Timeliness of splenic time-to-peak affects liver tumor CT perfusion measurements

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Research Article

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Abstract

Aim

In liver CT perfusion, the dual-input maximum slope (DI-MS) method is commonly used to estimate perfusion to aid diagnosis of tumors. The DI-MS method relies on a model that assumes the splenic time-to-peak (TTP) separates arterial and portal venous perfusion, and occurs prior to venous perfusion. In this preclinical study, we examined how the timeliness of splenic TTP affects DI-MS perfusion calculations of liver tumors.

Materials and Methods

We analyzed imaging data obtained from 11 New Zealand White rabbits bearing a single implanted VX2 tumor in liver. A liver 320-slice CT perfusion protocol (5,400 images per study) was used to generate images. Times for arterial and portal slopes were recorded, and hepatic arterial perfusion (HAP), portal perfusion (HPP) and perfusion index (HPI) for liver and tumor were separately calculated using manual and automated methods. T-test comparisons and Bland-Altman plot analyses were performed.

Results

Mean tumor TTP occurred at 9.79 s (SD=3.41) and splenic TTP at 9.75 s (SD=4.47, $p=0.98$). In 3/11 (27.27%) cases, tumor SP occurred prior to spleen (mean difference=1.33 s, SD=1.15 s). In these cases, mean automated HPP values were 43.8% (SD=52.48) higher compared to manually computed ones. There were statistically significant differences between automated and manual methods for normal liver and tumor HPI and HPP ($p<0.01$ and $p<0.0001$, respectively), but not HAP values ($p=0.125$ and $p=0.78$, respectively). There was also a statistically significant variation between methods for tumor HPP and HPI ($p=0.001$, respectively).

Conclusion

In 320-slice CT perfusion of liver in this preclinical model, we observed that tumor TTP occurred prior to splenic TTP in 27.27% of tumors in liver. This temporal relationship affects tumor perfusion calculations and should be identified to address potential deviations of model assumptions.

Introduction

The liver exhibits unique flow dynamics originating from its dual vascular supplies¹⁻⁴. Approximately 25% of the blood supply to the liver is arterial provided by the hepatic artery, and approximately 75% is venous provided by the portal vein from the spleen, gastrointestinal tract and associated organs⁴. By

contrast, hypervascular primary and metastatic liver tumors are supplied primarily by the hepatic artery, thus forming the basis of all intra-arterial interventions for these tumors⁵⁻⁷.

Diagnostic oncologic imaging and imaging-based evaluations of tumor response to therapy rely on accurate blood flow quantification⁸. Non-invasive blood perfusion measurements are conducted by imaging contrast changes occurring in tissues using a series of rapid sequential scans with X-ray computed tomography (CT) following intravenous delivery of iodinated contrast material^{8,9}. Imaging contrast increases and decreases as the concentration of contrast material within a tissue increases via arterial flow and subsequently decreases with its depletion by venous outflow¹⁰. Direct CT assessment of tissue perfusion is possible because the concentration of contrast material in tissue measured with CT and expressed in Hounsfield units (HU) is directly proportional to the local concentration of contrast material in the tissue¹¹. This quantitative information is unavailable from conventional contrast-enhanced CT where the degree of tumor enhancement at certain time points (i.e., arterial or portal venous phase) is assessed using qualitative criteria¹². Methods to calculate blood perfusion have evolved during the past two decades to include maximum slope (MS), tracer kinetic (single compartment), and deconvolution⁷⁻¹⁹.

Described by Peters *et al.*, the MS method was the first mathematical model applied to quantify tissue perfusion²⁰. Miles *et al.* used it to quantify hepatic perfusion by dynamic contrast-enhanced CT¹⁰, which they validated with dynamic colloid scintigraphy^{14,15}. The method was later modified by Blomley *et al.*¹⁶, and its simplicity has motivated its adoption for use in many studies involving quantification of liver perfusion for three decades⁸. The MS method assumes no venous outflow in the tissue, therefore only that portion of the time-density curve (TDC), which occurs before the start of venous outflow, is considered. Using this method, the time of peak enhancement of the tissue region of interest (ROI) is chosen as the beginning of the venous outflow and is defined as end phase (EP, measured in seconds). By definition, after EP the enhancement decreases, occurring only if contrast material is leaving the tissue, signaling venous outflow. Further, a start phase (SP, measured in seconds) is defined as the time when contrast material first enters the tissue, signified by the start of contrast enhancement. A short and fast contrast bolus is administered to ensure validity of the no-venous outflow assumption²¹⁻²⁵.

In a single supply organ, the maximum slope of the TDC, occurring between the SP and EP, is calculated and divided by the peak enhancement in HU of the supplying artery, to obtain the blood flow per unit volume. This is described by the following equation:

$$\frac{F}{V} = \frac{\left. \frac{d[c(t)]}{dt} \right|_{\max} \Big|_{t=SP}^{t=EP}}{a(t) |_{\max}} \quad 1)$$

where F is the blood flow rate in the tissue, V is the tissue volume, SP is the start phase, EP is the end phase, $c(t)$ and $a(t)$ are the concentration of contrast medium in the tissue and artery at time, t .

With liver being a dual blood supply organ, fed by hepatic artery and portal vein, a different approach is used. As the MS method is a derivation of Fick's principle, a generalized approach enables separate evaluation of each contribution, hepatic (arterial) and portal (venous), to the dual liver blood supply motivating its modification for the liver²⁶. It is generally assumed that contrast material supplied by the artery accumulates without venous outflow. Therefore, the slope of the TDC in a general sense is determined from the rate of intake of contrast material into the tissue, with the TDC of arterial supply providing information of the contrast concentration. It is also assumed that no contributions to contrast arise from other organs or tissues supplying the portal vein prior to the time of the splenic peak, i.e. peak contrast in spleen. In other words, observed increasing contrast in liver is explicitly assumed to be supplied only by arterial flow. Thus, the time of splenic peak enhancement designates the beginning of the portal phase (SP) and the time of peak liver tissue enhancement designates the end of the portal phase (EP). In subjects having normal blood circulation, peripherally intravenously injected iodinated contrast material arrives first within the hepatic artery, followed by the portal vein²⁴. Even though contrast medium from the splenic and pancreatic circulation arrives in the portal vein earlier than that from intestinal circulation, the contribution of the portal vein to hepatic enhancement is usually low within the arterial phase of the contrast injection^{25,27}.

To account for the dual input, the MS is thus modified to (DI-MS):

$$\left(\frac{F}{V}\right)_A = \frac{\left.\frac{d[c(t)]}{dt}\right|_{max}^{t=SM}}{a(t)|_{max}^{t=SP}} \quad 2)$$

$$\left(\frac{F}{V}\right)_P = \frac{\left.\frac{d[c(t)]}{dt}\right|_{max}^{t=EP}}{p(t)|_{max}^{t=SM}} \quad 3)$$

where $\left(\frac{F}{V}\right)_A$ is the arterial blood flow rate per unit tissue volume, $\left(\frac{F}{V}\right)_P$ is the portal blood flow rate per unit tissue volume, SM is the time at which spleen attains maximum enhancement, and $p(t)$ is the concentration of contrast medium in the portal vein at a time t .

For the DI-MS method to be valid for liver tumors, the SP should occur before the EP of a hypervascular liver tumor. An accurate estimation of the temporal relationship between tumor and splenic peak enhancement depends on various parameters, including individual patient characteristics and image acquisition parameters, curve fitting, motion artifacts, image processing and type of scanner. Moreover, manual and automated selection of SP and EP have been reported to lead to measurement discrepancies of CT perfusion values^{21,22}. Modern wide-array volume CT scanners can rapidly (<1 s) and simultaneously scan up to 16 cm of tissue, facilitating simultaneous visualization of the liver (with tumor) and spleen. This introduces fewer respiratory motion and misregistration artifacts²³. The aim of

this study was to evaluate the temporal relationship between tumor peak and splenic peak enhancements for calculation of perfusion parameters using the DI-MS method in a rabbit model bearing implanted tumor in liver, using a wide-array CT perfusion scanner.

Materials And Methods

Animal Model and Tumor Implantation

Eleven adult male New Zealand White rabbits (Robinson Services, Inc. Mocksville, NC), were used in this study. All rabbits weighed 3.5–4.2 kg prior to imaging. Rabbits were housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facility in compliance with the Guide for the Care and Use of Laboratory Animals²⁸. All procedures and protocol were approved by the Johns Hopkins Institutional Animal Care and Use Committee (IACUC). Male white New Zealand rabbits were selected for their relevance to intra-arterial procedures and liver tumor imaging as part of ongoing studies of liver cancer. The VX2 cell line was originally purchased from the American Type Culture Collection (ATCC) and has been maintained at the Johns Hopkins University via serial tumor cell implantations in New Zealand White rabbit thighs. As the VX2 tumor cell line is the only rabbit cell line maintained in our laboratory, we tested for species specificity by karyotyping to rule out contamination with human and other non-human cell lines (data available upon request). Karyotyping was routinely performed after thawing each batch of frozen cells and before publication.

At designated time points, individual animals were randomly selected for inclusion in study cohorts prior to implantation of VX2 tumor in the liver for subsequent CT perfusion imaging. Each animal received tumor implantation in the left lobe of the liver as detailed in previous publications^{29–31}. The tumors were allowed to grow in the rabbit livers for 13–15 days before imaging^{32,33}.

Wide-Array CT Perfusion Protocol

For CT perfusion imaging, each rabbit was first sedated with an intramuscular injection of ketamine (20 mg/kg) and xylazine (8 mg/kg) and subsequently scanned with a wide-array 320-slice clinical CT scanner (Aquilion ONE, Toshiba, Japan). The CT perfusion protocol included at least one non-contrast enhanced volume acquisition, followed by a series of contrast-enhanced CT acquisitions. Isoosmolar contrast iodixanol (1.5 ml/kg, 320 mg I/ml-Visipaque, GE Healthcare Inc., Princeton, NJ) was injected intravenously at a rate of 1 ml/s via a 21G butterfly needle inserted in a marginal ear vein, followed by a saline flush of 7 ml at the same rate. CT perfusion scanning parameters were: FOV = 22cm, KV = 120, mA = 80, slice thickness = 0.5mm, scan delay = 6 s. Based on the above information, the dose exposure for each animal was defined on the monitor to be $CTDI_{vol} = 164.70$ mGy for a total duration of 17.5 s. Total intermittent scanning time for each rabbit scan was 77 s. Wide-array CT scans were obtained at 2-s intervals for the first 25 s, and every 3 s thereafter for an additional 42 s. Each scan required 0.5 s (one volume acquisition equals a single gantry rotation at a speed of 0.5 s per 360°). A total of 27 acquisitions

with 5,400 images were obtained during each CT perfusion study. These were subsequently transferred to a dedicated workstation for image reconstruction and analysis.

Image reconstruction and registration

Following CT acquisition, images were reconstructed with adaptive iterative dose reduction 3D (AIDR 3D, Toshiba Medical Systems, Japan), the manufacturer's commercial hybrid iterative reconstruction algorithm that enables combining reconstruction in the raw data and image space domains. The reconstruction kernel (FC17), and the reconstruction pixel spacing (0.349 mm) were fixed for all studies.

For image registration, all imaging data from each study were exported to a dedicated workstation using manufacturer provided software (Body Registration; Toshiba Medical Systems, Tochigi, Japan) that corrects the spatially non-consistent position of each organ among the 27-image series of each study. The program adjusted the position of each organ three-dimensionally, i.e., proportionally along any axis and rotationally. For each registered study, a total of 25-image series was subsequently generated for body perfusion analysis.

CT Perfusion measurements

First, TDCs were derived from registered image series by placing circular ROIs over the aorta at the level of the porta hepatis, the main portal vein, the right and left lobes of the liver, as well as over the tumor at the level of longest axial diameter. The size of each ROI was at least 1.0 cm^2 or larger, except in the portal vein and the aorta, which were set to cover their shortest axis at the level of the hepatic hilum (and with a diameter of 1.0 mm^2). Each TDC comprised 25 time points.

Perfusion parameters were calculated by using: a) the dedicated commercial software, on a pixel-by-pixel basis that uses the dual-input MS model (Body Perfusion; Toshiba Medical System, Tochigi, Japan); and, b) MATLAB software (Version 9.0, Mathworks, Natick MA)⁹. For each organ or tissue of interest, care was taken to place each ROI at the same level as for TDC measurements. Flow measurements were expressed in mL per 100 mL per min. Hepatic arterial perfusion (HAP), hepatic portal perfusion (HPP) and hepatic perfusion index (HPI) were calculated for tumor, and left and right hepatic parenchyma.

A MATLAB algorithm was developed to implement the dual-input MS method and calculate the perfusion values. After background subtraction, curve fitting was performed for all the TDCs using a smoothing-spline function in MATLAB. The fitted TDCs were discretized using time steps of 0.1s for calculating maximum value or slope. For each ROI, the SP was selected as the time point where the enhancement reached 10% of the peak enhancement in the corresponding TDC²⁵. Similarly, the EP for each ROI was the time corresponding to the peak enhancement. The HAP and HPP values were calculated using Eq. 3 and 4, respectively. The perfusion value hence obtained has units of s^{-1} , which is then changed to $\text{ml} \cdot (\text{min}100\text{ml})^{-1}$ by multiplying the perfusion value with $100 \cdot 60 [\text{ml} \cdot \text{s} \cdot (\text{min}100\text{ml})^{-1}]$. The perfusion index was calculated using:

$$HPI = \frac{HPA}{(HPA + HPP)} * 100 \quad (4)$$

Statistical analysis

Statistical analysis included descriptive statistics for continuous variables (mean and standard deviation), *t*-test comparisons between liver and tumor normally distributed variables, Wilcoxon sign-rank test for non-parametric comparisons, as well as agreement between corresponding measurements obtained with the two different methods with the Bland-Altman analysis³⁴. The Levene's test was used to test for equality of variances and the Shapiro-Wilk test was used to test for normal distribution. The Pitman's test of variance for paired samples based on the bivariate normal distribution that compares the variance of the difference with the variance of the average was also performed and reported concurrent with each Bland-Altman plot³⁵. Stata 12 (StataCorp. 2011, *Stata Statistical Software: Release 12*, College Station, TX: StataCorp LP) was used for the statistical analysis.

Results

Mean time of occurrence of SP for aorta was 5.3 s (SD=2.59 s), portal vein 15 seconds (SD=3.97 s), spleen 9.75 sec (SD=4.47 s), tumor 9.79 s (SD=3.41 s), and liver 17.40 s (SD=1.05 s). There was no statistically significant difference between times of SP for tumor and spleen (*p* = 0.98). However, in 27.3% of cases (3/11), time of SP of tumor occurred prior to spleen (mean difference in SP, ΔSP = 1.33 s, SD = 1.15 s). Mean time for manual measurement of arterial slope in tumor was 14.86 s (SD = 3.65 s) and for liver 23.19 s (SD = 4.56 s). Mean time for manual measurement of portal slope in tumor was 24.92 s (SD = 7.20 s) and for liver it was 30.02 s (SD = 4.03 s). Mean time of occurrence of EP for tumor was 33.25 s (SD = 13.42 s) and for liver it was 42.42 s (SD = 5.07 s). Measured time-density curve (TDC) characteristics, including start phase, time to measure arterial and portal slope, as well as end phase, for aorta, portal vein, tumor, liver and spleen are shown in Table 1. A typical TDC of aortic, portal venous, splenic, tumor and hepatic enhancement is shown in Figure 1. Typical perfusion maps as calculated with the Body Perfusion software are shown in Figure 2.

Table 1
Time density curves (TDC) start (SP) and end (EP) phases measured for indicated organs.

Variable*	n	Mean	Std. Err.	[95% Conf.	Interval]
		(s)	(s)	(s)	
Aorta					
SP	11	5.31	0.78	[3.56	7.05]
EP	11	16.18	0.93	[14.11	18.26]
Portal Vein					
SP	11	15.00	1.20	[12.33	17.67]
EP	11	28.27	1.27	[25.45	31.10]
Liver					
SP	22	17.40	1.05	[15.22	19.58]
Arterial Slope	22	23.19	0.97	[21.17	25.22]
Portal Slope	22	30.02	0.86	[28.23	31.81]
EP	22	42.42	1.08	[40.17	44.67]
Tumor					
SP	11	9.79	1.03	[7.50	12.09]
Arterial Slope	11	14.86	1.10	[12.41	17.32]
Portal Slope	9	24.92	2.40	[19.38	30.45]
EP	11	33.25	4.05	[24.23	42.27]
Spleen					
SP	11	9.75	1.35	[6.75	12.76]
EP	11	23.52	1.37	[20.47	26.58]
Δ SP tumor-spleen	11	0.036	-0.319	[0.747	-0.674]
* SP=Start Phase, EP= End Phase, Δ SP=difference in SP times.					

Mean tumor HAP using the automated software was $87.5 \text{ ml} \cdot (\text{min}/100 \text{ ml})^{-1}$ (SD = 26.61) and mean tumor HAP with MATLAB (manual) calculation was $56.1 \text{ ml} \cdot (\text{min}/100 \text{ ml})^{-1}$

(SD = 17.01). Mean liver HAP using the automated software was $48.5 \text{ ml} \cdot (\text{min}/100 \text{ ml})^{-1}$ (SD=13.59) and mean liver HAP with manual method was $38.6 \text{ ml} \cdot (\text{min}/100 \text{ ml})^{-1}$ (SD=14.43). Overall, from each

method it was possible to differentiate tumor from liver for all variables, with the exception of HAP ($p = 0.78$ for the automated method and $p = 0.125$ for manual method). Table 2 lists mean HAP, HPP and HPI values calculated with each method for tumor and liver, as well as p-values for comparisons.

Table 2
Comparison of values obtained by Manual or Automated methods.

Analysis Method	Variable*	n	Mean	SD	95% CI		P-value
Automated	Liver HAP	11	90.36	6.56	[75.74	104.98]	0.78
	Tumor HAP	11	87.52	8.02	[69.64	105.40]	
	Liver HPP	11	301.53	12.09	[274.58	328.47]	0.003*
	Tumor HPP	11	41.42	5.79	[28.52	54.32]	
	Liver HPI	11	173.77	5.83	[160.79	186.75]	0.00001
	Tumor HPI	11	69.35	2.90	[62.88	75.81]	
Manual	Liver HAP	11	78.29	10.80	[54.22	102.36]	0.125
	Tumor HAP	11	59.15	5.13	[47.72	70.57]	
	Liver HPP	11	268.22	17.09	[230.14	306.30]	0.00001
	Tumor HPP	11	30.45	11.13	[5.66	55.25]	
	Liver HPI	11	44.56	4.56	[34.40	54.73]	0.0039
	Tumor HPI	11	71.50	6.89	[56.15	86.85]	
* hepatic arterial perfusion (HAP); hepatic portal perfusion (HPP); and hepatic perfusion indices (HPI). HAP and HPP values are expressed in mL per 100 mL per min and HPI in %. P-values are for t-test unpaired comparisons, except for (*), which denotes p-value of Wilcoxon signed-rank test.							

In animals with tumor time of SP preceding splenic time of SP, mean automated HPP values were increased by 43.8% (SD = 52.48) compared to manually calculated values. Tumors of these animals showed a negative portal phase duration, as calculated with the manual method, due to the splenic maximum enhancement occurring after the time of the EP of the tumor. By definition, the portal slope is calculated only from that portion of TDC that lies after the time of occurrence of the splenic maximum and before time of venous outflow (EP). Figure 3 shows representative data and corresponding fitted curves of time-dependent contrast dynamics measured in artery, portal vein, spleen, and left liver for a subject with EP of tumor occurring before EP of spleen. TDCs for healthy liver and tumor are plotted separately for comparison. The EP of left and right hepatic lobes occurred at 46.7 s and 45 s, respectively, which is greater than 13 s after the peak time of the spleen 30.5 s. The period between the peak splenic enhancement and EP is the period during which the portal flow dominates and this is when the maximum slope of the TDC curve is recorded. For tumor, EP occurs at 29.6 s, which precedes the time of peak

splenic enhancement. This suggests that by the time the portal flow begins to dominate, the tumor TDC has already gone beyond its maximum, signaling the start of the venous outflow. This violates the assumptions upon which the MS method is based and hence the MS cannot be used to calculate the hepatic portal flow of the tumor in such a case. A graph of average and standard deviation for liver and tumor SP, arterial maximum slope, portal maximum slope and EP, for all subjects is shown in Figure 4. Of note, another discrepancy observed was the violation of no venous outflow assumption, which was observed in one subject only. The TDCs for artery, portal vein, spleen, left/right liver and tumor of that subject animal are shown in Figure 5. The slope for the left/right liver and tumor are illustrated and the key time points of the TDCs are also shown. Although the EP for the tumor occurs at 44 s, this is 13.5 s after the peak splenic enhancement (30.5 s), consequently the no-venous outflow assumption has been violated. The TDC for liver attains its global maximum at 44 s and the maximum portal slope is calculated at 39.25 s. The portal slope was calculated where the no-venous outflow assumption was invalid.

Next, we assessed agreement of the two methods for perfusion calculations with the Bland-Altman plot analysis. Mean HAP, HPP and HPI values calculated using each method, as well as mean difference in variance and Pitman's test for difference in variance, are shown in Table 3. Overall, there was no statistically significant difference in the variances between the two methods for calculations of liver HAP, HPP and HPI ($p > 0.08$ for all comparisons), as well as tumor HAP ($p = 0.09$). There was, however a statistically significant difference in the measured variance between methods for tumor HPP and HPI ($p = 0.001$, respectively), indicating poor agreement between the two methods.

Table 3
Agreement between manual (MATLAB) and automated (Toshiba, Body Perfusion) methods.

			Mean	95% CI for	Pitman's	Pitman's
	Mean (SD)	Mean (SD)	Difference	Difference	r	p-value
Tumor*	59.14 (17.01)	87.51 (26.61)	-28.373	(-41.55 -15.19)	-0.532	0.092
HAP						
Tumor	30.45 (36.90)	41.41 (19.20)	-10.964	(-25.06 -3.13)	0.861	0.001
HPP						
Tumor	71.5 (22.84)	69.34 (9.62)	2.155	(-9.04 -13.35)	0.836	0.001
HPI						
Left	38.09 (10.74)	51.9 (15.64)	-13.809	(-26.31 -1.30)	-0.359	0.278
Liver						
HAP						
Left	123.65 (24.21)	156 (28.42)	-32.345	(-49.33 -15.36)	-0.189	0.577
Liver						
HPP						
Left	23.63 (5.27)	25.18 (7.30)	-1.545	(-6.80 3.71)	-0.325	0.33
Liver						
HPI						
Right	39.15 (17.91)	45.18 (10.88)	-6.036	(-15.62 -3.54)	0.546	0.082
Liver						
HAP						
Right	134.11 (28.34)	150.76 (20.06)	-16.655	(-35.21-1.90)	0.358	0.28
Liver						
HPP						
Right	22.28(7.56)	23.01(4.43)	-0.727	(-5.50, 4.04)	0.52	0.101
Liver						
HPI						
* hepatic arterial perfusion (HAP), hepatic portal perfusion (HPP) and hepatic perfusion indices (HPI) values of liver (left and right hepatic lobes) and tumor, according to Bland-Altman analyses.						

Discussion

The dual-input maximum slope method has been used in CT perfusion of liver for more than two decades^{9,10,14–16}. Earlier CT perfusion studies performed in helical CT scanners were hampered with limited field-of-view (i.e., 3–5 cm) of the scanner in the craniocaudal direction and presence of partial volume effects, among other factors⁸. A significant recent technological advancement is the development of wide-array CT scanners, which are capable of high temporal frequency imaging over a large tumor or body volume^{36,37}. For liver imaging, this is critical as wide-array CT technology enables rapid (<1s) scanning of the whole liver (up to 16 cm), providing temporal homogeneity and minimizing errors related to motion²³.

The DI-MS method was the first used to quantify hepatic perfusion parameters by dynamic CT¹⁰. Since this method was first described by Miles *et al*¹⁰ and modified by Blomley *et al*¹⁶, its simplicity has contributed to its use for many studies⁸. Many CT perfusion studies of liver using the DI-MS method have focused primarily on tumor diagnosis and differentiation, successfully demonstrating the ability of this method to distinguish perfusion differences between liver tumors and normal liver⁸. Very few studies have concentrated on evaluating the temporal relationship between SP of spleen and tumor SP, within the context of the DI-MS method. Since the spleen is the reference organ for separating arterial from portal flow with this method, SP timeliness and differences between the spleen and the organ or tissue of interest are crucial for accurately measuring perfusion values.

In this study, we demonstrated that the SP of spleen and therefore, the selection of splenic maximum, as a time point of reference for distinguishing the arterial and portal phases in the liver and tumor, might occur after the SP of tumor and subsequently lead to erroneous

CT perfusion calculations of tumor using the DI-MS method, as this occurrence violates one assumption upon which the DI-MS method is based. Nearly 30% of animals in this study bearing implanted VX2 tumors in liver showed SP occurring before the SP of spleen, leading to invalid calculations of the maximum slope of portal blood flow to tumor. Even in subjects for which peak splenic enhancement occurred before the EP of tumor, we observed very short time intervals (~ 2 sec) between the occurrence of peak splenic enhancement and EP of tumor enhancement.

A potential reason for this observation could be reduced blood supply from portal vein to tumors displaying these properties, which may indicate unexpected tumor growth. The VX2 tumor is a rabbit anaplastic squamous cell carcinoma that typically displays rapid growth and hypervascularity³⁹. Indeed, it is these properties that have made it indispensable to interventional radiologists in preclinical investigations of hepatocellular carcinoma. Thus, a 30% rate of *hypovascularity* cannot be ruled out from our results, though such an occurrence is unexpected. Regardless, our results suggest that a reliance on vascular perfusion imaging analysis using the DI-MS method, particularly automated analysis sequences that incorporate assumptions without additional checks, may be prone to a biasing error without inclusion of additional data.

Our pilot-scale study is the first to investigate the strength of the relationships and agreement between the manual (MATLAB-based) and automated (manufacturer-provided) methods for three CT perfusion measures (HAP, HPP and HPI) of healthy liver and tumor, in a large animal model. Through deployment of Bland-Altman plots and Pitman's test for variance, we discovered significant variances in tumor HPI and tumor HPP between the manual and automated methods, indicating a low level of agreement for these two measures.

As a pilot-scale study, it has several limitations. First, a small number of animals were enrolled. Despite the small number, we were able to observe limitations to conventional use of the DI-MS method in almost 30% of subjects. Second, our study was insufficiently powered to directly compare the two methods (manual vs. automated) of perfusion measurements. Third, a pathology gold standard reference was not employed for validation against each method, particularly with regard to vascular density (e.g. CD31 stained immunohistochemistry). We also did not use any other reference organ, such as the kidney¹⁰. We did not use the modified Blomely method, as in both methods, the time of peak splenic concentration is used as a surrogate to distinguish the arterial and the portal phases in the liver¹⁶. Moreover, as Miles *et al* have noted, measurement of portal flow might be inaccurate for measurement of venous outflow¹⁵. Deconvolution model method was not used for calculation of perfusion values in this study^{8,19,38}. This method assumes that the shape of $R(t)$ is a plateau with a single exponential wash-out. Though this assumption works well for most organs, it may be unsuitable for organs having complex circulatory pathways such as liver, for which it is preferable to use compartmental analysis. Deconvolution methods are appropriate for measuring lower levels of perfusion ($< 20 \text{ ml} \cdot (\text{min}/100\text{ml})^{-1}$) as they are able to tolerate greater image noise due to inclusion of the complete time series of images in calculation. Last, respiratory misregistration could not be completely avoided using the wide-array CT technology. This may have contributed to the observed discrepancy with the no venous outflow assumption, evidenced by the occurrence of several peaks on the tumor TDC in selected cases. Image registration alone may be insufficient to offset respiratory motion and respiratory gating has been proposed. Respiratory gating, however, requires the use of equipment often unavailable in a clinical setting. Some authors have proposed spatial and spatiotemporal filtering to reduce noise in perfusion CT images, and other algorithms to guarantee high fidelity of the time resolution²². Time-independent reference methods should be considered when calculating liver tumor perfusion values in wide-array CT scanners⁹.

Conclusions

CT Perfusion of liver is a functional technique becoming increasingly common as CT scanner technology advances^{8,12,19,23,38}. Wide-array CT technology offers true volumetric coverage of more than 10 cm of liver and tumor in a very short time ($<1 \text{ s}$), reducing image degradation to motion artifacts and providing true volumetric coverage. With such techniques, the start phase and peak enhancement of tumors may be more accurately recognized to occur prior to the reference organ time-to-peak, leading to miscalculations of perfusion with a reliance on a single method, i.e. DI-MS, without the benefit of additional data. This study demonstrates that the temporal timeliness between the start phase of an assumed hypervascular

liver tumor and spleen may differ from the assumptions upon which the analyzing methods are based. Furthermore, we demonstrated that two methods of calculating the same values purportedly using the same technique, can produce significantly different result. As CT technology advances, it is crucial to reduce variation between perfusion calculation methods, to increase further applicability of the CT perfusion technique.

Declarations

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Competing interests

E.L. and R.I. are inventors on patents issued or pending. All patents are assigned to Johns Hopkins University. In addition, R.I. is an inventor on patents assigned to Aduro Biotech; and, he is a member of the Scientific Advisory Board for Imagion BioSystems. All other authors declare no potential conflict of interest.

Author contributions statement

E.L., R.H., and R.I. conceived the experiments, and drafted the manuscript. S.M. and E.L. conducted the experiments in rabbits. E.L., R.I., R.H., A.A., and W.J.S. analyzed results. All authors reviewed and contributed edits to the manuscript.

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Figures

Figure 1

A) Plots of tissue-density curves (TDC) for aorta, portal vein, spleen and liver. Arrows point to the corresponding y-axis for each plot. Artery, portal vein, and spleen have y-axis on the left side with that for liver being plotted shown on the right side. **B)** TDCs for liver and spleen, illustrating the arterial and portal phase, separated by the time of peak splenic enhancement, and the corresponding maximum slopes. The TDC of tumor and liver can be divided into two phases, the arterial phase dominated by the arterial flow, and the portal phase dominated by the portal venous flow.

Figure 2

Axial views of liver CT perfusion maps, as generated by Body Perfusion software, with a circular region of interest around the tumor; (A) Hepatic Arterial Perfusion (HAP) map, (B) Hepatic Portal Perfusion (HPP) map, (c) Hepatic Perfusion Index (HPI) map, and (D) corresponding axial CT image.

Figure 3A

Figure 3B

Figure 3

A) TDCs for regions of interest (ROIs) in aorta, portal vein, liver, spleen and a tumor demonstrating SP earlier than the SP of spleen. TDC for aorta, portal vein, spleen and liver (left). The arrows in the figure show the corresponding y-axis for each plot. Aorta, portal vein and spleen have y-axis on the left side and liver's y-axis has been plotted separately on the right side. Aorta and portal vein attain a maximum enhancement value of 915.4 HU and 415.4 HU, at 21.75 s and 33.5 s, respectively. Note that peak splenic enhancement occurs at 30.5 s. **B)** TDC for spleen, liver and tumor. Tumor does not seem to have a portal phase as the spleen attains its maximum after the end phase of tumor. **C)** Rate of change of enhancement for liver and tumor.

Figure 4

Graph of average and standard deviation for liver and tumor SP, arterial maximum slope, portal maximum slope and EP, for all subjects.

Figure 5

TDCs for regions of interest (ROIs) liver, spleen and tumor, in an animal subject where violation of the venous outflow assumption occurred. The maximum enhancement of tumor occurs at 44 s, but it occurs after a dip in the TDC curve. A decrease in enhancement value signifies venous outflow and selecting EP after this time point violates the underlying assumption of maximum slope method. Therefore, any maximum slope occurring after a dip in the TDC should be rejected and only the initial maximum slope point should be selected, which occurs after a steady rise in the TDC. f average and standard deviation for liver and tumor SP, arterial maximum slope, portal maximum slope and EP, for all subjects.