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FULL PAPER

The prediction of radiofrequency ablation zone volume using vascular indices of 3-dimensional volumetric colour Doppler ultrasound in an *in vitro* blood-perfused bovine liver model

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ACL and SC performed the laboratory portion of the experiment and SC was the principal investigator of this study.

Objective: To determine the most reliable predictor of radiofrequency (RF) ablation zone volume among three-dimensional (3D) volumetric colour Doppler vascular indices in an *in vitro* blood-perfused bovine liver model.

Methods: 3D colour Doppler volume data of the local hepatic parenchyma were acquired from 37 areas of 13 bovine livers connected to an *in vitro* oxygenated blood perfusion system. Doppler vascular indices of vascularization index (VI), flow index (FI) and vascularization flow index (VFI) were obtained from the volume data using 3D volume analysis software. 37 RF ablations were performed at the same locations where the ultrasound data were obtained from. The relationship of these vascular indices and the ablation zone volumes measured from gross specimens were analyzed using a general linear mixed model fit with random effect for liver and backward stepwise regression analysis.

Results: FI was significantly associated with ablation zone volumes measured on gross specimens ($p = 0.0047$), but explained little of the variance ($R_b^2 = 0.21$). Ablation zone volume decreased by 0.23 cm^3 (95% confidence interval: $-0.38, -0.08$) for every 1 increase in FI. Neither VI nor VFI was significantly associated with ablation zone volumes ($p > 0.05$).

Conclusion: Although FI was associated with ablation zone volumes, it could not sufficiently explain their variability, limiting its clinical applicability. VI, FI and VFI are not clinically useful in the prediction of RF ablation zone volume in the liver.

Advances in knowledge: Despite a significant association of FI with ablation zone volumes, VI, FI and VFI cannot be used for their prediction. Different Doppler vascular indices need to be investigated for clinical use.

INTRODUCTION

Radiofrequency (RF) ablation is one of the most effective minimally invasive treatment modalities for small primary or secondary liver cancers. Its therapeutic effect is obtained through generation of coagulation necrosis of tumour tissue by local heat production from a probe placed within tumours. The predictability of the completeness of tumour ablation is a critical component of treatment planning, for which accurate prediction of ablation zone volume is essential. However, the RF ablation zone volume is markedly variable,¹ which is predominantly due to variable hepatic perfusion.² The variability in RF ablation zone volume demonstrates an inverse correlation with local hepatic

blood flow owing to heat sink effect. That is, the greater the local hepatic blood flow, the smaller the ablation zone.²

Based on the inverse relationship between flow and ablation size, the ability to measure local hepatic blood flow contemporaneously with an ablation procedure should enable the prediction of the size of ablation that will be obtained with an RF ablation device. There are several three-dimensional (3D) volumetric colour Doppler vascular indices developed to quantify blood flow, among which vascularization index (VI), flow index (FI) and vascularization flow index (VFI) are the ones most commonly used.^{3–5}

We hypothesized that RF ablation zone volume could be predicted by using these indices to measure regional hepatic blood flow just prior to performing a hepatic ablation. The goal of this study was to determine the most reliable predictor of RF ablation zone volume among the three 3D volumetric colour Doppler vascular indices.

METHODS AND MATERIALS

This study was exempt from approval by the Institutional Animal Care and Use Committee, as it did not use live animals.

Liver ablation model

There are few experimental models for RF ablation using bovine livers. The *in vitro* oxygenated blood-perfused bovine liver model is considered to be the closest to an *in vivo* model⁶ and was thus chosen as a surrogate model for human livers.

13 fresh bovine livers and their blood were procured from a local slaughterhouse (G&C Packing Co. Colorado Springs, CO). They were excised and flushed with 10 l of chilled Krebs–Henseleit solution and immersed in Dulbecco's phosphate buffered saline containing 30,000 IU heparin. 7 l of blood was also collected for each liver. The blood was filtered for clots and particulates and mixed with 30,000 IU of heparin. Livers underwent approximately 15 min of warm ischaemia. The livers and blood were placed on ice in a cooler to maintain cold ischaemia during the 2-h transport to the laboratory. The livers weighed 4.800 ± 0.668 kg [mean $\pm$ standard deviation (SD), range: 3.702–6.208 kg].

In vitro oxygenated blood perfusion

Immediately after the liver and blood arrived at the laboratory, the liver was cannulated with a 24-Fr Foley catheter into the main portal vein and connected to a perfusion system driven by a centrifugal pump (Bio-Medicus Bio-Console, model 540; Medtronic Inc., Minneapolis, MN). The autologous blood was passed through a 500-ml bubble trap and a membrane oxygenator and heat exchanger (NT Affinity; Medtronic Inc.) connected to a canister of 95% oxygen and 5% carbon dioxide (Airgas Inc., Radnor, PA) to achieve 40–50% oxygenation and a temperature of 37°C. The livers were perfused for approximately 1 h prior to ultrasound measurement and RF ablation.

The blood flow rate was controlled by the centrifugal pump and monitored by a flow meter (Bio-Medicus, model TX40; Medtronic Inc.). Targeting 30–60 ml min⁻¹/100 g tissue of normalized hepatic blood flow to mimic *in vivo* portal venous circulation,⁷ the blood flow was controlled by manipulating the revolutions per minute (range: 2010–3700 rpm) of the roller pump, with a resultant hepatic blood flow of 1000–3625 ml min⁻¹. Normalized hepatic blood flow rates varied between 18.0 ml min⁻¹ and 69.8 ml min⁻¹/100 g tissue. The inflow rates were calibrated by quantifying the blood from the draining hepatic veins. Intra-hepatic blood flow was confirmed prior to measurement and RF ablation using 3D colour Doppler ultrasound (IU 22, V6-2 3D volume transducer; Philips Healthcare, Andover, MA).

Three-dimensional colour Doppler ultrasound

Greyscale and colour Doppler ultrasound gain were adjusted by referring to the adjacent large vessels, to fill the entire

vascular lumens but to minimize colour artefacts outside the grossly visible vessels. The adjacent areas of the hepatic parenchyma selected were devoid of large vessels >3 mm in diameter and had significant depths >5 cm. Although all the circulating micro air bubbles were trapped out by the bubble trap of the *ex vivo* oxygenation model, the hepatic parenchymal areas with vessels containing already embolized air bubbles seen as reverberation artefacts or intravascular echogenicity on greyscale ultrasound were excluded. These areas were widely separated to prevent overlapping between ablation zones. The dynamic range of flow velocity for colour Doppler signal was in the range of ± 15.4 cm s⁻¹, the colour gain was approximately 57% and the wall filter was approximately 71 Hz. 3D volume colour Doppler scan was performed with a depth of 5 cm from the liver capsule by use of a 3D volume probe to obtain 3D colour Doppler volume data of the local hepatic parenchyma prior to ablation. A total of 37 areas of hepatic parenchyma were selected from the 13 livers: 4 areas in 5 livers, 3 areas in 3 livers, 2 areas in 3 livers and 1 area in 2 livers.

Post-processing and vascular index calculation

The 3D colour Doppler volume data were transferred to QLab 3D volume analysis software v. 8.1 (QLab®; Philips Healthcare, Andover, MA). For volume data analysis, a 4-cm³ spherical region of interest (ROI) was placed within the obtained volume, 1 cm away from the liver capsule to exclude the variable effects of manual compression with ultrasound probe on local perfusion in these superficial areas, although we made every effort to minimize the compression effects. The diameter of 4 cm was chosen to reflect the perfusion status of the hepatic parenchyma being ablated as much as possible, by including the usual maximum long axis diameter (LAD) of RF ablation zones, while excluding the perfusion status of the hepatic parenchyma that will not be ablated. For each ROI in each selected local hepatic parenchymal area, the VI (fraction of the colour pixels to the total number of pixels in the ROI), the FI (average intensity of the colour pixels in the ROI) and the VFI (average colour intensity in the ROI) were calculated from the following equations:

$$VI = \frac{C}{E}, \quad FI = \frac{\sum I_c}{C}, \quad VFI = VI \times FI = \frac{\sum I_c}{E}.$$

C represents the number of colour pixels in the ROI, E is the total number of pixels in the ROI and I_c is the intensity of colour. Because of post-processing in QLab, results for VI may not fall between 0 and 1.

Radiofrequency ablation

RF ablation was performed by a researcher who had 4 years' experience of RF ablation procedure of bovine livers, with a Cool-tip RF ablation system (Covidien, Boulder, CO), which consists of a 480-kHz, 200-W alternating electronic current generator (model CC-1-117), a 17-gauge Cool-tip 3.0-cm monopolar internally cooled applicator (model SWCT-1530), a roller pump (model PE-PM) and a dispersive electrode. The probe tip was cooled to 18–22°C by internal perfusion with chilled normal saline by the roller pump.

To obtain 3D colour Doppler volume data, the cool-tip probe was positioned under ultrasound guidance at the centre of the selected areas where the 3D volume scan was performed. The active segment of the probe was located at least 2 cm away from the large vessels >3 mm in diameter and at least 1 cm away from the capsule. Ablation was performed for 12 min using the impedance mode. The delivered energy recorded for 15 lesions was 25.59 ± 3.99 kJ (mean $\pm$ SD, range: 19.15–32.31 kJ). A total of 37 RF ablations of the hepatic parenchyma were performed.

Measurement of ablation zones on gross specimen

A wooden marker probe was inserted into the track of the RF probe when the RF probe was withdrawn after completion of RF ablation; the wooden marker was located along the long axis of the RF ablation zone. After the liver was disconnected from the perfusion apparatus, the bovine liver was cut along the wooden probe, allowing measurement of the long axis of the ablation zone, the maximum short axis diameter (SAD) and the LAD. Figure 1 shows how the ablation zones were measured from the outer margin to the outer margin of the red zone. The volume of the RF ablation zone was calculated using the formula for an ellipsoid:

$$V = (4/3) \pi (\text{LAD}/2) (\text{SAD}/2) (\text{SAD}/2).$$

Statistical analysis methods

The outcome of interest was ablation zone volume (cubic centimetre) on gross specimens (gross ablation zone volume). The predictor variables of interest were VI, FI and VFI. Using SAS® v. 9.3 of the SAS System for Windows (SAS Institute Inc., Cary, NC), we fit a general linear mixed model with a random effect for liver. Residual analysis was used to assess the normality assumption.

Figure 1. A cut gross specimen through the centre line where the radiofrequency (RF) ablation probe was located: the dotted circle delineates the RF ablation zone that includes white and red zones. The double-headed arrow shows the long axis diameter of an RF ablation zone and the double-headed arrow in the dotted line denotes the short axis diameter.

We tested the null hypotheses that (1) there is no association between gross ablation zone volume and VFI, (2) there is no association between gross ablation zone volume and FI and (3) there is no association between gross ablation zone volume and VI. To test those hypotheses, we performed a backward stepwise regression on all three of the candidate predictor variables with a Type I error rate of 0.05. Backward stepwise regression begins with a full model of all predictors and sequentially removes the predictor that has the least impact on the model fit, removing interaction terms before removing the main effect terms.^{8,9} The predictor with the least impact is defined as the variable with the smallest F-statistic, which results in the largest *p*-value. The process is complete when only “significant” terms, defined here as having *p* < 0.05, as well as all main effect terms that are parts of any significant interaction terms remain in the model.

The best predictive model is reported below, along with its parameter estimates, 95% confidence intervals and *p*-values. *R*² statistics for mixed models were determined using the technique from Edwards et al.¹⁰

RESULTS

3D volumetric colour Doppler vascular indices from 37 hepatic parenchymal areas in 13 bovine livers are summarized in Table 1. RF ablation zone volume of the gross specimen (*n* = 37) was 10.5 ± 3.7 cm³ (mean $\pm$ SD, range: 4.3–20.1 cm³).

Table 2 presents model estimates for the backward stepwise models for gross ablation zone volume as well as the *R*_B² values for each model assessed. VFI was eliminated first (*p* = 0.5793), followed by VI (*p* = 0.1122) during the second step of the elimination process. The finally tested model for gross ablation zone volume was FI (*p* = 0.0047). The *R*_B² value was 0.21, suggesting that this model explained 21% of the variance of the data. The fitted plot of the data with FI by volume and 95% confidence and prediction limits are shown in Figure 2.

DISCUSSION

Blood flow to the liver affects the size and volume of the RF ablation zones.^{13–18} An *in vivo* study using porcine livers demonstrated an inverse relationship of blood flow with RF ablation zone size, both in the presence of a large vessel close to the RF ablation zone and in the case of a diffuse decrease in blood flow to the entire liver by the Pringle manoeuvre.¹³ The variability in ablation zone size with fluctuations in hepatic blood flow makes accurate treatment planning difficult and can lead to undertreatment or overtreatment of a tumour. However, if interventional radiologists could predict the size of the RF ablation zone based on an accurate measurement of regional hepatic blood flow, the efficacy of the treatment might be improved.

Of the three vascular indices assessed in this study, VI, FI and VFI, only the FI demonstrated a statistically significant correlation with the ablation zone volume measured on gross specimens. However, FI could explain only 21% of the variability in the ablation zone volume, as seen on Figure 2

Table 1. Three-dimensional volume colour Doppler vascular indices of the hepatic parenchyma prior to radiofrequency ablation on 37 lesions

Vascular index	N	Minimum	First quartile	Third quartile	Median	Maximum	Mean	SD
VI	37	0.37	1.29	4.20	3.00	11.30	3.29	2.38
FI	37	2.39	9.10	17.30	13.80	32.10	13.74	7.31
VFI	37	0.02	0.12	0.85	0.37	1.95	0.51	0.50

FI, flow index; SD, standard deviation; VFI, vascularization flow index; VI, vascularization index.

(shaded area). The prediction limits of the ablation zone volumes (the area between the dotted lines) were too wide to be applied as predictors in clinical settings and therefore may not be clinically useful for the prediction of ablation zone volume.

Our results are similar to those of Dubiel et al,⁵ who reported a poor correlation of FI to flow velocity or volume of flow. For instance, in their study, a fourfold increase in flow velocity resulted in only a 10% increase in FI. This phenomenon may be due to the intrinsic limitation of the colour Doppler modality itself. Volume flow measurements on Doppler are also known to have significant interobserver variability and lack of reliability, which might occur simply owing to errors inherent in the acquisition process or data analysis.¹⁹ These measurement errors may also be associated with the limited scale of colour Doppler signal intensity and scan settings such as power, colour gain, pulse repetition frequency, dynamic range of flow velocity translated into colour intensity, wall filter and interaction with greyscale gain and vascular angle. Raine-Fenning et al⁴ reported that VI, FI and VFI were all affected by ultrasound power and gain settings and had a significant but complex relationship with pulse repetition frequency and wall filter settings in their *in vitro* experiment. Since FI and VFI contain the same component of the sum of

colour intensity of all colour voxels in their formula, both vascular indices are affected by these factors and will have measurement errors.

This study has several limitations. Although the bovine oxygenated blood-perfused liver model is considered the best *in vitro* model, there are some limitations to its use as a surrogate *in vivo* liver model. For example, the bovine livers were perfused only through the portal vein; thus, the overall perfusion was 20–25% less than that seen in live animals owing to lack of hepatic arterial flow. The difference in blood flow between the experimental system and *in vivo* system may result in a smaller RF ablation zone volume *in vivo*. Although colour Doppler ultrasound was used to assess regional blood flow in our model, these measures cannot ensure that its microcirculation is normal because most of the colour signal reflects grossly visible or nearly visible vessels. Although we made every effort to create the ablation zones within the hepatic parenchyma where 3D volume data were obtained from, their margins could have extended out of the area. Given that the average ablation zone volume is much smaller than the area of volume data measurement, however, its probability is thought to be low.

In conclusion, FI demonstrated a statistically significant correlation with RF ablation zone volume measured on

Table 2. Mixed model estimates, standard errors (SE) and *p*-values for radiofrequency ablation zone volume on gross specimens

Covariance structure: random intercept		Covariance estimates						
Model	Fixed effect	Estimate	SE	<i>p</i> -value	95% CI ^a	Random effects	Error	R _B ^{2b}
1	Intercept	15.530	2.567	<0.0001		0.122	1.817	0.27
	FI	−0.282	0.205	0.1785				
	VI	−0.785	0.711	0.2779				
	VFI	2.768	4.942	0.5793				
2	Intercept	14.349	1.315	<0.0001		0.034	1.763	0.28
	FI	−0.180	0.087	0.0490				
	VI	−0.417	0.256	0.1122				
3	Intercept	13.663	1.179	<0.0001	11.269, 16.058	— ^c	—	0.21
	FI	−0.230	0.076	0.0047	−0.384, −0.076			

CI, confidence interval; FI, flow index; VFI, vascularization flow index; VI, vascularization index.

^aFor final model fixed effects only.

^bR_B² calculated using the technique from Edwards et al.¹⁰

^cThe estimated G matrix is not positive definite.¹¹ Model 3 does not indicate enough variation in gross volume to attribute any variation to the effect of liver.¹² Subsequently, Model 3 was fit without a random effect term.

Figure 2. The fitted plot of the data with flow index (FI) by volume with 95% confidence and prediction limits.

gross specimens across different perfusion levels in this perfused bovine model of the liver. However, relatively wide prediction intervals of ablation zone volume limit its clinical applicability. There is a need for further refinement or development of new vascular indices that can accurately measure local hepatic blood flow and thus be used for the accurate prediction of ablation zone volumes.

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