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● *Review*

POTENTIAL OF CONTRAST-ENHANCED ULTRASOUND AS A BEDSIDE MONITORING TECHNIQUE IN CEREBRAL PERFUSION: A SYSTEMATIC REVIEW

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Abstract—Contrast-enhanced ultrasound (CEUS) has been suggested as a new method to measure cerebral perfusion in patients with acute brain injury. In this systematic review, the tolerability, repeatability, reproducibility and accuracy of different CEUS techniques for the quantification of cerebral perfusion were assessed. We selected studies published between January 1994 and March 2017 using CEUS to measure cerebral perfusion. We included 43 studies (bolus kinetics n = 31, refill kinetics n = 6, depletion kinetics n = 6) with a total of 861 patients. Tolerability was reported in 28 studies describing 12 patients with mild and transient side effects. Repeatability was assessed in 3 studies, reproducibility in 2 studies and accuracy in 19 studies. Repeatability was high for experienced sonographers and significantly lower for less experienced sonographers. Reproducibility of CEUS was not clear. The sensitivity and specificity of CEUS for the detection of cerebral ischemia ranged from 75% to 96% and from 60% to 100%. Limited data on repeatability, reproducibility and accuracy may suggest that this technique could be feasible for use in acute brain injury patients. **(E-mail: Astrid.Hoedemaekers@Radboudumc.nl)** **© 2017 World Federation for Ultrasound in Medicine & Biology. All rights reserved.**

Key Words: **Contrast-enhanced ultrasound, Cerebral blood flow, Acute brain injury, Ischemia, Perfusion.**

INTRODUCTION

An adequate supply of blood containing oxygen and nutrients is crucial for the recovery and survival of brain tissue. Monitoring of cerebral perfusion is essential in prevention of secondary brain damage in patients with acute brain injury. The severity of the brain injury frequently obscures clinical changes and limits the reliability of clinical neurologic examination. Direct monitoring of the cerebral perfusion enables the detection of changes in brain perfusion at a stage before irreversible damage has occurred. In addition, the effects of therapeutic interventions can be monitored to evaluate and adjust therapy (Dagal and Lam 2011).

Contrast-enhanced ultrasound (CEUS) has been suggested as a new method to measure cerebral perfusion in patients both with acute brain injury at the ICU and in the acute state of cerebral ischemia. Ultrasound is an attractive technique because it is non-invasive, has high temporal resolution and can be used at the bedside. Ultrasound contrast agents (UCAs) are used for visualization of the cerebral vasculature to overcome the restricted level of acoustic intensity caused by the physical obstacles of the skull and leading to a limited signal-to-noise ratio. For CEUS, three different approaches can be used to measure cerebral perfusion (Meairs and Kern 2015). These approaches are based on bolus, refill (replenishment) and depletion kinetics. After a bolus injection, microbubbles enter the insonation field, and the acoustic intensity in this plane increases. The amount of non-linear scattering or microbubble concentration can be represented by a time–intensity curve (TIC). Different parameters of the TIC can be extracted for quantification of parenchymal perfusion. Refill kinetics are based on the reappearance of UCA after complete destruction of the microbubbles. By destroying the contrast agent within the scanning plane using high-mechanical-index (MI)

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flashes, an absence of contrast is created locally, and new microbubbles enter the plane. Depletion kinetics are based on the destruction of contrast agent at a constant frame rate with a high MI. The perfusion status is analyzed by destruction curves and the difference in acoustic intensity before and after the destruction of UCA (Seidel and Meyer-Wiethe 2007).

Several CEUS methods have been used to monitor cerebral perfusion in acute brain injury. Main differences between the methods are differences in kinetics used and differences in contrast imaging modes. The aim of this systematic review was to assess the tolerability, repeatability, reproducibility and accuracy of the different CEUS techniques for the quantification of cerebral perfusion.

METHODS

We performed a systematic review in accordance with the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines (Stroup et al. 2000).

Search strategy and study selection

An online literature search was conducted by E.J.V. and A.J.K. on 1 March 2017 using the electronic database Medline *via* PubMed. The search terms included are listed in Table 1. To be included, studies had to involve human adults, had to report original data published in English between 1994 and 2017 and had to have used transcranial CEUS to measure cerebral perfusion or related parameters. CEUS studies merely reporting blood flow velocities in the cerebral arteries, characterization and visualization of tumors, visualization and characterization of vasculature (*e.g.,* aneurysms) and determination of stenotic or occluded arteries were excluded. Only studies reporting semiquantitative or quantitative CEUS parameters were included. Reviews and general discussion papers not reporting original data were excluded. For all studies cited, informed consent had been obtained from each study participant, and the protocol had been approval by an ethics committee or institutional review board.

Manual selection was performed by selecting relevant references from the reference list of included articles. Two reviewers (E.J.V. and A.J.K.) checked the titles and abstracts identified by the search strategy and examined any publication that potentially met the inclusion criteria. Final inclusion/exclusion decisions were made after independent duplicate examination of the full articles of selected references.

Table 1. Search terms

		AND
OR	Cerebral blood flow	Contrast-enhanced ultrasound
	Cerebral circulation	Contrast enhanced ultrasound
	Brain perfusion	Contrast-enhanced ultrasonography
		Contrast enhanced ultrasonography
		Contrast ultrasound
		Ultrasound perfusion imaging
		Ultrasound contrast agent
		Ultrasound contrast agents

Outcome measures

To compare tolerability, repeatability, reproducibility and accuracy of the different ultrasound techniques, the studies were categorized according to the underlying kinetic principles used: bolus kinetics, refill kinetics and depletion kinetics. For each category, the execution and data analysis characteristics were compared. Execution characteristics included operator, study population, UCA type, UCA dosage, duration of measurement and insonation approach. Data analysis characteristics, including reference method, ultrasound method, acquisition time, temporal and spatial resolution and outcome parameters, were recorded.

Tolerability was assessed by registration of side effects of both the UCA and application of high mechanical indexes (>1.0). Repeatability was defined as the variation in repeat measurements in the same subject by the same operator under identical conditions (Bartlett and Frost 2008). Reproducibility referred to the variation in measurements in the same subject under changing conditions (mainly inter-operator agreement). The accuracy of CEUS was assessed by comparison of this technique with a gold standard.

RESULTS

Study characteristics and population characteristics

We identified 407 publications in our primary search and added 9 articles by review of references. After exclusion of 373 publications, 43 publications were eligible for review. Main reasons for exclusion were subject, lack of perfusion parameters, measurement of flow velocity, stenosis/occlusion characterization, visualization or characterization of cerebral arteries or tumor or the fact that the CEUS measurements were not performed transcranially (Fig. 1).

The 43 studies were categorized into studies using the bolus kinetics (n = 31), refill kinetics (n = 6) and depletion kinetics methods (n = 6) (Table 2). A total of 861 patients were included (395 healthy control patients and 466 patients). In 309 (78.2%) healthy control patients, bolus kinetics was used; in 26 (7.0%) patients, refill kinetics; and in 60 (15.2%) patients, depletion kinetics. Most patients were studied after ischemic stroke.

Most studies assessed the diagnostic potential of the technique (23/43), mostly by comparing the technique directly with computed tomography (CT) or magnetic resonance imaging (MRI) (15/43). Other studies assessed feasibility of the technique in the healthy brain

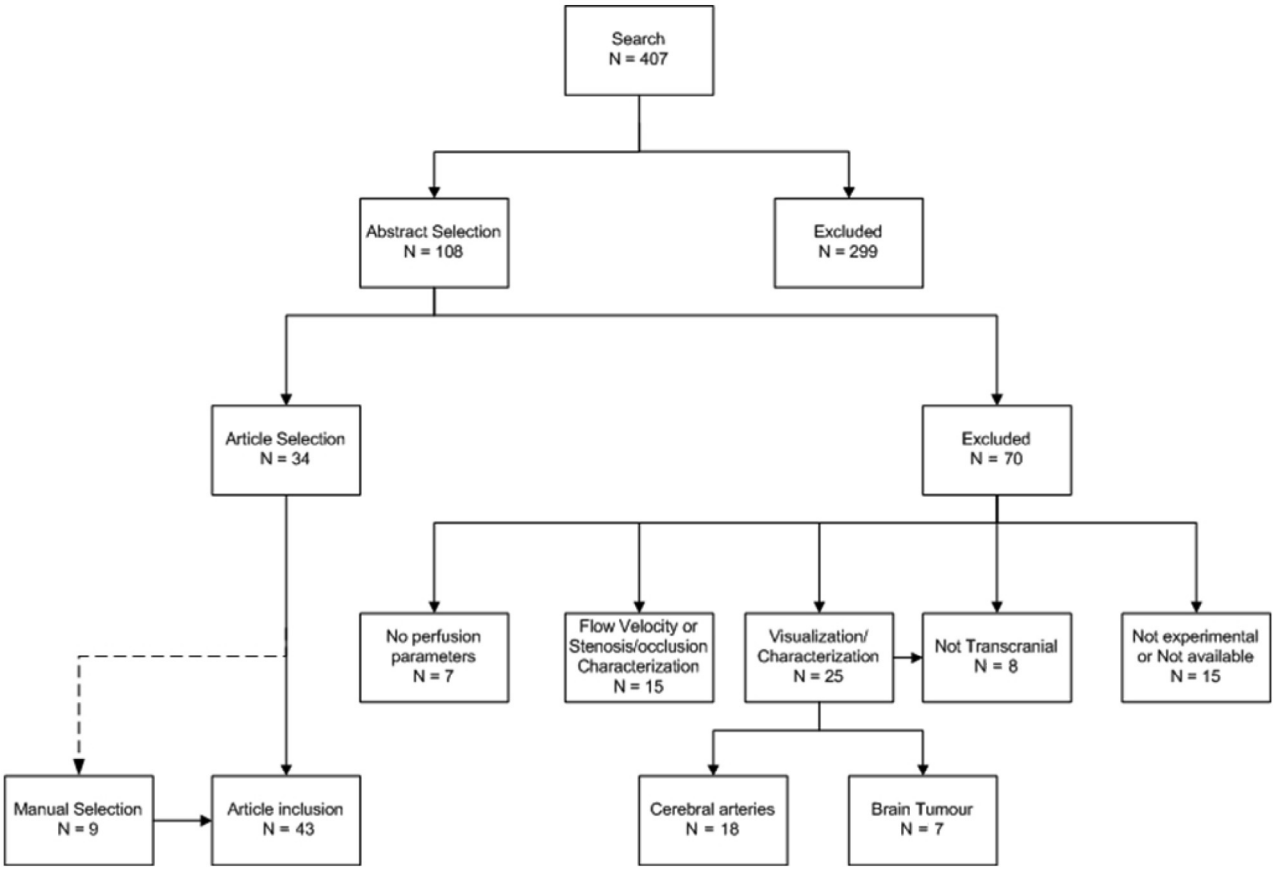

Fig. 1. Diagram of article selection.

(8/43), compared specific imaging settings (7/43), studied quantification of cerebral blood flow by comparing the technique with CT or MRI (3/43), estimated reproducibility between two hospitals (1/43) or determined inter- and intra-observer variability (1/43). The primary aim and outcomes of the individual studies are summarized in Supplementary Tables S1A, S1B and S1C (online only, available at http://dx.doi.org/10.1016/j.ultrasmedbio.2017.08.935).

Two studies compared the bolus kinetics parameters with the refill kinetics parameters (2/43).

Exclusion criteria were mainly associated with the contraindications for the UCA as indicated by the manufacturer, including right–left shunt, severe pulmonary hypertension, acute respiratory distress syndrome and pregnancy and lactation.

UCAs and ultrasound systems

Until 2003, Levovist (Schering) and Optison (Amersham Health) were the most commonly used contrast agents, in 13 of 43 and 9 of 43 studies, respectively.

Table 2. Overview of the demographic data and kinetic approaches in patients

	No. (%) of patients			
	Bolus kinetics	Refill kinetics	Depletion kinetics	Total
Ischemic stroke	212 (57.8%)	72 (100%)	15 (55.6%)	299 (64.2%)
Cerebrovascular disease	56 (15.3%)	0	0	56 (12.0%)
Brain tumor	21 (6.2%)	0	0	21 (4.5%)
Intracerebral hemorrhage	5 (1.3%)	0	12 (44.4%)	17 (3.6%)
Vascular dementia	40 (12.0%)	0	0	40 (8.6%)
Neurologic disorder	13 (3.5%)	0	0	13 (2.8%)
Diabetes type II	20 (5.4%)	0	0	20 (4.2%)
Total	367 (78.7%)	72 (15.4.5%)	27 (5.8%)	466 (100%)

Table 3. Overview of the characteristics of the different ultrasound contrast agents

	Levovist	Optison	Sonovue	Definity
Gas	Air	Octafluoropropane	Sulfur hexafluoride	Octafluoropropane
Shell	99.9% galactose and 0.1% palmitic acid	Albumin	lipid	Lipid
Size	2–4 μm	2–4.5 μm	2–3 μm	1.1–3.3 μm

In more recent years, SonoVue (Bracco) has mainly been used (22/43 studies). Characteristics of the different UCAs as documented by the manufacturers are summarized in Table 3. All 861 patients received at least one dose of UCA. The bolus dose, speed of injection of the bolus and infusion rate of the continuous infusion differed between patients and studies. Tolerability of the UCA was reported in 28 studies describing 861 patients. Side effects were reported in 12 patients (12/861), all healthy controls (4 studies). Eight patients received the UCA Levovist and 4 received Optison. The side effects reported included mild transient headache (1 subject), mild burning sensation in the right upper abdominal quadrant (1 subject) and mild local side effects at the injection site, such as local paraesthesia (10 patients).

The Siemens Sonoline Elegra (16/43) and Philips SONOS 5000 (15/43) were the ultrasound systems most frequently used. The remaining studies were performed used the HDI 5000, iU22 (both Philips Medical Systems), Acuson Seqouia 512 (Siemens) and Vivid 7 (GE) (Supplementary Tables S2A, S2B and S2C, online only, available at http://dx.doi.org/10.1016/j.ultrasmedbio.2017.08.935).

The spatial resolution depends on the transducer frequency, where an increasing frequency on average results in improved spatial resolution. However, increasing the transducer frequency decreases the depth of penetration. The transducers were set at frequencies between 1.8 and 5 MHz and yielded a field of view between 100 and 160 mm. The most relevant imaging characteristics of the studies included in this review are summarized in Supplementary Tables S1A, S1B and S1C.

Imaging

Harmonic imaging was the most commonly used ultrasound imaging method, performed in 14 of 43 studies, combined with the integrated backscatter modus in 6 of these studies. Pulse inversion harmonic imaging (a method that is more sensitive to destruction) was used in 10 of 43 studies. Other imaging methods included contrast burst imaging, time variance imaging, power modulation imaging, contrast pulse sequencing and power Doppler. Three studies used both time variance imaging and contrast burst imaging.

The MI ranged from 0.9 to 1.8 for the bolus kinetics studies. For refill kinetics studies, the MI for the triggered registration was set at 0.7–1.47 for the destruction of UCA and at 0.17–0.29 for the subsequent continuous registration. For depletion kinetics, a MI of 1.2–1.8 was applied (Supplementary Tables S1A, S1B and S1C)

The temporal resolution of the techniques depends on the frame rate. For the bolus kinetics study, the frame rates for registration of UCA varied between 0.5 and 5 Hz, with one study using a frame rate of 11 Hz, and another study, 30 Hz. In six studies, electrocardiogram-triggered pulsing intervals were used of one frame every four cardiac cycles. For both the refill and depletion kinetics studies, frame rates ranged from 0.5 to 15 Hz.

The acquisition time in the bolus technique was approximately 45 s. In the refill kinetics study, the acquisition time was mentioned in three of six studies. In two studies, the acquisition time was 10 s, and in one study, 16 min. In the depletion studies, the acquisition time ranged from 1.4 to 35 s.

No adverse events related to the specific imaging modality or settings were reported in any of the studies.

Data recording and analysis

After acquisition, data were transferred to an external evaluation unit and analysed offline. Analysis was performed using commercially available software or in-house developed software. Quanticon was the most frequently used commercially available software (in five studies). In seven studies, in-house developed software was used. The analysis software used in the other studies are listed in Table 4.

The most frequently used perfusion parameters for bolus kinetics studies were peak intensity (PI) and time

Table 4. Overview of the analysis software

Analysis software	No. of studies
Not mentioned	16
Densitometry unit	2
DataPro	3
Quanticon	5
BHI-view	2
HDI-Lab	1
Qontrast software	1
Qlab	3
Optimas	1
Echo Tech System	1
Own developed software	7
VueBox	1

to peak (TTP). Four studies determined the cerebral transition time (CTT), defined as the time difference between arrival of contrast in the posterior cerebral artery and arrival in the vein of Galen. The plateau of echo enhancement (A), the rate constant determining the rising rate of echo enhancement (β) and flow ($F = A\beta$) were measured in the refill kinetics studies. The perfusion coefficient (PC [1/s]), minimal observation time, relative error (RE) and destruction coefficient (DC) were the main parameters for depletion kinetics studies.

Repeatability, reproducibility and accuracy

Repeatability of the technique was assessed in 3 of 43 studies, all using the bolus kinetics approach. One study reported a high repeatability, defined as a Spearman correlation coefficient of $r = 0.76$ of the CTT, in 60 patients with dementia and 25 control patients (Puls et al. 1999b). A second study by the same group confirmed the high repeatability of the CTT in 57 patients with cerebral microangiopathy and 30 healthy controls as measured with multiple regression analysis (Puls et al. 1999a). The third study compared the repeatability between two sonographers with different levels of experience in 10 healthy control patients. The repeatability was high for the experienced sonographer, whereas the less experienced operator had poor repeatability (Harrer et al. 2011), suggesting that this technique is highly operator dependent and requires adequate training and experience.

Reproducibility was assessed in 2 of 43 studies. One study tested the reproducibility of the bolus kinetics between two operators with different levels of experience, resulting in a Spearman correlation coefficient of $r = 0.34$ (Harrer et al. 2011). The other study compared the bolus technique in a group of healthy controls in two centers using a standardized protocol. Mean perfusion parameters did not significantly differ between the two centers (Holscher et al. 2005). However, the centers used different groups of patients differing significantly in age, thus limiting the validity of this study.

The accuracy of CEUS was assessed in 19 of 43 studies by comparison with MRI or CT parameters, mostly in patients after ischemic stroke. Several studies used MRI or CT as a reference for the localization of perfusion deficits to define the regions of interest (ROIs) and/or to evaluate the perfusion parameter results in these regions.

In 7 of 43 studies, the sensitivity and specificity of the detection of ischemia were assessed. In these studies, the sensitivity and specificity of the detection of size and localization of infarction ranged from 75% to 96% and from 60% to 100% respectively, compared with CT or MRI. In 1 of 43 studies, localization of the infarct was compared with localization on CT. In 4 of 43 studies, the affected area was compared with non-affected area (stroke vs. non-stoke and tumor vs. non-tumor) and this was compared with CT or MRI. In 4 of 43 studies, perfusion characteristics of the stroke area were compared with those of the stroke area as determined with CT.

In 3 of 43 studies, ultrasound perfusion parameters were directly compared with MRI perfusion parameters. With the bolus technique, relative values of time-related ultrasound parameters were similar to those of MRI perfusion parameters in 12 healthy control patients (Meves et al. 2002). In contrast, the more volume-related ultrasound parameters such as peak intensity differed significantly from MRI volume-related measures, depending on the insonation depth. Intensity can vary in response to many parameters (*e.g.,* attenuation, total blood volume, amount of contrast, speed of injection) which are uncontrolled.

One study compared bolus kinetics parameters with perfusion MRI parameters, namely, mean transit time (MTT), regional cerebral perfusion and regional cerebral blood volume. The parameters were compared in 16 patients. A weak positive correlation was found between real-time TTP and MTT ($R = 0.51$), and a weak negative correlation between real-time TTP and regional cerebral perfusion ($R = -0.31$). The remaining correlation between parameters in MRI and CEUS was not significant (Kern et al. 2011).

One study compared the bolus technique with MRI of CT in the detection of normo-, hypo- and non-perfused brain areas in 30 patients with acute middle cerebral artery stroke. Time-to-peak intensity of ischemic ROIs was compared with that of four standardized non-ischemic ROIs of the same patient. The correlation in TTP delay between ultrasound and MRI or CT for the detection of ischemia was high, with a high sensitivity for the detection of hypo-perfusion and non-perfusion by ultrasound (Reitmeir et al. 2017).

Two studies compared the bolus technique with the refill kinetics approach. One study was performed in 24 patients with acute ischemic stroke, and the other, in 23 patients with acute ischemic stroke. The bolus parameter TTP was equally as sensitive as the refill parameter β for discrimination between hypo-perfused and normal brain tissue (Bolognese et al. 2013; Kern et al. 2011).

DISCUSSION

Contrast-enhanced ultrasound is a promising technique for bedside monitoring of cerebral perfusion in ischemic stroke patients and ICU patients with acute brain injury. Several requirements need to be met before such a technique can be implemented in routine critical care. The technique must be tolerable and without significant side effects. In addition, a new method should be repeatable, reproducible and accurate with sufficient temporal and spatial resolution.

The tolerability of CEUS is related mainly to the UCA, the acoustic output of the transducer or their interaction. No severe adverse events were documented in the 43 studies included in this review. Overall, the incidence of serious adverse events related to the administration of UCA is estimated at 0.013%, and serious hypersensitivity reactions are observed in fewer than 1 in 10,000 exposures (Tang et al. 2017). Although hypersensitivity reactions to UCAs can occur, the incidence of anaphylactic reactions is very rare and only reported as case reports in the literature (Geleijnse et al. 2009; Piscaglia et al. 2006; Solivetti et al. 2012).

Thermal and non-thermal effects like cavitation as induced by ultrasound can result in local tissue injury, and these effects are generally enhanced by UCAs. In the brain, CEUS may compromise the integrity of the blood–brain barrier (BBB). Ultrasound levels within diagnostic limits in combination with UCA in healthy humans did not lead to MRI-detectable BBB changes or focal brain damage (Jungehulsing et al. 2008; Meairs and Alonso 2007). Although no adverse events were reported in this review, possible secondary injury induced by CEUS may occur in patients with acute brain injury and remain indiscernible from the primary insult. Studies addressing this issue are needed to ensure the tolerability of this technique in patients with acute brain injury.

Data on repeatability, reproducibility and accuracy were scarce in our review. Repeatability was high for well-trained operators, but significantly lower for less experienced sonographers. As CEUS is highly operator dependent, reliable CEUS requires appropriate training and experience. The reproducibility of CEUS remains unclear, as the only two studies addressing this issue were difficult to interpret because of differences in the experience of the sonographers or differences in the study populations. The accuracy of CEUS was studied in less than 50% of the studies, mainly by comparison of regions of ischemia detected by ultrasound with CT or MRI. The sensitivity and specificity of CEUS for the detection of cerebral ischemia ranged from 75% to 96% and from 60% to 100%, respectively. However, in only three studies were (semi)quantitative perfusion parameters directly compared with MRI parameters.

Repeatability and reproducibility are strongly affected by the variability of the technique. This variability may be related to specific characteristics of the subject, such as attenuation of the echo signal by the skull, heterogeneity of the temporal bone density and physiologic fluctuations of cerebral perfusion. In addition, differences in volume and speed of contrast administration, imaging settings, transducer frequencies, frame rate, data recording and acquisition will also strongly influence the results. Although subject-related heterogeneity is an intrinsic feature of clinical measurements, uniformity of UCA and ultrasound settings may reduce the variability of the results. Heterogeneity in technique in terms of imaging, transducer and analysis parameters prohibits direct comparisons between studies.

The bolus kinetics technique was the most frequently used. One study directly compared the bolus and refill kinetics methods (Bolognese et al. 2013). The time-related parameters of the bolus and refill kinetics methods were equally sensitive for the detection of perfusion impairment in acute MCA stroke; however, they did not discriminate between qualitative and (semi-) qualitative impairment. Major difference between the two techniques were the high MI (1.47) used in the bolus technique versus the lower MI (0.17) used in the refill technique. Although this lower MI may decrease the risk of tissue damage, the refill kinetics were of poor quality in 12.5% of the patients, despite acceptable insonation conditions with the bolus kinetics.

From a theoretical point of view, time-dependent parameters like TTP (bolus kinetics), perfusion coefficient (depletion kinetics) and β (refill kinetics) are the most useful for the quantification of cerebral perfusion. This is because the amplitude depends on both the insonation depth and the attenuation of the skull. In accordance with this theoretical point of view, studies investigating the depth and ultrasound dosage dependence of the different perfusion parameters reported higher dependence of these external factors in the amplitude parameters than the time parameters. It is concluded by most studies that the time-dependent parameters are the most suitable (semi)quantitative perfusion parameters.

Limitations of this study include the incomplete description of the technique in several studies. The sensitivity of the perfusion parameters was studied using perfusion CT or diffusion- or perfusion-weighted MRI as reference method. Yet the gold standard for quantifying cerebral blood flow is positron emission tomography with $H_2{}^{15}O$. However, high cost and limited accessibility have restricted the widespread clinical use of this technique. Although perfusion CT and MRI are validated techniques, small differences in the approximation of cerebral blood flow may occur. Most studies concentrated on the detection of hypo-perfusion of the brain. In acute brain injury, hyper-perfusion may be as deleterious as hypo-perfusion. The accuracy of CEUS for the detection of hyper-perfusion has not yet been assessed.

CONCLUSIONS

Although no severe adverse effects were documented in the studies included in this review, the tolerability of CEUS in patients with acute brain injury has not been systematically assessed. So far, most studies have compared CEUS with MRI or CT for the detection of regions

of ischemia, with acceptable sensitivity and specificity. For patients after ischemic stroke, CEUS may thus serve as an additional clinical tool for the bedside evaluation of brain tissue perfusion and response to recanalization therapy. A (semi)quantitative measurement of cerebral perfusion may be of great value in acute brain injury patients at risk for decreased cerebral perfusion, for example, during vasospasm after subarachnoid hemorrhage. Data on repeatability, reproducibility and accuracy of the (semi)quantitative application of CEUS are scarce, and results are heterogeneous. More efforts to reduce operator dependency, for example, by using headsets, automated attenuation assessment tools, optimization of UCA administration and imaging settings or innovative data analysis tools may in the future improve the reliability of CEUS.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ultrasmedbio.2017.08.935.

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