, to enable comparison of imaging techniques, to evaluate new UCA applications, to quantify tissue and tumor enhancement in order to characterize focal lesions, to evaluate therapeutic response, and to limit variability in clinical diagnosis [3]. Tissue perfusion is a relevant functional imaging parameter with pathophysiological and clinical relevance in different clinical settings and can be assessed with different imaging techniques, e. g., brain perfusion in stroke imaging using magnetic resonance imaging (diffusion) or dynamic contrast-enhanced computed tomography or myocardial perfusion using dynamic contrast-enhanced echocardiography for the heart.

An objective and quantitative diagnosis of perfusion characteristics is of particular relevance in the follow-up of cancer patients but can also be used for the diagnostic assessment of other pathological changes associated with alterations in tissue perfusion. This applies, for example, to the noninvasive diagnosis of the progression of parenchymal liver disease, liver cirrhosis, and portal hypertension [10, 11, 12, 13, 14, 15, 16] and for the noninvasive evaluation of chronic kidney disease [17, 18, 19] and subclinical kidney transplant rejection [17, 20, 21, 22, 23, 24]. There are partially contradictory data regarding the evaluation of inflammatory activity and response to biologic therapy in inflammatory bowel disease [25, 26, 27, 28, 29, 30, 31, 32, 33, 34]. A relatively new field of research is the application of DCE-US for the differential diagnosis, grading of the biological behavior, and outcome assessment of malignant tumors [35, 36, 37, 38, 39, 40, 41]. This position paper is focused on the assessment of tumor perfusion.

DCE-US as a dynamic examination is based on relatively long video sequences that measure changes in contrast signal over time from the bolus transit in the body. For precise diagnostic evaluation, such data need to be analyzed to extract biomarkers and other parameters that are related to relevant physiologic and

patho-physiologic properties and presented in a form that is compatible with the imaging process (e. g., color coded maps). It may be anticipated that such quantitative measures may play a major role in big data analysis and the development of machine learning, which itself may influence diagnostic approaches. Thus DCE-US has the potential to strengthen the role of CEUS in future diagnosis and follow-up [42, 43].

Current assessment of response to cancer treatment is still mainly based on interval evaluation of the tumor size according to the Response Evaluation Criteria In Solid Tumors (RECIST) [44]. Unfortunately, RECIST only reflects tumor size changes (which are often delayed, if they occur at all) and is unable to identify non-responders at an early time-point, when novel cytostatic biologic agents are employed [45]. A patient may be misclassified as a non-responder because the tumor size remains unchanged, or even increases in the early stages of treatment due to hemorrhage, necrosis, or edema, in spite of a decrease of the viable tumor. To add functional assessment, new methods that also reflect tumor perfusion have been introduced in the form of modified RECIST (mRECIST) criteria [46]. This has highlighted the need for alternative accurate and reproducible quantitative techniques to assess changes in tumor vascularity, a question which is not addressed satisfactorily by current standard diagnostic evaluation.

Clinical Applications

DCE-US quantification has been used to monitor changes induced by anti-angiogenic [47, 48] and anti-inflammatory [49, 50, 51, 52, 53] therapies, both as a potential marker of response and as a tool to enable dose optimization of therapy in individual patients [54]. Early clinical trials assessing tumor response in gastrointestinal stromal tumor (GIST) were based on the subjective and qualitative assessment of enhancement dynamics. Subsequent studies assessed response in renal cell carcinoma, hepatocellular carcinoma (HCC), breast cancer, pancreatic cancer, and colorectal metastases using semi-quantitative techniques [55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65]. Additional studies [50, 51, 56] used quantitative techniques to derive parameters related to the time course of contrast enhancement, in comparison to clinical endpoints such as Progression Free Survival (PFS) and Overall Survival (OS) following anti-angiogenic treatment. Techniques such as respiratory gating [59, 66, 67] and motion correction have been shown to improve the reproducibility of DCE-US measurements. A number of clinical trials have since evaluated DCE-US in therapy monitoring or intervention guidance, also demonstrating the potential of this technique in comparison to other imaging techniques such as DCE-MRI [53, 68, 69, 70, 71, 72, 73, 74, 75, 76], CT perfusion [77], or positron emission tomography [78]. Preliminary results have also been reported in children [79].

The number of clinical studies on DCE-US has increased since the initial publication in 2012, as well as the variety of technical approaches used to acquire and analyze contrast enhancement dynamics. The selection of these techniques may influence the reliability of reported results and possibly explain contrasting observations between studies. The following sections attempt to explain the available DCE-US techniques and parameters, with the

aim of establishing a more standard approach to DCE-US examinations.

General considerations

Clinical DCE-US is usually performed with pure blood pool agents, such as SonoVue/Lumason [sulfur hexafluoride with a phospholipid shell, Bracco spa, Milan, Italy], or Definity [Octafluoropropane with a phospholipid shell, Lantheus Medical Imaging, Billerica MA, USA]. Quantitative contrast techniques can also be applied to agents, which are targeted to accumulate through specific biological interactions or to be extracted by a specific process (such as phagocytosis), but they require more complex multi-compartment kinetic models and are beyond the scope of this paper [43].

DCE-US can be performed using two different administration methods, an intravenous bolus injection or an infusion of UCA. The latter is followed by a disruption-replenishment technique and is much less commonly used for the assessment of tumor perfusion than the bolus injection.

The dual blood supply of the liver complicates blood flow quantification. After a bolus injection, the arterial blood supply is responsible for the initial enhancement of the normal parenchyma and of focal lesions, as the microbubbles arriving through the portal blood supply are delayed by 5 to 10 seconds. With the infusion technique, the replenishment reflects a combination of arterial and portal flow inputs.

After a bolus injection of UCA with wash-in/wash-out (bolus-transit) analysis, single-plane imaging at a low mechanical index (MI) is usually performed at about 10 frames per second for the duration of the enhancement. Frame rates that are too high should be avoided to prevent bubble destruction. Three-dimensional acquisition (corresponding to a volume) rather than a single plane would be preferable to overcome some limitations related to single plane analysis, but it is currently not feasible with the currently available commercial hardware (in terms of transducers and computing speed of available equipment). The average CEUS signal intensity within a region of interest (ROI) is calculated in linear units and is displayed as a function of time, i. e., a time-intensity curve (TIC), which describes the phases of progressive increase in enhancement of the contrast agent in the ROI (also termed wash-in) and the subsequent phase of slow decrease in contrast signal intensity (termed wash-out phase). Additional ROIs can be placed in a reference tissue for comparison purposes or in different areas of the lesion.

UCA administration

The approved doses are for bolus injection of SonoVue 2.4 mL for examinations of the macro- or microvasculature, and 2 mL SonoVue or Definity (10 $\mu\text{L}/\text{kg}$) in echocardiography. However, this dose may be reduced to 0.6–1.5 mL in most ultrasound systems, or increased up to the dose of two bolus injections (4.8 mL of SonoVue) under certain conditions depending on the sensitivity of the equipment, the transducer type and central frequency, the degree of vascularity, and the depth of the target lesion [80]. With more recent and sensitive equipment, the lower doses are adequate and should be preferred except when high-frequency transducers are

used. For example, the dose may be reduced to 1 mL when scanning the kidneys (and particularly in renal transplants), while it can be increased to 4.8 mL in the case of a superficial lesion using a high-frequency linear array or endoscopic transducers [81, 82, 83]. All microbubbles tend to go up in saline and should be shaken from time to time – or a pump should be used.

The bolus injection in general and also for quantitative DCE-US using SonoVue should be quick and be performed with a short angio-catheter typically 20G (never a smaller diameter than 22G to avoid disruption of the contrast microbubbles when they cross a too narrow catheter lumen), placed in an antecubital vein, without using a long extension line. A 3-way stop valve may be used at the end of the catheter to allow controlled access. In this case, it is preferable to connect the contrast syringe to the lock directly in line with the intravenous tract (not the perpendicular one) to avoid microbubble disruption that could occur when injecting contrast bolus against the stop valve tube wall. A saline flush (5 mL) should immediately follow to further sharpen the injected bolus and to limit the volume of UCA remaining in the angio-catheter and stop valve.

For infusion studies up to 2 vials (9.6 mL) have been infused at a rate of about 1 mL/min (or less) depending on the enhancement level required [84]. Slow infusion requires either a drip bag that is gently shaken from time to time or a pump that can be placed vertically or a specific rotating pump to continuously agitate the microbubbles [84]. Analysis should be performed during a steady UCA concentration in the blood. An acceptable steady state situation is usually achieved after about 2 minutes of infusion depending on the infusion rate. An initial faster injection rate can be used to achieve steady state earlier.

CEUS time intensity curve parameters

CEUS time intensity curve parameters have been summarized in the EFSUMB position paper describing the bolus-transit of the contrast microbubbles in the ROI [85, 86]. Time-related parameters can be differentiated from signal intensity-related parameters [3]. Several derived TIC parameters are purely descriptive/empirical. Reliability and potential sources of errors have been described [80].

Time-to-peak (TP), rise time (RT), mean transit time (MTT), peak intensity (PI), and area under the curve (AUC) have been proposed as primary parameters and all others are derived from those parameters [87]. In the EFSUMB position paper parameters such as time zero offset (T_0), time-to-peak (TP), wash-in time (WIT), wash-out time (WOT), mean transit time (MTT), full width half max (FWHM) are explained in detail [3]. Different from the other parameters, MTT can be calculated only in combination with a fitted mathematical model, while the other parameters are curve-descriptive parameters and thus can be derived also without a dedicated model. Since it is assumed that the signal intensity in DCE-US is proportional to the number of microbubbles (see below, linearized image data), and the microbubbles remain strictly intravascular, the TIC parameters are related to the vascularization of the analyzed region. Some signal-related parameters (peak intensity, area under the curve) are more correlated to the

local blood volume of the region (~ mL), while other time-related parameters are more reflective of blood flow (TTP, WIT, AUC is also related to blood flow according to the Steward-Hamilton relationship). All time and intensity values should be calculated from a curve fitted to the linearized echo intensity values and not from image data.

Signal intensity-related parameters are given in arbitrary units [a.u.], with the most important being peak intensity (PI) and area under the curve (AUC). Both are described in detail in the already mentioned paper. The whole AUC describing the area under the curve may be divided into two components: the AUC of the wash-in phase up to peak intensity PI (WIAUC) and of the wash-out from peak intensity until the predefined time of end (WOAUC). The total AUC is the sum of WIAUC + WOAUC.

Other parameters include the wash-in rate (WIR) [a.u./s], which describes the slope of the TIC curve during wash-in [signal intensity/s]. The *maximum* slope of the TIC curve or the *mean* slope of a certain wash-in time interval (e. g., from 5 % to 95 % signal intensity) is used for this empirical parameter that is related to the blood flow. In a similar way, change during wash-out (WOR) can also be derived. In addition, combinations of the above parameters exist, in particular ratios between a signal intensity and a time-related parameter such as the wash-in perfusion index WIPI, which is the wash-in AUC divided by the wash-in time (WIAUC/WIT).

Refilling kinetics describe the replenishment of microbubbles during the infusion of UCA. UCA is first imaged without being disrupted at a low MI, then a few frames are acquired at a high MI (often at the highest available) causing bubble disruption in the image plane. Immediately thereafter, the MI is reverted to its low setting and the arrival of fresh microbubbles is imaged. Refilling kinetics are described by parameters that are different from those after bolus injection. T_0 has an identical definition as for the TIC curves after bolus injection. TP, WIT, and MTT can also be calculated using a mathematical model that describes the refilling process. In contrast to bolus injection TIC curves, the maximum signal here is no longer reached at a peak but rather in the plateau phase. I_p is the *maximum signal* reached at the plateau (complete replenishment), often also called A, and is proportional to the local blood volume. The *rise of the replenishment curve* [1/s], often called B or β (based on the model by Wei *et al.*, see below), is a parameter that is proportional to the local blood flow velocity. Since the replenishment curve usually has a sigmoidal shape, this parameter varies with time, and its concrete definition depends on the model used. In principle, A and B are parameters of the replenishment curves that are directly related to the blood volume and flow velocity (and its product is directly related to the blood flow, $F = A \cdot B$). They can be extracted from the curves even without using a specific mathematical model that requires a closed form analytical expression, and thus they can easily be calculated and may be less prone to model-dependent limitations.

In 1998, Wei *et al.* [85] were the first to introduce the disruption-replenishment method and the development of the mono-exponential model. Krix *et al.* [88, 89, 90] used a similar approach as Wei *et al.* However, the modified formulas were no longer based on empiric assumptions and were based on a multi-vessel model incorporating differences in the acoustic field properties when using

high- and low-MI imaging. This model was found to be at least equivalent to the mono-exponential model, but it is nevertheless used much less frequently. Wei's model was improved by Arditi's model [91], which was subsequently further improved by Hudson *et al.* [92]. This model has 3 components that were not present in Wei's model: accounts for tissue perfusion through realistic microvascular geometry (Lognormal perfusion model), considers the ultrasound field properties of the destruction beam, and also considers the ultrasound imaging field. With the Arditi-Hudson model, it is possible to calculate the relative mean flow rate.

For repeated DCE-US exams, identical contrast protocols, DCE-US parameters, and analysis models have to be used in order to facilitate inter- or intra-patient comparison. Standardization and harmonization of software-based solutions and the various solutions integrated in the US platform are desirable but don't exist yet. For a detailed description of the equipment settings and patient-based factors, we refer to the published position papers [3]. Most studies focusing on AUC and wash-out recorded 3-minute loops [68]. In studies using infusion of UCA and the destruction-replenishment protocol, a shorter loop of the replenishment of the lesion or organ is sufficient (15–60 s) with the option to repeat it.

Clinical aspects of a DCE-US protocol

Choice of DCE-US parameters

A key question is which DCE-US technique and parameter should be used and evaluated in the various clinical settings. As described above, some parameters are more related to the blood volume (like the peak intensity I_p or the plateau A in replenishment kinetics) while others are more related to the dynamics of the blood supply, the blood flow (like the MTT). This is a first relevant aspect when choosing a certain DCE-US parameter. Furthermore, like with other imaging methods that analyze tissue vascularization (e. g., CT or MRI perfusion imaging in stroke) also a set of parameters and the identification of a potential mismatch between them may be useful to evaluate. In oncology treatment, monitoring or even outcome prediction are key aspects for use of DCE-US. This means parameters that could allow early assessment or prediction of treatment success or failure are the candidates of choice. In theory, changes in vascular dynamics (blood flow) would occur before a change in the vascular morphology (blood volume) becomes evident, but this has not yet been clearly demonstrated with DCE-US. Still, a widely used approach in research projects using DCE-US is to calculate more or less all feasible parameters and then to analyze if there is a correlation between these parameters and the specific clinical efficacy/outcome parameters. A few studies have suggested a certain parameter to be preferable in a specific setting (e. g., the MTT in bevacizumab therapy of metastases [53]) but a general broad consensus is lacking. AUC may be the most robust parameter in terms of technical errors.

DCE-US study design

Future studies should report DCE-US results in a more specific manner, related to certain parameters. Results should also be set in the context of the concrete tumor and treatment being assessed.

“DCE-US for chemotherapy monitoring” may be a too broad and unspecific term. It should be clarified to which specific treatment or drug group study results are reported. In general, confirmatory studies are still needed to determine the crucial DCE-US parameters that should be focused on for the various clinical scenarios. It means an a priori hypothesis is to be proven in a prospective multicenter approach— such as “change of AUC tumor/AUC liver at time point x compared to baseline provides decisive information for therapy management with drug xy”. So, a very narrow study hypothesis focusing on concrete parameters, time points, etc. and using a valuable clinical endpoint should be applied. Several studies so far have been explorative and have only used another biomarker for comparison such as perfusion in MRI or microvessel density in pathology.

Here, a comparison between classic early RECIST and DCE-US results is per se of no or low additional clinical value. Studies should rather focus on the potential additive value of DCE-US compared to standard diagnostics, i. e., on the predictive value of the method at early time points. The use of long-term outcome data as the standard of reference should be preferred to demonstrate whether DCE-US performs better at follow-up compared to RECIST.

DCE-US exam time points

This is related to the question at which clinical time point(s) a DCE-US examination should be performed. Treatment monitoring requires follow-up examinations while predictive messages or data for guidance of interventions can be derived from a single, early exam. Clinical trials using DCE-US in monitoring have often focused on standard time points, i. e., before the start of treatment and follow-up exams performed at standard time points in parallel to established imaging (e. g., for RECIST). Early time points for follow-up sometimes have been added, in particular a DCE-US exam after a first cycle of chemotherapy. Currently, further studies are still needed to determine the optimum monitoring regime for a specific treatment. Not only the duration after the general start of treatment can be relevant, but also the duration after a certain cycle of chemotherapy can have a considerable impact. Anti-angiogenic effects may be observable already within a short period of time, maybe the optimum only within a specific period of time after administration. DCE-US should clearly report how the used exam time points have been chosen and further studies can increase knowledge for optimization of monitoring schemes.

How to perform DCE-US, how to interpret the results, technical advice

To optimize the machine settings for DCE-US, the following issues are important. One single focus position should be set in a deeper region of the scanning plane, which must include by large all the regions of interest. The lowest but still reliable mechanical index (MI) should be used to avoid any unnecessary bubble disruption. The most convenient MI value varies depending on the specific equipment. The receive gain should be set so that it is usually aligned in the middle position. The persistence mode

should be turned off and the dynamic range should be kept tentatively high despite the fact that these two adjustments may not provide the best ultrasound images.

The conditions of the patient and surrounding factors (including posture, resting time, heart rate, blood pressure) and also of the scanning plane (acoustic window, probe position) at each acquisition, to help explain discrepancies in unexpected findings taken at different sessions during follow-up should be standardized and recorded. For adequate reproducibility, the follow-up examinations require the scanning plane to be exactly the same. This is often very difficult to achieve, even for expert users. A clear description of the probe position for examining the lesion, with landmarks in relation to the skin surface and documentation of representative anatomical structures, e. g., liver segment(s), major vessels, as well as the CEUS acquisition parameters, such as the depth of the lesion, mechanical index, etc., are essential to ensure standardization of these subsequent studies. It is important to keep all imaging (machine) parameters unchanged after the baseline scan to allow the comparison of the effects of therapy in subsequent scans.

Find a tumor in conventional B-mode and choose a tumor plane to study. Inject the appropriate dosage of microbubble contrast agents and scan in contrast mode (side-by-side). In order to keep the probe stationary, be aware of and compensate for any motion. Some examiners have also used an articulated arm to stay on the same plane. Scan continuously for 2 up to 5 minutes (depending on the clinical application) avoiding bubble destruction. Commercially available software (e. g., Vuebox) also allows the merging of smaller videos into one video, which can be helpful in the case of motion but also to reduce bubble destruction. Save the DICOM video loop in a format that allows data linearization. If more than one TIC curve may be recorded, then rotate the probe to select a different tumor plane (to evaluate tumor heterogeneity) and repeat the steps above for both infusion and replenishment.

The data analysis involves the use of a software package that allows forming of the TIC from linearized data from ROIs in the lesion and one in the normal parenchyma. One ROI should cover the whole tumor, and the placement of optional additional ROI(s) should follow representative areas of the “whole tumor” guided by highly vascularized parts of the tumor. For early relapse prediction, focusing on highly vascularized ROIs may be useful. In partially necrotic tumors, this guidance can make an important difference. For some of the mentioned recommendations, no consensus has been reached so far.

Next, a curve is fitted to the TIC data and the important perfusion parameters (rise time RT, mean transit time MTT, peak intensity PI, and area under the curve AUC) are extracted. Interpretation of the results involves statistically correlating the perfusion parameters with physiological data and clinical outcomes.

Further technical and methodological aspects

Technical considerations also contribute to the choice of an optimum DCE-US parameter. Reproducibility is an important factor, and DCE-US exams can be influenced by various aspects. Thus, the most robust parameters can be preferable. Time-related parameters (rise time) are robust since they do not depend on

the acoustic signal level – if the bolus arrival time is subtracted to avoid circulation time dependencies. Integrals are per se more robust than single values, thus the AUC or also parameters based on a mathematical integral and a closed form analytical expression (MTT) can be beneficial in the clinical routine, but this has been a topic of controversial discussion between the authors. However, the quality of the fit must be recorded to avoid misinterpretation. Even then, all parameters related to the CEUS signal intensity can crucially be influenced and biased by the various acoustic and patient conditions which may drastically limit inter- or longitudinal intra-patient comparison. This is mainly related to signal-related parameters. Normalization is the key to reducing this variability. Instead of using parameters of a single ROI (in oncology usually this is the tumor), values obtained from this ROI should be normalized, usually placing a second ROI in normal tissue adjacent to the relevant tissue (for instance the liver) and calculating the ratio of the parameters in these ROIs. Such normalized parameters are less prone to external bias. Time-related parameters are less influenced but also these parameters of a tumor can be compared with the surrounding tissue, e. g., as the difference in the rise time in the tumor compared to the liver.

The choice of the US plane may be affected by the visibility of representative tissue. The second ROI should be placed at the same depth. If no normal (healthy) parenchyma is present, other normal organs visible in the US plane could be used as an exception. Due to signal linearization, large vessels should not be used as the standard ROI for comparison. The focus should be positioned just at the level of the target lesion for most ultrasound scanners. Deeper focal zones might be used to achieve a more uniform acoustic field, which improves sensitivity to the agents and lessens the risk of bubble disruption. Detailed general technical recommendations have been published elsewhere in a consensus paper [93].

Perspectives

Modern oncologic therapies not only aim at a decrease in tumor size but may also focus on a “return to normal” situation, i. e., a tumor then may still have a high but relatively normal blood volume or perfusion. Thus, even more sophisticated DCE-US parameters beyond those related to blood volume or perfusion could be needed to describe DCE-US patterns correlated with the vascular architecture. Existing models are able to derive such additional information, but they are not used in clinical practice. Finally, the described DCE-US parameters provide a temporal analysis of DCE-US exams, not a spatial analysis. Vessel architecture analysis, however, also requires a spatial component. Placing more than one ROI in a tumor, e. g., in the periphery and the center, is the simplest approach to add a spatial analysis. When color-coded parameter maps are generated with suitable software, more complex approaches are feasible, up to a pixel-wise comparison and correlation of DCE-US parameters. A simple spatial approach is also to use the size of the colored area in a tumor as an additional parameter – it is not pure DCE-US but DCE-US is used here to create such spatial parameters. For instance, the size of the AUC above a certain threshold/size of the whole tumor can reflect the vascularization – similar to “% of vascularized tumor”. A combination of both a spatiotemporal analysis and the use of 3-D-US for

DCE-US may provide a more complete description of the UCA transport process and better characterization of perfusion, contrast dispersion, and vascular architecture [43]. In brain perfusion studies using MRI, such parameters are relevant – e. g., to identify a mismatch between perfusion parameters and to see if there is viable tissue at risk that justifies treatment after stroke. In regard to 3 D DCE-US, CEUS techniques are limited and publications are lacking [94], and these topics are beyond the scope of this document. Variability studies using phantoms and models across multiple scanners and quantification software have been described in detail; refer to [80, 87].

Although promising, all studies identified so far on AI in CEUS are single-center, retrospective studies, or studies on limited, selected case series using different algorithms for machine learning and with various clinical aims, even if characterization of liver lesions is the most frequent. Most often the algorithms are run in post-processing, making them less useful in a clinical workflow. There is a need to perform prospective, multi-center studies with clinically useful endpoints, preferably using open-access software in order to find the place for AI in the evaluation of CEUS cine loops.

Open questions

TIC curve analysis of CEUS bolus injections provides several parameters that reflect local blood flow. None of the parameters alone represent clear-cut tissue characterizing abilities, although differences are observed for e. g., neoplastic and non-neoplastic tissue [37, 95, 96, 97, 98, 99]. The ability to combine several parameters simultaneously using AI may provide improved characterization, but this must be shown in prospective multi-center studies using standardized technology. The dynamic contrast assessment methods need to be integrated in the clinical workflow, not requiring too much time, and the results should provide information influencing the clinical management of patients.

Concluding remarks

Results of recent monocentric and multicentric clinical trials propose that quantitative DCE-US may be useful in oncology, in particular in the assessment of response to targeted therapies beyond classic RECIST assessment. The current article provides general information about the technique and parameters utilized in DCE-US quantification and recommendations on its use to provide a standardized approach, which may improve clinical management.

Statements

STATEMENT 1

Compared to a purely subjective comparison of the phase-specific enhancement of different tissues or of the same tissue under different pathological or therapeutic conditions, DCE-US allows a more objective assessment when used in a standardized way.

STATEMENT 2

Using only tumor diameter changes (i. e., RECIST) is a suboptimal method for tumor response assessment. Treatment monitoring assessment of vascularization/perfusion adds relevant information both in the early and later phases after initiation of pharmacological treatments.

STATEMENT 3

Further research is recommended to investigate the potential of DCE-US to noninvasively improve the differential diagnosis of focal lesions in parenchymal organs, to graduate the biological aggressiveness of various malignant tumors, and to predict their outcome, as well as to record the temporal dynamics of pathological processes in parenchymal organs associated with changes in perfusion characteristics.

STATEMENT 4

DCE-US provides quantitative information about local blood flow and can be carried out with two main DCE-US modalities, which provide different information and parameters: the bolus technique and the infusion technique (using the disruption-replenishment method).

STATEMENT 5

The bolus technique quantifies the entire course of contrast kinetics, from wash-in to wash-out. The analysis is carried out along one single plane for each injection and a cineloop of at least one minute in duration is recommended. The disruption-replenishment method is carried out at a steady-state high signal enhancement level. The analysis requires a shorter cineloop (usually 10–25 seconds), so that multiple planes can be assessed. Parameters and information obtained with the two methods differ from each other.

STATEMENT 6

Relative quantification of perfusion using a reference area at the same depth should be preferred to absolute evaluation of contrast enhancement.

STATEMENT 7

In order to optimize machine settings for DCE-CEUS, the following recommendations are important: a) use a single focus

position to be set in a deeper region of the scanning plane that must include all regions of interest; b) use a low mechanical index (MI); c) set the receive gain high with TGC usually aligned in the middle position; d) turn off the persistence mode and keep the dynamic range tendentially high despite the fact that these two adjustments may not provide the best B-mode ultrasound images.

STATEMENT 8

The MI should be set as low as possible, with the goal of avoiding any unnecessary bubble disruption. The most convenient MI value varies depending on the specific equipment and the contrast agent being used.

STATEMENT 9

To assess tumor response in a patient, the same machine settings should be used for consecutive DCE-US examinations as for the baseline examination. It is recommended to keep a detailed record of patient conditions and surrounding factors (including posture, resting time, heart rate, blood pressure) and also the scanning plane (acoustic window, probe position) for each acquisition, to help explain discrepancies in unexpected findings taken in different sessions during follow-up.

STATEMENT 10

Suitable planning and choice of a representative imaging plane is crucial to avoid respiratory motion of the ROI which is a major source of error in the quantification of DCE-US. Especially out-of-plane motion cannot be corrected, and out-of-plane acquisitions must be excluded from the DCE-US analysis, which is a time-consuming and demanding process.

STATEMENT 11

Quantification software may be embedded in ultrasound equipment or may be work off-line on separate hardware. It is necessary to perform calculations on linearized data to maintain the linear relationship between microbubble concentration and signal intensity.

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Conflict of Interest

All other authors have received lecture honoraria from Bracco and from companies selling ultrasound systems. Martin Krix is employee of Bracco.

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