Effects of perfusion and vascular architecture on contrast dispersion: validation in ex-vivo porcine liver under machine perfusion

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Abstract—Dynamic contrast enhanced ultrasound (DCE-US) enables imaging of cancer angiogenesis by quantification of perfusion and dispersion. Although increased perfusion may be found in areas of active angiogenesis due to increased demands for blood supply, decreased perfusion may be caused by the decreased efficiency and functionality, typical of cancer angiogenic microvasculature. Contrast dispersion, mainly determined by the flow profile in large vessels and by the multipath trajectories in the microvasculature, may thus represent a suitable alternative to characterize cancer angiogenesis. Based on a model of the contrast transport kinetics as a convective-dispersion process, several DCE-US methods have been proposed estimating dispersion for characterization of cancer angiogenic vasculature. Although dispersion imaging has shown promising in a clinical context, its physical link with variations in flow and vascular architecture has never been shown. The objective of this work is thus to investigate the influence of flow and underlying vascular architecture on the estimation of dispersion in an ex-vivo machine-perfused pig liver.

Index Terms—Angiogenesis imaging, dynamic contrast-enhanced ultrasound, perfusion quantification, dispersion quantification, cancer.

I. INTRODUCTION

DYNAMIC contrast-enhanced ultrasound (DCE-US) has been shown to be a powerful tool for real-time in-vivo imaging of blood flow at both the macro- and microvascular level [1]. In DCE-US, ultrasound contrast agents (UCA) consisting of gas-filled, encapsulated microbubbles, providing strong back-scattering, are used in combination with contrast-specific imaging sequences to enhance the received acoustic intensity [2], [3]. Thanks to their size of 1-10 μm in diameter, which is similar to red blood cells, they can travel throughout the entire circulation remaining intravascular [2], [3].

These characteristics make DCE-US particularly interesting for imaging of angiogenesis, a fundamental process in cancer development by which additional blood supply is formed to feed the tumor and allow its spreading [2], [4]. The resulting tumor vasculature is characterized by a dense, irregular network of fragile microvessels, exhibiting increased permeability and tortuosity, arterio-venous shunts, and chaotic flow patterns [2], [4].

On the hypothesis that increased blood supply demands translate into increased perfusion, several DCE-US methods have been developed for assessment of cancer angiogenic vasculature by quantification of perfusion [2], [5], [6]. Time-intensity curves (TICs), obtained at each pixel or in a region-of-interest (ROI) in the DCE-US loops, can be fit by several mathematical models, enabling the estimation of quantitative or semi-quantitative perfusion parameters which have been related to the hemodynamic changes occurring in tumor vasculature due to angiogenesis [1], [2].

However, due to the complexity of tumor vasculature, a clear link between blood flow changes and disease progression is difficult to establish. While the higher microvascular density and the presence of arterio-venous shunts may cause low flow resistance and increased perfusion, the higher tortuosity, the increased interstitial pressure, and the lower functionality of angiogenic vessels may lead to increased resistance and lower perfusion; this makes perfusion-based assessment of angiogenesis a challenging task. Alternatively, the tortuosity and irregularity of the tumor vasculature can be related to dispersion features of the UCA transport [7], [8], [9]. In fact, while in large vessels UCA dispersion is mainly determined by the Brownian motion of contrast particles due to radial diffusion, in tissue the macroscopically observed UCA dispersion is mostly due to the multi-path trajectories of the contrast particles in the microvasculature [10].

Motivated by this, several DCE-US methods for dispersion quantification have been developed in recent years. Modeling the UCA transport as a convective-dispersion process, and fitting DCE-US derived TICs by the modified local density random walk solution (mLDWR) of the convective-dispersion equation enables dispersion quantification by the estimation of the parameter $\kappa = v^2/D$, where $v$ and $D$ are the local UCA velocity and dispersion, respectively [7]. Alternatively, dispersion can be indirectly assessed by spatio-temporal analysis of neighbouring TICs, providing similarity measures which have been mathematically related to $\kappa$ [8], [11]. More recently, a method for separate estimation of $v$ and $D$ has been proposed by Wiener system-identification of the convective-dispersion equation [9].

Although these methods have shown promising results in the clinical context, the physical dependency of dispersion on

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blood flow and the vascular architecture has never been proved. The aim of this work is thus to investigate the influence of flow and the underlying vascular structure on dispersion parameters derived by DCE-US. Validation is performed in an ex-vivo pig liver in machine perfusion [12], which was shown to be a promising testbed for DCE-US quantification [13].

II. Methods

A. Ex-vivo porcine liver in machine perfusion

Pig livers were harvested from a local slaughterhouse and were connected to a machine perfusion system to maintain it functional and viable. The machine perfusion system comprise a closed circuit in which a perfusate is pumped out of an organ chamber, where the liver is positioned, passing through an oxygenator. At the exit of the oxygenator, flow is split into two channels, one connected to the portal vein (PV) and one to the hepatic artery (HA), going through flow-meters which are used for flow control. Liver viability was monitored by measuring bile production, oxygen consumption, and sustained vascular perfusion at every half hour [12], [13]. Further details on liver procurement and the machine perfusion system can be found in [12].

In this study, the total flow rate was changed from 800 to 1600 ml/min by changing the flow in the PV from 600 to 1400 ml/min while maintaining the HA flow constant at 200 ml/min. At these flow rates, the HA and PV pressures are comparable to those found in humans.

B. Ultrasound imaging

After injection of a bolus of BR38 contrast agent (Bracco Suisse, Geneva, Switzerland), DCE-US was performed using an iU22 diagnostic US scanner (Philips Medical Systems, Bothell, WA, USA) with an L9-3 linear array probe held in a fixed position using a mechanical arm. Contrast loops were acquired in power modulation mode with the following settings: frequency = 3.1 MHz, MI = 0.05, focal depth = 3.5 cm, frame rate = 11 Hz, dynamic range = 50 dB. DCE-US loops of about 45 s were recorded for each value of the flow rate (800, 1200, 1600 ml/min).

C. Dispersion analysis

The theoretical basis of the proposed methods for dispersion quantification resides in the description of the transport of an UCA bolus in a blood vessel as convective-dispersion process:

$$\frac{\partial C(z,t)}{\partial t} = D \frac{\partial^2 C(z,t)}{\partial z^2} - \nu \frac{\partial C(z,t)}{\partial z}, \tag{1}$$

where \(C(z,t)\) is the UCA concentration at time \(t\) and position \(z\); \(D\) is the dispersion coefficient; and \(\nu\) is the convective velocity.

The modified local density random walk (mLDRW) solution of (1) provides a local characterization of the UCA dispersion process as [7]

$$C(t) = \alpha \sqrt{\frac{\kappa}{2\pi(t-t_0)^2}} e^{-\frac{(t-t_0)^2}{\sigma(t-t_0)^2}}, \tag{2}$$

where \(\alpha\) is the time-integral of \(C(t); t_0\) is the theoretical contrast injection time; \(\mu\) is the mean transit time of the contrast particles between injection and detection sites; and \(\kappa\) is the dispersion parameter, given by the local ratio between contrast convection (squared velocity \(\nu^2\)) and dispersion (dispersion coefficient \(D\)).

Fitting DCE-US derived TICs by (2) enables the assessment of dispersion by estimation of the local dispersion parameter \(\kappa\). After spatial filtering by a Gaussian filter with \(\sigma = 0.25\) mm and time-filtering by a low-pass filter with cut-off frequency of 0.5 Hz, parameter estimation is performed as explained in [7].

Under the observation that dispersion greatly influences the degree of similarity between TICs in neighbouring pixels, shown by simulation of (1) [8], spatio-temporal analysis is also performed for indirect assessment of dispersion. TIC similarity is estimated in the time domain by calculation of the linear correlation coefficient \(\rho\) between TICs within a ring-shaped kernel with inner radius of 0.1 cm and outer radius of 0.25 cm, as explained in [8].

Finally, a model-based Wiener system identification approach is used for separate estimation of \(\nu\) and \(D\) [9]. The vascular network is considered as a dynamic linear system, whose impulse response can be locally identified by the input-output analysis of TICs in a kernel, which is shaped as a ring with inner and outer radius of 0.5 mm and 0.8 mm, respectively. The input-output response is modeled by (1) and a Wiener filter is constructed for identification of the local dilution system. Parameter estimation is performed with a maximum likelihood approach as explained in [9].
Fig. 2. Changes in $\kappa$, $\rho$, $v$, $D$ with varying flow rates in the PV (blue triangles) and Pa (green squares) with corresponding fit (dotted black line). Error bars represent the standard deviation.

To investigate the influence of flow on contrast dispersion, the parameters $\kappa$, $\rho$, $v$, and $D$ are estimated for three different values of input flow rate (800, 1200, 1600 ml/min). The ability of dispersion to distinguish between different vascular architectures is then assessed by comparing the parameter values in two ROIs (Fig. 1), representing a large vessel (PV) and the Parenchyma (Pa).

III. RESULTS

The changes in $\kappa$, $\rho$, $v$, and $D$ in the PV and Pa for different values of the input flow rate are shown in Fig. 2a-d. The plots are obtained by calculating the mean and the standard deviation (error bars) of the parameters within the ROIs, representing a large vessel (PV) and perfused tissue (Pa) (Fig. 1). The quadratic dependency of $\kappa$ on contrast velocity can be well observed in Fig. 2a for the PV (coefficient of determination $R^2$ of quadratic fit is 0.97). As expected, lower in-plane velocity $v$ is found in the Pa compared to the PV. This is shown in Fig. 2b, which also suggests a linear relationship between $v$ and flow ($R^2$ of linear fit equals 0.91 for PV and 0.96 for Pa); on the other hand, the changes in $\rho$ and $D$ with the input flow rate were not significant, as shown by the low angular coefficient of the linear fit (in the order of $10^{-5}$), suggesting that these parameters are mostly dependent on the underlying vascular structure.

IV. DISCUSSION AND CONCLUSIONS

In this work, we investigated the influence of flow rate and vascular architecture on dispersion parameters measured by DCE-US in an ex-vivo pig liver under machine perfusion.

Dispersion was assessed by estimation of the local dispersion parameter $\kappa = v^2/D$, based on mLDRW model fitting; by estimation of the linear correlation coefficient $\rho$, indirectly assessing dispersion by spatio-temporal similarity analysis; and by separate estimation of velocity $v$ and dispersion $D$, based on Wiener local system identification of the convective dispersion equation. As expected from the direct relationship between flow and velocity, the parameters $\kappa$ and $v$ are influenced by the input flow. Therefore, although possible, the distinction between different vascular architecture is more
challenging, especially for \( \kappa \) at lower flow rates. On the other hand, the parameters \( \rho \) and \( D \) show little to no dependence on input flow, and they were able to distinguish between different underlying vascular architecture: a large vessel and the parenchyma.

Lower dispersion values were observed in the portal vein compared to the parenchyma. In large vessels, dispersion is mainly determined by the flow profile, while in the microcirculation it is mainly due to the multipath trajectories across the microvascular bed. In case of a parabolic flow profile, which is expected in the portal vein, the contrast particles are dragged by the carrier fluid at different velocities depending on their radial position across the vessel [14]. As a result, dispersion increases with the peak velocity. In the parenchyma, although lower dispersion can be expected in each single micro-vessel due to the smaller size and lower peak velocity [15], the distribution of contrast transport across multiple trajectories in the microvascular bed leads to higher microscopically-observed dispersion. Accordingly, the local dispersion parameter \( \kappa = \frac{v^2}{D} \) showed smaller values in the parenchyma compared to the portal vein.

Despite the promising results, there are some limitations in this study. The estimation of \( v \) and \( D \) is challenging due to the fact that only the 2D projection of the 3D velocity vector onto the US imaging plane is actually estimated. This is particularly critical in the PV, where the flow was almost perpendicular to the imaging plane. The reliability of the estimated parameters might also be affected by an encountered issue with the microbubbles which tend to stick to the pig liver [13]. This issue, normally not occurring in humans, might be due to the absence of trans-pulmonary passage, filtering out the larger bubbles, and/or to some partial collapse of the microvessels in the \textit{ex-vivo} liver. The "sticking” effect, hiding the TIC wash-out, may affect the estimation of \( \kappa \) and \( \rho \), for which the analysis of part of the wash-out is typically included, and it is even more critical for the estimation of \( v \) and \( D \) by Wiener system-identification, whereby the full TIC is used.

Finally, in this study the investigation was limited to the comparison between a large vessel and a vascular network including smaller vessels and capillaries. Further validation comparing different vascular networks, showing typical features of malignant and benign tissue, is necessary to establish the ability of dispersion analysis to highlight those changes in the microvasculature due to cancer angiogenesis, and to clarify the theoretical rationale underlying dispersion imaging, needed to support the promising results obtained in the clinical context. In future work, this could be achieved by fluid-dynamic simulations, by \textit{in-vitro} experiments with vascular phantoms, and by \textit{in-vivo} studies with controlled tumor models in animals.

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