Quantitative time intensity curve analysis of contrast-enhanced ultrasound (CEUS) examinations for the assessment of focal liver lesions

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INTRODUCTION

Contrast enhanced ultrasound (CEUS) has a well-established place in the characterization of focal liver lesions (FLLs), as it enables a high spatial and temporal resolution as well as a dynamic assessment of macro- and microvascularization. When examining the liver, CEUS allows dynamic visualization of the three vascular phases: arterial, portal venous and late phase, differentiating benign from malignant FLLs with an accuracy similar to that of contrast enhanced computer tomography (CECT) and magnetic resonance imaging (CEMRI) [1,2]. Nevertheless, CEUS has the disadvantage of being examiner-dependent, so quantitative analysis of time-intensity curves in dynamic CEUS (D-CEUS) could provide further information. The aim of this article is to provide a general review of the current literature regarding the usefulness of D-CEUS in the assessment of FLLs.

Abstract

Contrast enhanced ultrasound (CEUS) is well-established for the characterization of focal liver lesions (FLLs). By using intravenous ultrasound contrast agents, followed by specific low mechanical index examinations, CEUS enables a high spatial and temporal resolution as well as a dynamic assessment of macro- and microvascularization down to the capillaries. Nevertheless, CEUS has the disadvantage of being examiner-dependent, so quantitative analysis of time-intensity curves in dynamic CEUS (D-CEUS) could provide further information. The aim of this article is to provide a general review of the current literature regarding the usefulness of D-CEUS in the assessment of FLLs.

Keywords: dynamic CEUS; time intensity curve (TIC) analysis; focal liver lesions (FLL)

Technical considerations

D-CEUS is performed after standard CEUS examinations, using analysis software that can be embedded either directly in the ultrasound system or on external computers. They generate TICs by calculating change in mean signal intensities within a defined region of interest (ROI). Mean signal intensity within ROI is calculated
in linear units, displayed as function of time. Therefore, using the bolus injection of the ultrasound contrast agent (UCA), TICs describe the wash-in and wash-out of the contrast agent in the ROI. Additional ROIs can be placed in reference tissue or different lesion areas for comparison purposes. It is also important to note that all time and intensity values are calculated from the fitted curve and not from raw image data [4].

Regardless of the software used to perform quantitative perfusion analysis, the TICs obtained describe the bolus kinetics when using the bolus injection of UCA, from wash-in to wash-out of the contrast agent, using time-related, intensity-related and combined parameters.

The most important TIC parameters are described in Table I and Table II. The name based on the representation on the time intensity curve as shown in figure 1 is mentioned for each parameter as well as potential synonyms / equivalents encountered in the literature [5].

Novel perfusion parameters focusing on signal intensities within the lesion and the background tissue in the late phase, expressed as either a difference (washout value - WOV) or a ratio (late phase ratio - LPR) are also described in recent clinical trials [6,7].

Table I. Time intensity curve parameters related to blood volume [5].

<table>
<thead>
<tr>
<th>Parameter name</th>
<th>Synonym</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak enhancement (PE)</td>
<td>Peak intensity (PI), Maximum intensity (IMAX)</td>
<td>The maximum value of the intensity in arbitrary units</td>
</tr>
<tr>
<td>Area under the curve (AUC)</td>
<td>Wash-in wash-out area under the curve (WiWoAUC)</td>
<td>The area under the time-intensity curve above baseline and is calculated numerically between the starting time and a predefined end time.</td>
</tr>
<tr>
<td>Relative blood volume (RBV)</td>
<td></td>
<td>Allows to estimate the relative blood volume regardless of the time of arrival and flow velocity of the bubbles in ROI. Particularly important in lesions with irregular contrast enhancement.</td>
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</tbody>
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Table II. Time-related parameters - in relation to blood flow [5].

<table>
<thead>
<tr>
<th>Parameter name</th>
<th>Synonym</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time zero offset</td>
<td>Arrival time (AT), Time of arrival (ToA)</td>
<td>Time from the UCA injection to the first appearance of any UCA signal within the ROI - the point on the abcissa where the TIC curve starts the uprise</td>
</tr>
<tr>
<td>Time to peak (TTP)</td>
<td>TtoPk</td>
<td>Time to achieve the maximum intensity</td>
</tr>
<tr>
<td>Rise Time (RT)</td>
<td>Wash-in time (WIT)</td>
<td>Time from UCA appearance / TI to maximum intensity time. Depending of the software used, RT can be described as time from 5% (10%) to 95% (90%).</td>
</tr>
<tr>
<td>Mean transit time (MTT)</td>
<td>Mean transit time (mTT)</td>
<td>The mean time taken by the bubbles to pass through the region of interest.</td>
</tr>
<tr>
<td>Wash-in rate - maximum slope (WiR)</td>
<td>Ascending slope, Rise slope RS</td>
<td>Characterizes the rate of UCA accumulation in the region of interest.</td>
</tr>
<tr>
<td>Wash-out rate - minimum slope (WoR)</td>
<td>Descending slope (DS) K</td>
<td>Characterizes the rate of UCA wash-out in the region of interest.</td>
</tr>
<tr>
<td>Fall time (FT)</td>
<td>Wash-out time (WOT)</td>
<td>Time from maximum intensity to TO – the point where the minimum slope tangent intersects the x-axis</td>
</tr>
</tbody>
</table>

UCA, ultrasound contrast agent
ble to that of contrast-enhanced CT/MRI [8]. Hypoenhancement of solid lesions in the late and postvascular phases characterizes malignancies, almost all metastases and typical hepatocellular carcinomas (HCCs) showing this feature [9,10]. However, there are certain situations when an examination based purely on visual assessment can lead to misdiagnoses. Benign entities, such as hemangiomas, adenomas, focal nodular hyperplasias (FNHs) might show mild washout in the late phase, while inflammatory lesions such as immature abscesses can present an early and marked washout mimicking the enhancement pattern of non-hepatocellular malignant tumors [11-14]. On the contrary, some HCCs might not show washout, especially well-differentiated ones [15].

Since hypoenhancement in the late phase represents the main characteristic of malignant FLLs, recent studies focused on the quantitative analysis of CEUS recordings obtained during the late phase, in order to further characterize the washout phenomenon [6,7].

Mulazzani et al retrospectively analysed 39 late phase CEUS recordings of FLLs in patients at risk for HCC and proposed the use of a novel parameter: WOV. This is expressed as the median value of all frame-by-frame differences in nodule to parenchyma signal intensity in order to increase the sensitivity of CEUS in the detection of washout. All 30 confirmed HCCs included in the study were hypoenhanced during the late phase (considering negative values of WOV), while the simple visual assessment of the operator using CEUS noted washout in only 23/30 HCCs [6].

Schwarz et al proposed the use of a different parameter: late phase ratio of signal intensities within the lesion and the background tissue (LPR) and found that it differed significantly between malignant and benign entities. With a cut-off at 1 (ROI/REF), every measurable hypoenhancement can be considered suspicious for malignancy, with a diagnostic accuracy of 85.8% [7].

Existing clinical trials showed varying results when it comes to analysing CEUS perfusion quantification parameters related to the arterial and portal-venous phases. Thus, Beyer et al found that peak intensity (PI) and regional blood volume (RBV) are significantly different between malignant and benign FLLs, with higher values corresponding to the latter. However, Goertz et al found no significant difference between malignant and benign FLLs in peak intensity (PI) [16,17]. It is important to notice that in the study conducted by Beyer et al, perfusion quantification parameters were obtained by continuously measuring over a determined time period of 3 minutes, up until the late phase, allowing a better characterization of the blood volume by calculating AUC, while peak enhancement (PE) can be influenced by external factors like the contrast medium injection speed as shown by previous studies [16].

Schwarz et al found that rise time (RT) showed a large variation in the benign FLLs group. While hemangiomas and adenomas are characterized by a longer RT, FNHs tend to have a shorter RT. Schwarz et al also showed that RT has only a moderate diagnostic performance in differentiating between all benign and malignant FLLs, most likely due to the particularly distinct behaviour of FNHs compared to the other benign liver lesions [7]. The fast contrast inflow of FNH was also described by Pei et al who found a significant shorter time to peak (TTP) in FNH compared to HCC, results congruent to those obtained by Zheng et al [18,19].

**Differentiation between malignant FLLs**

The role of CEUS in distinguishing between malignant FLLs, particularly HCC and intrahepatic cholangiocarcinoma (ICC) remains controversial [20]. The WFUMB/EFSUMB guideline update state that arterial hyperenhancement, followed by a late onset mild washout is a key feature for the diagnosis of HCC in liver cirrhosis, while rim-like enhancement followed by a more rapid (<60 s) and pronounced washout is typical to other primary tumors, such as ICC and liver metastases [9]. Similar patterns of enhancement have been described in multiple studies, for both ICC (fig 2) and HCC (fig 3) [21-23]. Sensitivity of CEUS for diagnosing HCC is 88% according to a meta-analysis including 1333 HCCs from 19 different studies [24].

Overlapping features are also frequently observed depending on the tumor size, histological variant, grade of differentiation, making it difficult to establish the definitive diagnosis solely on subjective analysis [25-27].

Clinical trials comparing CEUS perfusion quantification parameters of the arterial phase of HCC and ICC showed varying results without finding significant differences. While Wildner et al found no statistical difference of the arterial D-CEUS parameters between HCC and ICC, Li et al found that PE was lower in ICC compared to HCC [28,29].

The results of quantitative analysis of the portal venous and late phase CEUS recordings of both HCC and ICC are characterized by a more homogenous pattern among existing studies. Wildner et al showed a more rapid and intense loss of signal intensity for ICC compared to HCC, with lower values of MTT, FT and higher percentage loss of intensity at definite time points after PE [28].

A recent study by Qiu et al aimed to compare the visual and D-CEUS based interpretations of the CEUS
LI-RADS -Liver Imaging Reporting and Data System (v.2017), regarding consistency, nodule enhancement intensity and washout onset in patients with liver nodules at high risk for HCC. The LI-RADS visual and D-CEUS interpretation results differed significantly in detecting early washout (<60 s) and the LR-5 washout onset [30].

A retrospective study by Lu et al analysed the value of CEUS perfusion parameters in the differential diagnosis of HCC and hypervascular liver metastases and found that washout time (WT) proved to be the most useful parameter to differentiate between these tumors, with values significantly lower for liver metastases compared to HCC [31]. It is important to note that WT represents the duration between PE and the point of TICs in tumor meeting with that in references (the moment when the washout starts) and its value is dependent on tumoral drainage [32].

In conclusion, D-CEUS can also be used as a starting point for optimizing already implemented standardized systems for technique, interpretation, reporting and data collection for CEUS examinations in patients at risk for HCC, such as CEUS LI-RADS v2017 [30].

Correlation between quantitative parameters and histological aspects in HCC

Multiple studies have previously focused on the relationship between histological grading and imaging findings (CE-CT, CE-MRI). Even though definite differentiation among HCCs only by imaging is impossible, it has been reported that tumor pathological differentiation correlates well with imaging findings [33,34]. A few studies thus reported that enhancement patterns observed during CEUS may be related to pathological aspects of HCCs [35,36].

A study by Pei et al, using Sonoliver® quantification software, reported that WT is the only perfusion parameter that correlates with histological grading, longest WTs corresponding to well-differentiated HCCs [32]. The findings are in agreement with a previous study by Liu et al which showed that the timing of HCC becoming hypoenhancing on CEUS is correlated with tumour cellular differentiation, well-differentiated HCCs washing out slower than moderately/poorly differentiated ones [35].

No correlation between RT, TTP, RS or maxim intensity / peak intensity (IMAX / PI) and the histological grading was found by Pei et al [32]. The same findings were reported by Liu et al, who showed there was no significant difference between hyperenhancing, iso-enhancing and hypoenhancing lesions of distinct cellular differentiation in the arterial and portal phase [35]. Contradictory results were also presented by Jang et al who reported that moderately differentiated tumors are mostly hypervascularized and therefore appear more frequently as hyperenhanced in the arterial phase, when compared to well-differentiated and poorly differentiated tumors [26]. A correlation between blood supply and the grade of malignancy of HCC nodules was demonstrated by previous radiological and pathological studies. During the multistep hepatocarcinogenesis process, the vascularization of hepatic nodules changes from a predominantly portal supply to an exclusively abnormal arterial supply [36,37]. A previous study by Pei et al showed that RT, TTP, RS and IMAX/PE did correlate with the number of unpaired arteries, reflecting the possible role of D-CEUS in monitoring the effects of anti-angiogenic therapy on HCC [38].

A relationship between the presence of microvascular invasion (MVI) and pathological grade of the tumor...
was also described, moderately and poorly differentiated tumors having relatively higher incidences of MVI, even at smaller tumor sizes [39]. Damage of the intravascular fronds and rupture of arterioles could contribute to arterio-venous shunting, which in turn leads to even faster washout. It is hypothesized that the faster the washout is, the more frequent the MVI would be [40]. The potential role of D-CEUS in the prediction of MVI in HCC had only been studied in a couple of clinical trials. A retrospective study by Dong et al found that the WiR and WoR are significantly higher in invasive tumors, previously described in former studies as the “fast in, fast out” enhancement pattern [41].

Xu et al retrospectively analyzed 40 arterial phase CEUS recordings from patients with histologically proven ICCs in order to investigate the correlation between enhancement patterns and pathological findings. They reported that tumoral hypenrenancement during the arterial phase always indicated increased density of tumoral cells [42]. It is important to also take in consideration that continuous dynamic registration until the late phase might be needed since blood volume is best evaluated by calculating AUC and not PE [16].

**Monitoring treatment response-systemic and locoregional treatments**

Molecular targeted therapies with multi-kinase inhibitors (MKI) are used as first and second line treatments for patients with advanced-stage HCC and preserved liver function [43]. Considering their antiangiogenic effect, MKI induce changes in tumor vascularity and structure, before triggering changes in tumor size, therefore assessing the response to treatment using purely dimensional criteria might not be adequate. Consequently, D-CEUS is currently being evaluated as a potential method to appreciate response to antiangiogenic therapies in patients with HCCs [44].

Lassau et al conducted a prospective study which included 539 patients with different tumors, including HCCs and confirmed that the percentage changes of the AUC are predictive of response to antiangiogenic therapy [45]. The utility of assessing AUC variation for predicting response to antiangiogenic therapy has been confirmed in a number of other clinical trials. Frampas et al conducted a study on 11 patients with HCC treated with sorafenib and sunitinib and showed that a decrease in AUC of more than 40% at 1 month using D-CEUS predicted non-progression with reference to RECIST at 2 months estimated using CECT [46]. Sugimoto et al included 37 patients with HCC treated with sorafenib and found that wash-in AUC (WiAUC) on day 14 was the most relevant perfusion parameter for predicting tumoral response to antiangiogenic therapy [47].

Wang et al showed that TTP is the best differentiator between responders and non-responders, continuing to increase in the responder group, suggesting an impact of antiangiogenic therapies on blood flow velocity [48]. The same tendencies were observed in an earlier clinical trial by Zocco et al [49].

Thermal ablation (TA) is regarded as one of the best treatment options for patients with early-stage HCCs, who are not appropriate candidates for liver resection or transplantation. D-CEUS applications in this area are limited, and studies have predominantly focused on radiofrequency ablation (RFA) since its energy distribution is known to be influenced by tumor perfusion. Previous studies reported that a low PE before RFA is a significant risk factor for intrahepatic recurrence, theorizing a thermal sink effect and consecutive incomplete ablation, due to rapid blood outflow from a drainage route [50]. The predictive value of TTP values before RFA remains controversial to this day. Thus Gao et al found that lower values of TTP before RFA are associated with an increased risk of HCC recurrence after ablation, contrary to the findings of Han et al [51,52].

The role of D-CEUS in evaluating post-transarterial chemoembolization (TACE) success control has been investigated in a small number of clinical trials [53-56]. Nam et al found a significant PI reduction in the complete treatment group, results in agreement with findings from a previous study by Moschouris et al [53,54]. No consensus has been reached regarding the usefulness of TTP and MTT in evaluating TACE treatment response [55,56].

**Computer-aided color parametric imaging**

Computer-aided color parametric imaging (CPI) is a novel technique that can be used to improve the characterization of FLIs, highlighting their hemodynamic features [57-61]. It measures the differences in arrival time of the UCA between the target lesion and reference points, such as intrahepatic arteries, portal vein or tumor vessels (fig 4). In color mapping, delays in the arrival of the UCA at the target site compared with that at the reference point (0 seconds) are represented in colors, usually from red to navy blue, the time interval set between each color being set at around 0.5 seconds. It is also important to mention that starting from the color mapping (CM) images obtained, by setting a ROI, quantitative parameters can be acquired, such as the mean arrival time of the UCA in the ROI from the reference point (MT), therefore allowing for a more objective, quantitative analysis [57,58].
Wu et al. conducted a retrospective study that included 42 patients with a history of colorectal cancer (CRC) in order to compare the ability of CEUS and CPI to that of routine CEUS alone in differentiating atypical hemangiomas from CRC liver metastases. They found that CPI improved the diagnostic performance for both resident and senior radiologists. Due to its higher capacity to demonstrate temporal changes in contrast enhanced imaging findings, the combination of CEUS and CPI proved to be better at detecting peripheral nodular enhancement, mosaic enhancement and feeding artery, characteristics which were significantly different between liver hemangiomas and liver metastases [59]. Wakui et al. showed that CEUS combined with CPI was better at detecting the spoke wheel patterns of FNHs less than 3 cm in size, overcoming the limits of CEUS, which cannot detect tiny changes in UCA dynamics in lesions that are either too small or enhanced very shortly [60].

A recent study by Wang et al. who performed CEUS with CPI analysis on 121 patients with HCC prior to RFA showed that a centrifugal perfusion CPI pattern correlates with well to moderate differentiated HCCs, while a centripetal one correlates with poorly differentiated HCCs. Aside from correlating with histopathologic features, Wang et al. also found that the CPI perfusion pattern was an independent risk factor for progression-free survival post RFA, patients with HCC and a centripetal perfusion CPI pattern before RFA having a poorer prognosis compared to those with an initial centrifugal CPI pattern [61].

The role of CEUS-CPI in evaluating early response to antiangiogenic therapy in patients with HCC has been investigated in a limited number of studies. Shiozawa et al. suggested the possibility of quantification and objective evaluation of changes in tumoral blood flow velocity visually detected in CM images in 2 previous studies [57,58].

**Conclusion**

In conclusion, D-CEUS has emerged as a promising tool for assessing dynamic changes in the vascularity of FLLs. From helping establish a diagnosis to monitoring therapeutical response, D-CEUS has the potential of becoming a valuable instrument in the multistep evaluation process of patients with various FLLs. Nevertheless, quantitative analysis of CEUS perfusion (D-CEUS) can provide more objective, reliable and reproducible results, overcoming the limits of a purely visual analysis - interobserver variability and low reproducibility of results.

**Conflict of interest:** none

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**References**


