

● *Original Contribution***QUANTIFICATION OF TUMOR MICROVASCULARITY WITH RESPIRATORY GATED CONTRAST ENHANCED ULTRASOUND FOR MONITORING THERAPY**MICHALAKIS AVERKIOU,^{*} MARIOS LAMPASKIS,^{*} KONSTANTINA KYRIAKOPOULOU,[†]DIMOSTHENIS SKARLOS,[§] GEORGIOS KLOUVAS,[§] COSTAS STROUTHOS,^{*} and EDWARD LEEN[‡]^{*}University of Cyprus, Nicosia, Cyprus; [†]Henry Dunant Hospital, Athens, Greece; [‡]Hammersmith Hospital, Imperial College, London, UK; and [§]Metropolitan Hospital, Athens, Greece

(Received 3 February 2009; revised 1 July 2009; in final form 7 July 2009)

Abstract—The aim of this feasibility study was to evaluate the response to cytotoxic and antiangiogenic treatment of colorectal liver metastasis using respiratory gated contrast enhanced ultrasonography. Seven patients were monitored with contrast enhanced ultrasound. Sulfur hexafluoride filled microbubbles (SonoVue; Bracco S.P.A., Milan, Italy) were used as contrast agent and the scans were performed with a nonlinear imaging technique (power modulation) at low transmit power (MI=0.06). The mean image intensity in the metastatic lesion and in the normal liver parenchyma were measured as a function of time and time-intensity curves from linearized image data were formed. A novel respiratory gating technique was utilized to minimize the effects of respiratory motion on the images. A reference position of the diaphragm (or other echogenic interface) was selected and all frames where the diaphragm deviated from that position were rejected. The wash-in time (start of enhancement to peak) of metastasis and adjacent normal liver parenchyma was measured from time-intensity curves. The ratio of wash-in time of the lesion to that of the normal parenchyma (WITR) was used to compare the perfusion rate. In a reproducibility study (five patients), the average deviation of WITR was found to be 9%. There was an increase in the WITR for patients responding to treatment (mean WITR increase of 17% after first dose of treatment and 75% at the end of the therapy). In four out of five patients (80%) responding to therapy WITR predicted their response from the first treatment. All six patients that responded to therapy by the end of the therapy cycle (6–9 doses) were correctly predicted by using WITR. The WITR may be a new surrogate marker indicative of early tumor response for colorectal cancer patients undergoing cytotoxic and antiangiogenic therapy. (E-mail: maverk@ucy.ac.cy) © 2010 World Federation for Ultrasound in Medicine & Biology.

Key Words: Ultrasound contrast agents, Perfusion quantification, Respiratory gating, Liver metastasis, Therapy monitoring.

INTRODUCTION

Liver metastases are the most frequent liver lesions in western countries. These may originate most frequently from tumors in the gastrointestinal tract but also from breast, ovarian, lung or prostate cancer. Colorectal cancer is the second leading cause of cancer death in the western world (Bekradda and Cvitkovic 1999; Jemal et al. 2006) and colorectal metastasis is the major type of metastatic disease in the liver.

As outlined in Hanahan and Weinberg (2000) tumor progression is a multistage process that depends on

a sequence of alterations in cell physiology, which collectively dictate cancerous growth. Angiogenesis, which is the complex process of generating new vessels as a result of branching and sprouting of arteries from pre-existing blood vessels to supply nutrients to the tumor, is a prerequisite to the fast expansion of cancer associated with the formation of macroscopic cancers (Folkman 1990). Therefore, angiogenesis has become an object of intense efforts to identify and understand cancer and has emerged as one of the principal targets for novel therapeutic strategies in oncology. Over the last few years many drugs that specifically target angiogenesis, the crucial process in cancer metastasis, have been developed and clinically evaluated (Folkman 2007; Browder et al. 2000; Kerbel 2006). According to Jain (2008) a balanced combination of antiangiogenic and chemotherapeutic drugs can lead to better treatment.

Address correspondence to: Michalakis Averkiou, Mechanical and Manufacturing Engineering, University of Cyprus, 75 Kallipoleos Avenue, P.O. Box 20537, Nicosia, 1678, Cyprus. E-mail: maverk@ucy.ac.cy

All therapy regimes need feedback so as to optimize their effectiveness, adjust doses or change to a different therapy if deemed necessary. The measurement of angiogenic activity may have important prognostic potential. To evaluate the effectiveness of antiangiogenic therapies, an assessment of perfusion through measurements of hemodynamic parameters related to blood volume and blood flow is needed. This may be achieved by employing fast, high resolution, noninvasive imaging methods. Various imaging modalities such as positron emission tomography (PET), X-ray computed tomography (CT), magnetic resonance imaging (MRI), ultrasound and optical imaging have different advantages and disadvantages but none can complete the task of perfusion monitoring (Miller *et al.* 2005; Pandharipande *et al.* 2005).

Ultrasound is the only imaging modality that has a blood pool contrast agent, which effectively disregards diffusion and leakage because the microbubble contrast agents are similar in size with red blood cells (Wei *et al.* 1998; Averkiou *et al.* 2004). Additional advantages of contrast enhanced ultrasound (CEUS) are short examination times, relatively lower costs involved compared with MRI and CT and the absence of ionizing radiation. In combination with nonlinear imaging techniques (Averkiou *et al.* 2003; Ferrara *et al.* 2000), CEUS is capable for detection and characterization of liver lesions (Leen *et al.* 2006a, 2006b; Quai *et al.* 2006) and for monitoring changes of hemodynamic parameters during therapy (Lassau *et al.* 2007). CEUS has also been used for imaging lesions in other organs and tissues such as the myocardium (Wei *et al.* 1998), kidney (Correas *et al.* 2006), spleen (Görg 2007) and breast (Rizzato *et al.* 2001). For moderate microbubble concentration, the intensity of the scattered ultrasound signal is proportional to the concentration of microbubbles (Schlosser *et al.* 2003). This allows for the quantification of perfusion in lesions and normal tissues by extracting various blood flow and blood volume related parameters from time-intensity curves (Lassau *et al.* 2007; Correas *et al.* 2006; Rizzato *et al.* 2001; Arditi *et al.* 2006).

In this article we propose a new surrogate marker for monitoring the response to treatment (cytotoxic and antiangiogenic) of colorectal cancer liver metastases using CEUS. Our hypothesis is that cytotoxic and antiangiogenic treatment leads to microvascular changes that we can image and measure with CEUS. We assess perfusion changes of liver lesions in comparison to that of normal liver parenchyma. Previously, various physiologic parameters have been extracted from time-intensity curves (Elie *et al.* 2007; Blomley and Dawson 1997). We focus on the wash-in time because it is linked with blood flow rate (Wei *et al.* 1998). At large blood flow rates the microbubbles move fast into a region-of-interest (ROI), implying a short wash-in and vice versa. In addition, the effects of the

microbubbles recirculation on the wash-in part of a time-intensity curve are negligible compared with the wash-out part. In particular, we form the ratio of wash-in times of blood flow in the lesion and normal parenchyma (WITR) and use it as a surrogate marker to monitor therapy outcomes.

MATERIALS AND METHODS

Clinical study

Seven patients (three male, four female; 47–76 years old) undergoing a combination of antiangiogenic and cytotoxic treatment for colorectal metastasis in the liver were monitored with CEUS. Approval was obtained by the ethics review board of our hospital and informed consent was obtained from all patients at the time of scanning after the nature of the procedure was fully explained. The scanning of the patients took place on the same day a few hours before the administration of each biweekly dose. The dosage, which was controlled by an oncologist, consisted of Bevacizumab (Avastin, a monoclonal antibody that targets angiogenesis) and a regimen of cytotoxic agents (oxaliplatin or irinotecan combined with capecitabine). The type and dose of drugs are different for each patient as prescribed by the oncologist. A total of 60 contrast ultrasound examinations were performed. Out of seven patients, five completed the study; one had surgery after the third treatment and one died due to disease progression.

A reproducibility study was performed to estimate systematic variations introduced in our method by the variability in the administration of the intravenous bolus injection of the contrast agent, various imaging parameters and the patient's cardiac output. Five patients with liver lesions not undergoing any form of therapy, either benign lesions not requiring treatment or malignant lesions before the initiation of treatment were examined. A total of 12 contrast enhanced ultrasound examinations were performed as shown in detail in Table 1. The intravenous bolus administration was varied both in amount (1.2 and 2.4 mL of SonoVue; Bracco S.P.A., Milan, Italy) and/or duration of injection (1 and 2 s) to induce changes to the wash-in time and to monitor their effect on the WITR (ratio of wash-in times between lesions and normal parenchyma). It was necessary to demonstrate that WITR is not changing unless either the therapy or the disease is progressing.

Ultrasound examinations

All ultrasound examinations were performed on a Philips iU22 (Bothell, WA, USA) ultrasound scanner with the curve-linear array probe C5-2. The imaging parameters were: power modulation (PM 3 pulses) transmit frequency 1.7 MHz at low transmit power (mechanical index <0.06), at approximately 7–10 frames per s and one focus well

Table 1. Reproducibility study results

Subject	Examination No.	Lesion WIT (s)	Deviation (%)	Normal parenchyma WIT (s)	WITR	Deviation (%)
A	1	5.77	30	11.92	0.48	4
	2	10.61	30	20.51	0.52	4
B	1	11.41	0	20.84	0.55	10
	2	11.22	2	23.17	0.48	4
	3	10.01	13	21.97	0.46	8
	4	13.14	15	25.63	0.51	2
C	1	7.91	11	20.91	0.38	10
	2	9.85	11	21.62	0.46	10
D	1	16.81	20	30.70	0.55	16
	2	11.22	20	28.16	0.40	16
E	1	7.82	8	21.74	0.36	15
	2	9.09	8	18.67	0.49	15

WIT = Wash-in time; WITR = Wash-in time ratio.

below the level of the target lesion to ensure a more uniform pressure field. “Contrast side/side” (contrast/tissue) image loops of approximately 120 s were acquired and the “native” data (machine data) before compression were saved. Effort was made to have a uniform gain across the image and to avoid gain saturation. The time gain compensation (TGC) was set such that before contrast arrival a uniform black image with “a hint of noise” was shown. As long as image saturation was avoided, the measurement of WITR was not affected by the TGC. The contrast injection consisted of an intravenous bolus of 2.4 mL of contrast agent (SonoVue) injected in 2 s followed by a saline flush of 5 mL. Patients were instructed to breathe normally (no breath holding or deep breaths). The radiologist maintained a constant image plane with the aid of the tissue (fundamental image) of the “contrast side/side” imaging mode.

Region-of-interest selection and analysis

The main image analysis tasks are: (1) segmentation of metastatic lesion area, (2) selection of a representative region of normal parenchyma, (3) measurement of lesion size and (4) formulation of time-intensity curves. Once a loop of images was collected (a typical image is shown in Fig. 1a and b), time-intensity curves were extracted using commercial quantification software (Q-LAB version 6; Philips Medical Systems, Bothell, WA, USA) as shown in Figure 1c. This software allows for manual ROI selection, measurement of the selected ROI area and provides linear (logarithmic compression removed) data for the time-intensity curves. The lesion ROIs were selected manually and based on the contrast at the arterial (Fig. 1a) and late portal phase (Fig. 1b). For the ROI in the normal parenchyma effort was made to place the region in an area without large vessels and especially without arterial branches (see Fig. 1a) and also in a depth similar to that of the lesion. The lesions monitored were only a few centimeters long and covered a small part of the overall image depth. The ROI spanned only in 1 to 2 ranges of the overall TGC.

Measurement of wash-in time

To accurately and consistently measure blood flow parameters from the time-intensity curves, it is preferable to fit the acquired data to a similarly shaped function such as in the example in Figure 2. This is necessary because the data sets for intensity vs. time from liver lesions are inherently noisy. In Figure 2 we show a time-intensity curve from a liver metastasis as dots and a fitted mathematical function as a solid line. The wash-in time (WIT) is defined as the time taken for the intensity to reach the peak (taken the 5% to 95% point). Another important parameter is the peak intensity I_p occurring at time t_p which as suggested by others (Blomley and Dawson 1997) is proportional to the blood volume in the ROI. The value I_0 (at time t_0) is the initial intensity before the contrast arrival and it is usually zero, unless there is a small amount of tissue signal that is not perfectly cancelled by the nonlinear pulsing scheme. For our work we developed a custom MATLAB (MathWorks, Inc., Natick, MA, USA) function for the measurement of the wash-in time from time-intensity curves. This was done by fitting the data to a sigmoid curve based on the error function defined as

$$I(t) = A[\text{erf}\{(t-t_0)/T\}] + I_0 \quad (1)$$

where $I(t)$ is the linear intensity at time t , A is the maximum intensity over the baseline offset, T is the rise time parameter, which is linearly proportional to WIT, t_0 is the bolus arrival time and I_0 is the baseline offset. Typical curves generated by eqn (1) are shown in Figure 3 for WIT = 1, 5, 10 and 20 s. As we will show in the next section, eqn (1) fits with precision the wash-in part of the time-intensity curves for both normal parenchyma and the tumor. The reason for using a mathematical model to fit our data is to enable us to easily and accurately measure the WIT from this mathematical function instead of trying to perform this measurement with noisy image data.

The WIT measured in a lesion is normalized with that of a region considered to be normal liver parenchyma (*i.e.*,

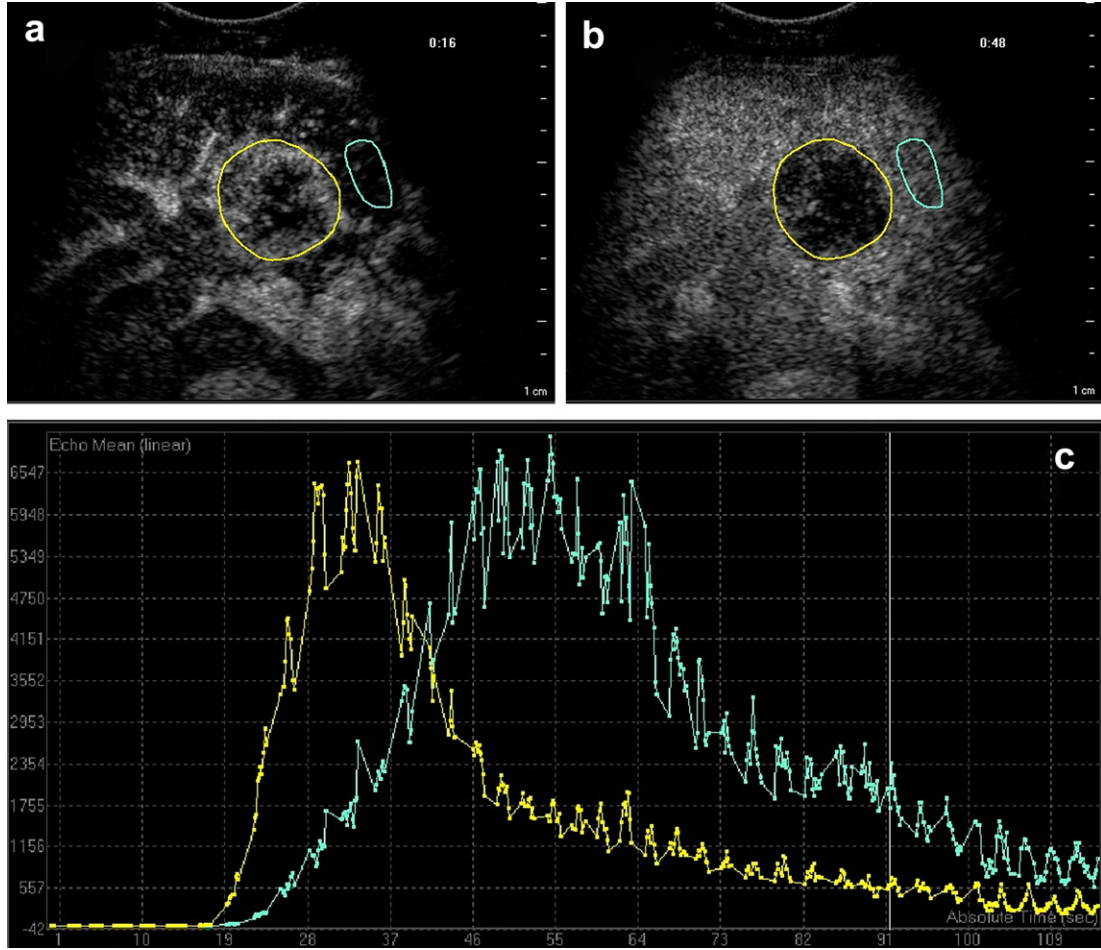


Fig. 1. Placement of region-of-interest (ROI) and typical images in arterial phase (a) and late portal phase (b). Examples of time-intensity curves (c) with a ROI in a metastasis and in normal liver parenchyma.

where there is no evidence for a metastatic or any other focal lesion). We define the wash-in time ratio, WITR as

$$\text{WITR} = \frac{\text{Lesion wash-in time}}{\text{Normal parenchyma wash-in time}}, \quad (2)$$

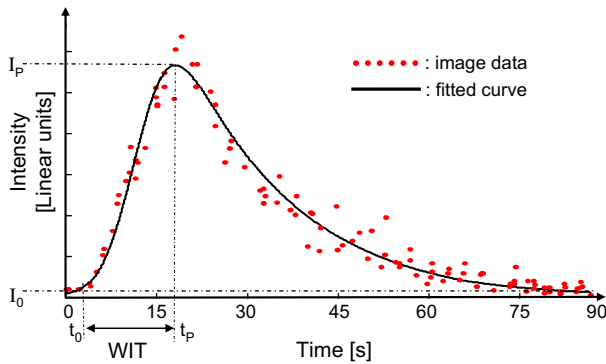


Fig. 2. Example of a time-intensity curve fitting. Dots are uncompressed image data and the solid line is a fitted curve. WIT is the wash-in time, t_0 the start of enhancement time, t_p the peak time, I_0 the baseline intensity and I_p the peak intensity.

and it is indicative of the difference in the rate at which the lesion is perfused as compared to normal liver parenchyma.

Respiratory gating and motion compensation

Even though respiratory motion can be compensated by the operator (in his/her mind) for visual diagnosis, it is a major source of error when quantification of the image data is desirable. Techniques that attempt to compensate for the motion by moving the ROI are inherently flawed

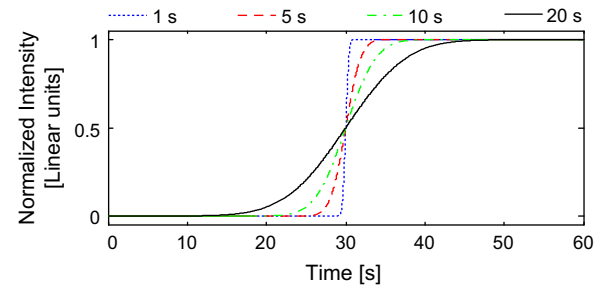


Fig. 3. Example of plots of eqn (1), with the parameter T varying to produce sigmoid curves with wash-in times between 1 and 20 s.

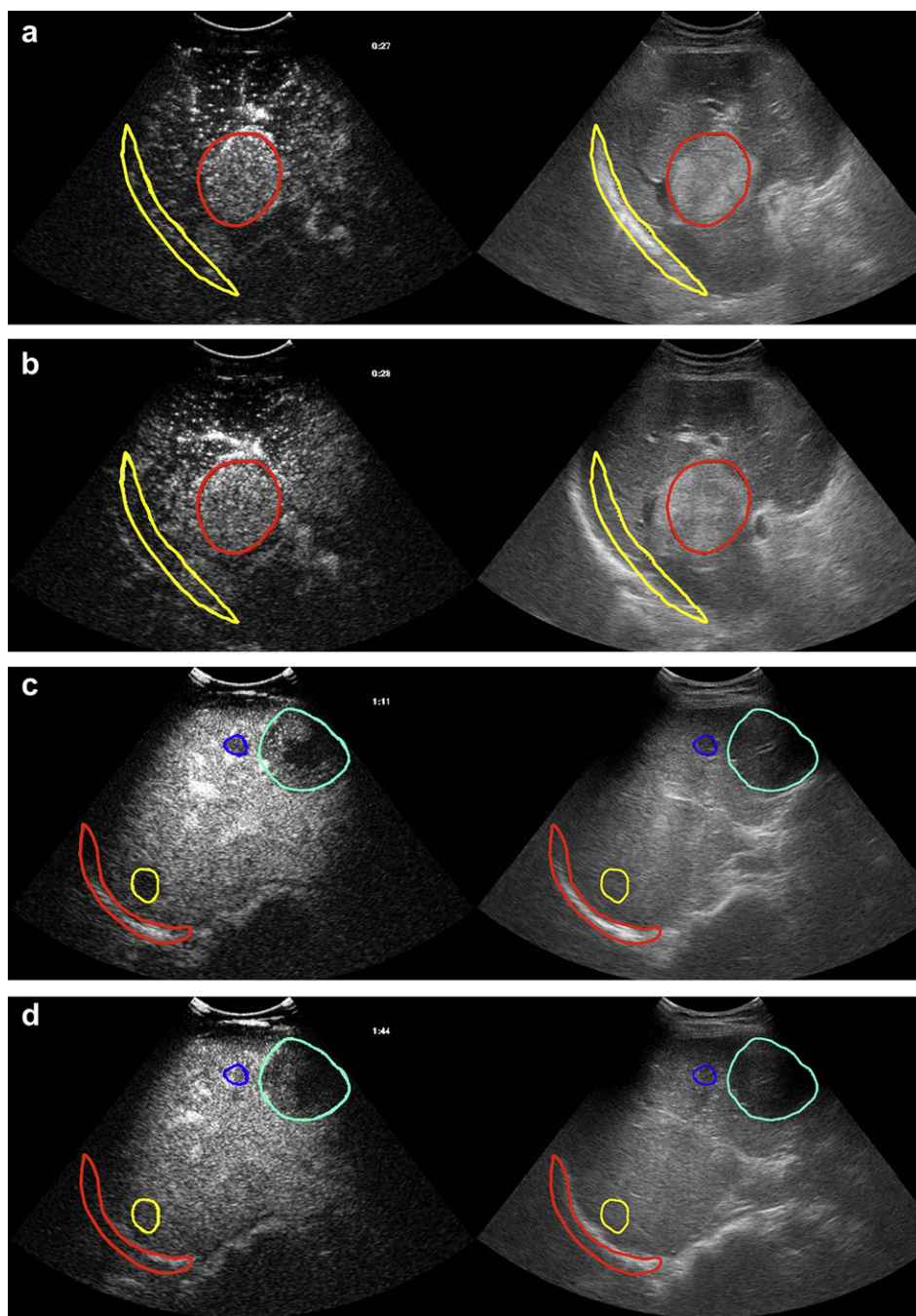


Fig. 4. Respiratory gating implementation for two different patients. In (a) the reference position of the diaphragm is shown with the lesion being in the center of the region-of-interest (ROI). In (b) where the diaphragm has moved from the reference point, the lesion is not centered in the ROI. The small lesions in (c) are completely lost in (d) when the diaphragm moves away from the reference position.

for out of plane motion, which in the case of the liver, is always present to some degree in abdominal imaging. A respiratory gating technique proposed by Renault et al. (2005) performs independent component analysis to estimate the respiratory motion component. For the work presented here, respiratory gating is performed by selecting a reference position for the diaphragm (on the tissue

side image) and rejecting (manually) all frames where the diaphragm deviated from that.

In Figure 4 we show two sets of images taken from a loop at two different instances of the respiratory cycle, where in (a) and (c) the diaphragm was placed in the center of the ROI and in (b) and (d) respiratory motion has caused the diaphragm to be outside the ROI. As

a consequence of this motion, the lesion (the red ROI) in (a) is exactly within the ROI and in (b) has shifted to the side and also possibly out of plane. With larger lesions such as the one shown in Figure 4a and b, the effect on the time-intensity curve is not very dramatic. However, the small lesions of Figure 4c move completely out of plane in (d) when the diaphragm is outside the reference position. The time intensity curves of smaller lesions that move completely out of plane with respiratory motion have more pronounced oscillations such as those in Figure 5a. In cases where the diaphragm is not recorded in the loop, any other bright interface in the tissue image may be used.

The oscillations in the time-intensity curves make it difficult, if not impossible, to accurately measure any parameters from the time-intensity curve, such as WIT, peak intensity or area under the curve. We also note that the respiratory motion is not coplanar with the scan plane and the time-intensity curve of Figure 5a is actually presenting information of different planes of the lesion (or even outside the lesion) at different times. However, once we apply our respiratory gating algorithm and we remove any frames where the diaphragm was outside its reference ROI (e.g., Fig. 4b or d), then we end up with only the frames where the lesion was stationary in the same plane and the time-intensity curves become smoother as shown in Figure 5b. If we used a curve fitting algorithm to the data in Figure 5, we would find a different curve to fit Figure 5a vs. Figure 5b. We note that before we had applied the respiratory gating algorithm to our clinical data for therapy monitoring, more than 50% of the acquired loops were not quantifiable due to excess noise caused by the motion.

Patient classification

Patients were classified as good or bad responders with conventional means (not our WITR measurements) based on a combination of the following criteria:

- (1) The number of lesions and the lesion size increased or decreased. The lesion size was measured from the ultrasound image loops and other imaging techniques such as MRI and CT. If the lesion is not visible in any imaging technique we classify it as “complete response”. If there is a decrease $>30\%$ in the size of the biggest diameter we classify it as “partial response”. If there is an increase $>25\%$ in the size of the biggest diameter it is classified as “disease progression”. “Stable disease” is assumed when neither the partial response criterion nor the disease progression criteria are fulfilled.
- (2) Blood tests for serum tumor markers such as carcino-embryonic antigen (CEA) and CA 19.9.
- (3) Liver function tests.

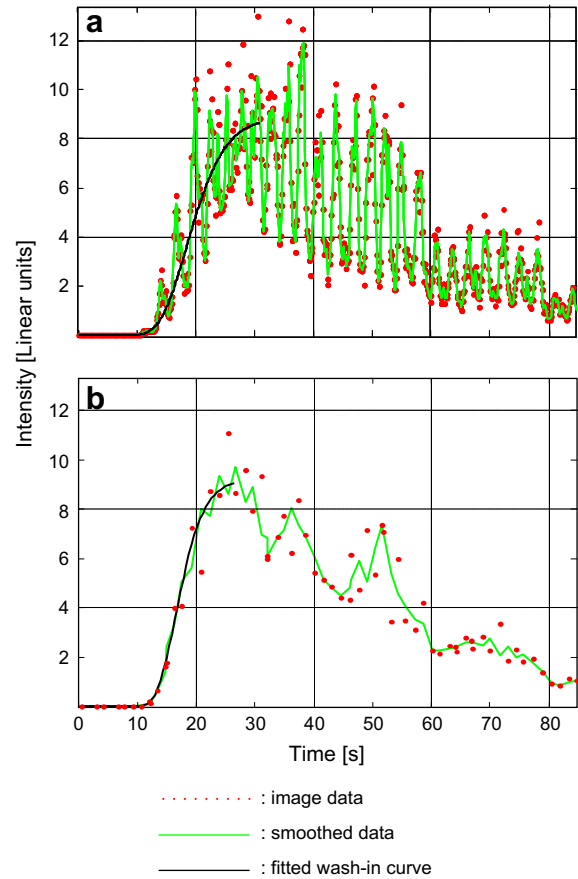


Fig. 5. Example of time-intensity curves before (a) and after (b) respiratory gating is applied. Notice the difference of the resulting fitted curve.

Our hypothesis for good responders is that the WITR increased with time (as therapy progressed) and remained close to one, implying that the perfusion rate in the lesion is approximately equal to that of the normal parenchyma. For bad responders the WITR remained below unity and the perfusion rate in the lesion remained larger than the perfusion rate in normal parenchyma implying that the lesion microvasculature is still “active”.

RESULTS

Reproducibility study

For the subjects of the reproducibility study, the WITR variation (same patient, different injection) was 9% on average with a maximum deviation of 16% and a minimum of 2%. The detailed results of the reproducibility study are shown in Table 1. The results confirm our hypothesis that WITR is a “normalized” parameter that is not affected by imaging settings or contrast agent bolus variations. As shown in the table, WITR remained constant between different loops even though between injection 1 and 2 (and 3 and 4) the bolus was changed from 1.2 mL to 2.4 mL. More detailed description of the

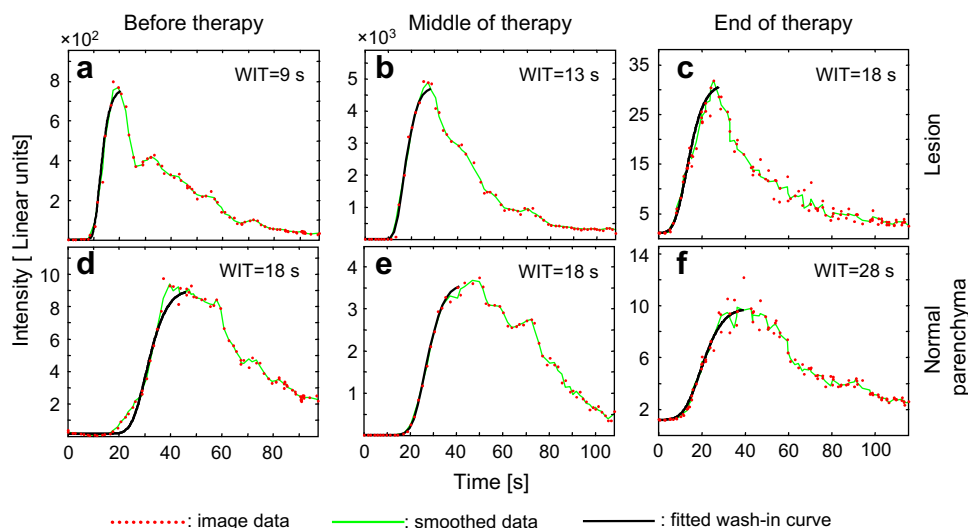


Fig. 6. Series of time intensity curves for a single patient over the course of therapy both for lesion (a)–(c) and for normal liver parenchyma (d)–(f).

reproducibility study is shown elsewhere (Averkiou et al. 2009).

Response to therapy

In Figure 6 we show examples of time-intensity curves corresponding to either a lesion (top row) or normal liver parenchyma (bottom row) on the same patient over the course of therapy. We have fitted the sigmoid curve eqn (1) to the image data and the results are shown as a solid black line starting from zero intensity and extending to the peak of the curve. In this specific case, the WIT increased with the number of therapy cycles, starting at 9 s and ending at 18 s for the lesion and going from 18 to 28 s for the normal parenchyma. This change is a result of the differences in the patient condition and in contrast boluses on different days. These WITs result in WITRs of 0.5, 0.72 and 0.64, respectively. According to the conventional indicators used for the evaluation of the patient (tumor markers and other imaging such as CT and MRI), the oncologists suggest that this is a partial responder and this also validates our hypothesis that increase of WITR (towards unity) suggests partial response to therapy.

The variation of the WITR as a function of time (number of therapy doses) of one partial (patient CD) and one bad responder (patient DRb) are shown in Figure 7. The WITR for the partial responder slowly but steadily rises until it approximates unity implying some kind of “normalization” of the microvasculature suggesting that the arterialization present during tumor angiogenesis is regressing. A straight dashed line is shown in the figure to indicate the value of WITR=1. We have also observed that the lesion size (total area in imaging plane) for the partial responder has decreased considerably from the initial size, whereas the lesion area of the bad

responder stayed roughly the same (had a very small decrease). In the case of the bad responder, the WITR remained approximately the same (well below unity) with some fluctuations in its value during the course of therapy (seven sessions). All the results for the response to therapy are depicted graphically in Figures 8 and 9, for WITR and lesion area, respectively. In Figure 8 the WITR as a function of time is plotted and in Figure 9 the lesion size in the image plane in square millimeters is plotted.

The clinically established criteria were compared with our hypothesis for good/bad responders based on the WITR and are tabulated in Table 2. The first column of Table 2 is the patient identifier, the second column is the comparison of the WITR first week result with conventional classification at the end of the therapy (lesion size and number and tumor markers) and the third column is the WITR at the end of the therapy. A tick mark indicates agreement of our proposed method with conventional evaluation whereas an “x” indicates disagreement.

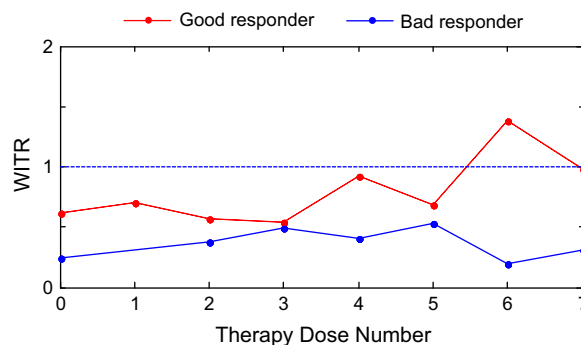


Fig. 7. The wash-in time ratio as a function of time (over the course of therapy) for a good (dotted line) and a bad (solid line) responder.

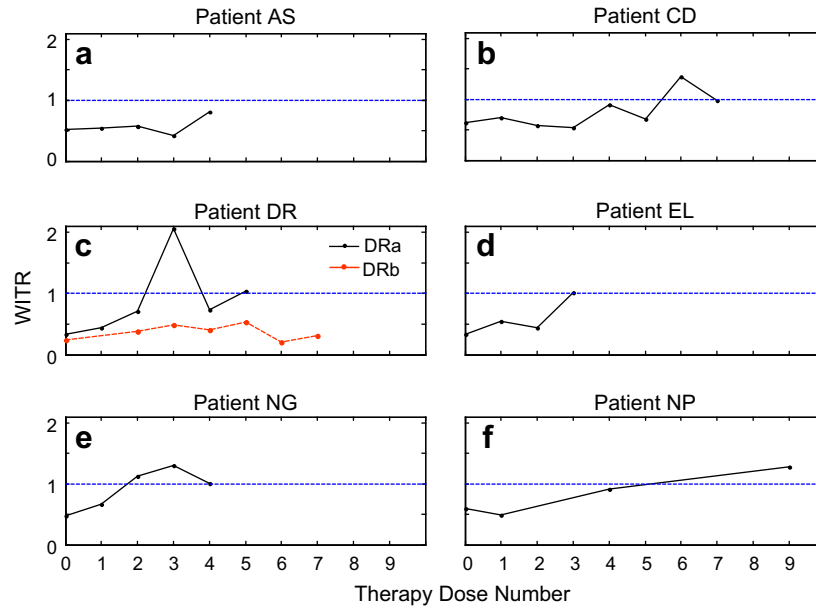


Fig. 8. Plot of wash-in time ratio over the course of therapy for six of the seven patients that took part in this study.

It is important to note that with the results of just one therapy cycle we have successfully predicted the outcome for four out of five (80%) partial responders or stable disease patients. For the four patients that their response was correctly predicted from the first dose (tick marks in second column of the table), the average WITR increase was 17%. In addition, when considering all the measurements (during each cycle of therapy) our results agree with the conventional evaluation in six out of seven (86%) patients and have predicted all five patients (100%) that

responded to therapy. For the five patients that responded to therapy at the end of all doses (tick marks in third column), the average increase of WITR was 75%.

DISCUSSION

A method was developed for the quantification of blood flow in the microcirculation of metastatic liver lesions from colorectal cancer with contrast enhanced ultrasound imaging. This method enables us to assess

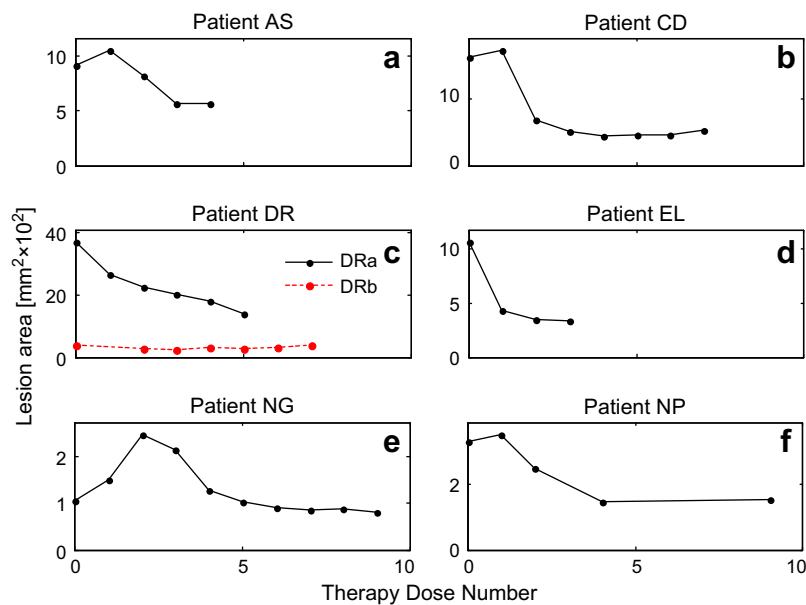


Fig. 9. Plot of metastatic lesion area over the course of therapy for six of the seven patients that took part in this study.

Table 2. Comparison of CEUS results with conventional classification

Patient	WITR result – first therapy	WITR result –overall	Conventional evaluation
AS	✓	✓	Partial response
CD	✓	✓	Partial response
DP	✓	✓	Partial response
DR	x	✓	Progressive disease
EL	✓	✓	Partial response
NG	x	x	Stable disease
NP	x	✓	Partial response

CEUS=contrast enhanced ultrasound; WITR = wash-in time ratio.

Tick marks denote agreement between WITR and conventional patient classification (as outlined in Materials and Methods) and “x” denotes disagreement.

perfusion changes caused by regression of tumor angiogenesis (or any other changes to microcirculation) during a combination of antiangiogenic and cytotoxic therapy. Time-intensity data were fitted with a sigmoidal function based on the error function (*erf*) and the variation of the wash-in time in cancerous and healthy normal liver parenchyma regions was measured. The use of a mathematical model enabled us to accurately measure the WIT. Various other models have been suggested in the past to curve fit the data from time-intensity curves of organ and tumor perfusion. Examples are the gamma-variate function (Mischi et al. 2008), the lagged normal function (Fisher et al. 2002), the local density random walk model (Mischi et al. 2003) and the lognormal function (Tienman et al. 2000; Qian and Bassingthwaite 2000).

An important aspect of the work is the use of linear data (*i.e.*, with logarithmic compression removed) from the diagnostic ultrasound images that enabled us to form the WITR (linear data is needed for forming ratios). In essence, the formation of WITR is a normalization scheme to deal with variations caused by the intravenous bolus administration (amount of contrast and injection rate), patient cardiac output and machine settings and was confirmed with a reproducibility study. We compared our proposed parameter WITR with conventional means of therapy evaluation such as the variation of the tumor size during the course of treatment and found good agreement between the two.

The movement of the scan plane due to the respiratory motion is one of the issues we resolved in this work. This problem was severe when the lesion was relatively small and completely moved out of plane. This issue was resolved with a novel respiratory gating scheme based on tracking the position of the diaphragm (or other echogenic interface). This technique lends itself to automation and can be easily implemented in commercial quantification packages. In addition, with our proposed scheme, the clinician no longer needs to ask the patient

to control his breathing in any way. It is indeed recommended that the radiologist monitor the tissue side image only (with systems offering the dual display with contrast in one and tissue in the other) where no large changes of the contrast wash-in are seen with the fundamental tissue mode. Another possible source of motion induced error is the sonographer's hand motion and a solution would be the use of a surgical-type articulated arm to fix the probe to the patient and avoid those errors.

Based on a short reproducibility study (12 examinations), we have seen that our approach is repeatable when used on subjects not undergoing therapy (where no perfusion changes are expected especially in short time intervals between injections). The average deviation of WITR was 9% whereas that of WIT was 14%, thus, confirming our normalization approach and the choice of WITR as the surrogate marker. In the reproducibility study the deviations in the WITR were significantly smaller compared to the measured deviations caused by changes due to therapy. In patients with colorectal liver metastasis we observed that the WITR increases when the patient is responding to treatment, with an average increase of 17% after the first dose which is larger than the percent deviation found in our reproducibility study. The average increase of WITR at the completion of all therapy doses for these patients was 75%, a value that is eightfold the average deviation found in the reproducibility study. The patients' response to treatment was also evaluated by magnetic resonance, computed tomography and serum tumor markers and was found to be in good agreement with our method based on CEUS. The total number of lesions was also considered in the evaluation of patients. For example, patient DR that had progressive disease had more than five lesions and no reduction in the number of lesions was observed. However, patients EL and AS classified as partial responders had three and two lesions, respectively, and the number was observed with CT to be the same in the middle and end of therapy.

Other parameters have also been suggested in the past for use of therapy monitoring such as peak intensity, area under the curve (AUC) and mean transit time (MTT). We have chosen the WITR because based on our reproducibility results it was the most reproducible parameter with the smallest percent deviation. The peak intensity may be influenced by machine settings (analog and digital gain, compression, focus placement). The AUC and MTT require a complete wash-in and wash-out curve; often the image loops collected do not extend out to 2 min to cover the complete wash-out as it is difficult to maintain the same image plane for such a prolonged time. WIT may be accurately measured with shorter time-intensity curves. In addition, the wash-out part of time intensity curves is often affected by the recirculation of the contrast agent.

CONCLUSIONS

The ratio of lesion to normal parenchyma wash-in time (WITR) may be a new surrogate marker indicative of early tumor response for colorectal cancer patients undergoing cytotoxic and antiangiogenic therapy. Respiratory motion compensation and a normalization scheme are required for accurate derivation of WITR.

Acknowledgements—This work was supported by the Marie Curie Chair of Excellence, TUMOURANGIO, FP6-2005-Mobility-10-042255 and Cyprus Research Promotion Foundation (YGEIA/0506/06).

REFERENCES

- Arditi M, Frinking PJA, Zhou X, Rognin NG. A new formalism for the quantification of tissue perfusion by the destruction-replenishment method in contrast ultrasound imaging. *IEEE Trans Ultrason Ferroelectr Freq Control* 2006;53:1118–1229.
- Averkiou M, Powers J, Skyba D, Bruce M, Jensen S. Ultrasound contrast imaging research. *Ultrasound Q* 2003;19:27–37.
- Averkiou MA, Bruce M, Jensen S, Rafta P, Brock-Fisher T, Powers J. Pulsing schemes for the detection of nonlinear echoes from contrast microbubbles. Rotterdam, The Netherlands: 9th European Symposium on Ultrasound Contrast Imaging; January 2004. 17–24.
- Averkiou MA, Lampaskis M, Kyriakopoulou KN, Klouvas G, Skarlos D, Leen E. Quantification of tumor microvasculature with respiratory gated contrast enhanced US for monitoring therapy, ESGAR. Valencia, Spain 2009 (in press).
- Bekradda M, Cvitkovic E. New possibilities in chemotherapy for colorectal cancer. *Annals of Oncology* 1999;(Suppl. 6):S105–S111.
- Blomley MJK, Dawson P. Bolus dynamics: Theoretical and experimental aspects. *Br J Radiol* 1997;70:351–359.
- Browder T, Butterfield CE, Kraling BM, Shi B, Marshall B, O'Reilly MS, Folkman J. Antiangiogenic scheduling of chemotherapy improves efficacy against experimental drug-resistant cancer. *Cancer Res* 2000;60:1878–1886.
- Correas JM, Tranquart MCF, Helenon O. The kidney: Imaging with microbubble contrast agents. *Ultrasound Q* 2006;22:53–66.
- Elie N, Kaliski A, Pronneau P, Opolon P, Roche A, Lassau N. Methodology for quantifying interactions between perfusion evaluated by DCE-US and hypoxia throughout tumor growth. *Ultrasound Med Biol* 2007;33:549–560.
- Ferrara K, Merritt C, Burns P, Foster FS, Mattrey RF, Wickline SA. Evaluation of tumor angiogenesis with us: Imaging, Doppler and contrast agents. *Acad Radiol* 2000;7:824–839.
- Fisher NG, Christiansen PC, Leong-Poi H, Jayaweera AR, Lidner JR, Kaul S. Myocardial and microcirculatory kinetics of BR14, a novel third-generation intravenous ultrasound contrast agent. *J Am Coll Cardiol* 2002;39:530–537.
- Folkman J. What is the evidence that tumors are angiogenesis dependent? *J Nat Cancer Inst* 1990;82:4–6.
- Folkman J. Angiogenesis: An organizing principle for drug discovery? *Nat Rev Drug Discov* 2007;6:273–286.
- Görg C. The forgotten organ: Contrast enhanced sonography of the spleen. *Eur J Radiol* 2007;64:189–201.
- Jain RK. Taming vessels to treat cancer. *Sci Am* 2008;298:56–63.
- Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, Thun MJ. Cancer statistics, 2006. *CA Cancer J Clin* 2006;56:106–130.
- Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100:57–70.
- Kerbel RS. Antiangiogenic therapy: A universal chemosensitization strategy for cancer? *Science* 2006;312:1171–1174.
- Lassau N, Chami L, Benatsou B, Peronneau P, Roche A. Dynamic contrast-enhanced ultrasonography (DCE-US) with quantification of tumor perfusion: A new diagnostic tool to evaluate the early effects of antiangiogenic treatment. *Eur Radiol Suppl* 2007;17(Suppl. 6):F89–F98.
- Leen E, Ceccotti P, Moug SJ, Glen P, MacQuarrie J, Angerson WJ, Albrecht T, Hohmann J, Oldenburg A, Ritz JP, Horgan PG. Potential value of contrast-enhanced intraoperative ultrasonography during partial hepatectomy for metastases: An essential investigation before resection? *Ann Surg* 2006a;243:236–240.
- Leen E, Ceccotti P, Kalogeropoulou C, Angerson WJ, Moug SJ, Horgan PG. Prospective multicenter trial evaluating a novel method of characterizing focal liver lesions using contrast-enhanced sonography. *Am J Roentgenol* 2006b;186:1551–1559.
- Miller CM, Pien HH, Shani D, Sorensen AG, Thrall JH. Imaging angiogenesis: Applications and potential for drug development. *J Nat Cancer Inst* 2005;97:172–187.
- Mischi M, Kalker T, Korsten E. Videodensitometric methods for cardiac output measurements. *EURASIP J Appl Signal Proc* 2003;5:479–489.
- Mischi M, Den Boer JA, Korsten HHM. On the physical and stochastic representation of an indicator dilution curve as a gamma variate. *Physiol Meas* 2008;29:281–294.
- Pandharipande PV, Krinsky GA, Rusinek H, Lee VS. Perfusion imaging of the liver: Current challenges and future goals. *Radiology* 2005;234:661–673.
- Qian H, Bassingthwaite JB. A class of flow bifurcation models with lognormal distribution and fractal dispersion. *J Theor Biol* 2000;205:261–268.
- Quaia E, Palumbo A, Rossi S, Degobbi F, Cernic S, Tona G, Cona M. Comparison of visual and quantitative analysis for characterization of insonated liver tumors after microbubble contrast injection. *Am J Roentgenol* 2006;186:1560–1570.
- Rizzatto G, Martegani A, Chersevani R, Macorig D, Vrtovec M, Aiani L, Tufarulo. Importance of staging of breast cancer and role of contrast ultrasound. *Eur Radiol* 2001;11(Suppl. 3):E47–E52.
- Renault G, Tranquart F, Perlberg V, Bleuzen A, Herment A, Frouin F. A posteriori respiratory gating in contrast ultrasound for assessment of hepatic perfusion. *Phys Med Biol* 2005;20:4465–4480.
- Schlosser T, Pohl C, Kuntz-Hehner S, Omran H, Becher H, Tiemann K. Echoscintigraphy: A new imaging modality for the reduction of color blooming and acoustic shadowing in contrast sonography. *Ultrasound Med Biol* 2003;29:985–991.
- Wei K, Jayaweera AR, Firoozan S, Firoozan S, Linka A, Skyba DM, Kaul S. Basis for detection of stenosis using venous administration of microbubbles during myocardial contrast echocardiography: Bolus or continuous infusion? *J Am Coll Cardiol* 1998;32:252–260.