

Dynamic contrast enhanced ultrasound assessment of the vascular effects of novel therapeutics in early stage trials

Edward Leen · Michalakis Averkiou · Marcel Arditi ·
Peter Burns · Daniela Bokor · Thomas Gauthier ·
Yuko Kono · Olivier Lucidarme

Received: 22 June 2011 / Revised: 23 November 2011 / Accepted: 24 November 2011 / Published online: 3 February 2012
© European Society of Radiology 2012

Abstract Imaging is key in the accurate monitoring of response to cancer therapies targeting tumour vascularity to inhibit its growth and dissemination. Dynamic contrast enhanced ultrasound (DCE ultrasound) is a quantitative method with the advantage of being non-invasive, widely available, portable, cost effective, highly sensitive and reproducible using agents that are truly intravascular. Under the auspices of the initiative of the Experimental Cancer Medicine Centre Imaging Network, bringing together experts from the UK, Europe and North America for a 2-day workshop in May 2010, this consensus paper aims to provide guidance on the use of DCE ultrasound in the measurement of tumour vascular support in clinical trials.

Key Points

- DCE ultrasound can quantify and extract specific blood flow parameters, such as flow velocity, relative vascular volume and relative blood flow rate.

- DCE ultrasound can be performed repeatedly and is therefore ideally suited for pharmacokinetic and pharmacodynamic studies evaluating vascular-targeted drugs.
- DCE ultrasound provides a reproducible method of assessing the vascular effects of therapy in pre-clinical and early clinical trials, which is easily translatable into routine clinical practice.

Keywords Ultrasonography · Contrast agents · Perfusion quantification · Vascular-targeted therapy · Therapy response assessment

Introduction

Angiogenesis plays a key role in tumour growth and its inhibition may arrest local growth and distant metastasis

E. Leen (✉) · T. Gauthier
Imaging Department, Imperial College London NHS Trust,
Hammersmith Hospital Campus, Du Cane Road,
London W12 0NN, UK
e-mail: edward.leen@imperial.ac.uk

M. Averkiou
Department of Mechanical and Manufacturing Engineering,
University of Cyprus,
75 Kallipoleos Str.,
1678 Nicosia, Cyprus

M. Arditi
Bracco Suisse SA,
Geneva Research Center and Manufacturing Site,
31 route de la Galaise, Plan-les-Ouates,
CH-1228 Geneva, Suisse, Switzerland

P. Burns
Sunnybrook Health Sciences Centre, University of Toronto,
2075 Bayview Avenue, Room S660,
Toronto, ON M4N 3 M5, Canada

D. Bokor
Bracco Imaging SpA,
Via XXV Aprile 4,
20097 Milan, Italy

T. Gauthier
Philips Healthcare,
22100 Bothell Everett Highway,
Bothell, WA 98021-8431, USA

Y. Kono
Department of Medicine, Division of Gastroenterology
and Hepatology, University of California,
San Diego, 200 W. Arbor Drive, MC8756,
San Diego, CA 92103, USA

O. Lucidarme
Radiology Department, Pitie Salpetriere Hospital,
47-83 Bd de l'hôpital,
75013 Paris, France

[1–3]. Hence, there has been extensive research in identifying pro-angiogenic factors and in developing strategies to inhibit their action for cancer treatment and the prevention of metastasis.

Imaging techniques, such as computed tomography (CT), magnetic resonance imaging (MRI) and ultrasound, are routinely used to monitor the response to cancer therapy. Current assessment of response is based on interval evaluation of the tumour sizes using the Response Evaluation Criteria In Solid Tumours (RECIST) [4]. However, as these anti-vascular therapies are predominantly cytostatic, current criteria for monitoring response are inadequate as they reflect late changes and are unable to identify non-responders at an early time point. Furthermore, the development and clinical usage of these targeted agents are also costly; accurate, reproducible and non-invasive imaging methods of assessing their effectiveness at an earlier stage are required.

The advent of novel therapies targeting tumour vascularity over the last decade has highlighted the need for more accurate and reproducible quantitative techniques to assess the alteration in tumoural vascularity. Imaging techniques such as dynamic contrast enhanced CT [5, 6], MRI [7] and positron emission tomography (PET) [8] have been used to assess perfusion changes in monitoring anti-vascular therapies. However, these investigations have disadvantages such as ionising radiation (CT/PET), lack of availability and high costs, which may limit their application in routine clinical practice. Furthermore, assessment of early response has invariably been disappointing to date compared with pre-clinical studies [9].

Ultrasound provides an alternative method of assessing the vascular effects of these therapeutics in pre-clinical and early clinical trials, and may be more translatable into routine clinical practice. DCE ultrasound involves the use of ultrasound contrast agents (UCA), which are widely approved for clinical use worldwide for radiology and cardiology applications. Advances in non-linear imaging techniques have enabled ultrasound imaging to visualise the macro- and microvasculature in real time. Currently UCAs are routinely used in radiology for improved lesion detection (Fig. 1) and characterisation as well as in monitoring response to physical ablative/embolic therapies [10, 11]. UCAs are gas-filled microbubbles 1–10 μm in diameter and are pure blood pool agents, i.e. they are truly intravascular by virtue of their larger size with no leakage into the interstitial space [10]. This represents an advantage in enabling the imaging and haemodynamic quantification of the microcirculation but without diffusion and permeability information. Furthermore, DCE ultrasound is real-time imaging (at 10–50 frames per second) with resolution of 0.2–2.0 mm and portable, allowing for bedside evaluation, combined with the absence of ionising radiation and harmful side effects, such as renal toxicity. DCE ultrasound can be



Fig. 1 Dynamic contrast enhanced ultrasound image of a colorectal liver metastasis, which appears hypervascular in the arterial phase

performed repeatedly as the UCA can be administered many times at 5- to 10-min intervals. It is therefore ideally suited for pharmacokinetic and pharmacodynamic studies evaluating vascular-targeted drugs. Also, the microbubbles can be intentionally disrupted by the ultrasound waves, providing alternative approaches to quantification [12–15].

In most mid- to high-end ultrasound devices, manufacturers offer contrast-specific imaging, which uses non-linear modes to cancel the echo from tissue, allowing specific visualisation of microbubbles. At present, most systems offer 2D acquisition only, but 3D contrast-specific imaging is becoming available with the advent of matrix array transducers. As ultrasound is performed with handheld transducers, it is therefore operator-dependent and not amenable to automation procedures available in other imaging techniques. Few validation and reproducibility studies have been published on anti-angiogenesis/anti-vascular treatment [16–19], and no consensus discussions for its appropriate use in this area have taken place until now.

Current approaches of DCE ultrasound

With the current diagnostic ultrasound equipment and various quantification software packages, it is possible to quantify and extract specific blood flow parameters, such as flow velocity, relative vascular volume and relative blood flow rate [15, 17]. Although research into extracting absolute measures of blood flow and volume is ongoing, DCE ultrasound can identify relative changes in these parameters at present. The perfused area may be measured and the overall perfused volume may be deduced from reconstruction of multiple planes. In future, 3D ultrasound will allow the perfused volume to be assessed directly. In addition, useful information on the vascular organisation of tumours can be inferred owing to the high temporal and spatial resolution of

DCE ultrasound [17]. However, no permeability information can be obtained because of the pure “blood pool” nature of microbubbles.

Quantification of tumour vascularity with DCE ultrasound can be performed using two different approaches namely: (a) an intravenous bolus injection using wash-in/wash-out analysis, and (b) steady intravenous infusion of agent using disruption–replenishment analysis.

Bolus injection of UCA and wash-in/wash-out analysis

The bolus injection method is more widely used than the infusion method. Multiple repeated injections of small volumes (0.05 to 2.4 mL) of UCA are feasible for multiple data acquisitions during the same ultrasound examination. Once the UCA is administered intravenously, the vascular compartment can be visualised a few seconds later using contrast-specific imaging. Imaging is usually performed at 10–20 frames per second and a single plane is imaged over the whole duration of the UCA enhancement, which lasts for 3–5 min depending on the dose of UCA used. The average intensity within a region of interest (ROI) can be displayed as a function of time, in the form of a time–intensity curve, which describes the wash-in and wash-out of the UCA in the ROI (Fig. 2). Alternatively, UCA echo levels can be monitored at the pixel level to allow parametric imaging, providing a spatial rendering of perfusion parameters across the contrast enhanced image. A model function derived from the indicator–dilution theory is fitted to the time–intensity curve(s) to allow extraction of haemodynamic parameters [16, 20]. Furthermore, another ROI can be placed in reference tissue for comparison (Fig. 3). Various other commercial software packages can also perform motion correction and other automated measurements, in addition to performing the above analyses. The rationale for choosing a reference ROI is twofold: first, a suitable arterial input function is usually unavailable in the bolus administration mode; second, perfusion parameters are influenced by ultrasound attenuation in the tissue path from the transducer.

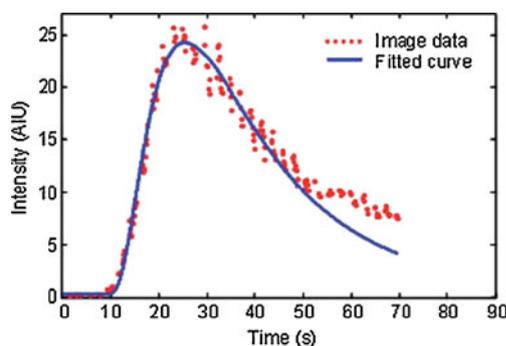


Fig. 2 Bolus injection technique showing wash-in/wash-out curve in a colorectal liver metastasis undergoing targeted therapy

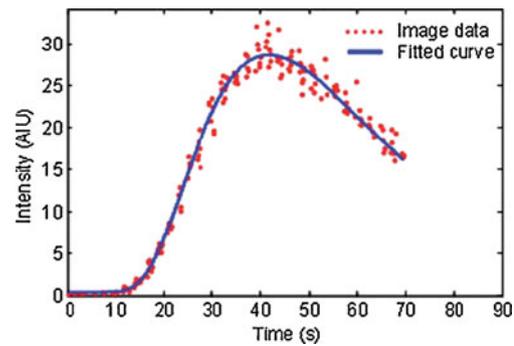


Fig. 3 Bolus injection technique showing wash-in/wash-out curve in reference tissue

Information from the reference ROI can serve to normalise the echo intensity parameters in tumour.

Table 1 outlines a practical clinical protocol that can be used for the bolus technique. It is fast, easily performed and yields measurements related to relative flow velocity, relative vascular volume, relative flow rate and vascularised fraction; however, it is limited to 2D acquisition and in-plane motion compensation. In future, real-time 3D contrast-specific ultrasound may mitigate these issues.

Infusion of UCA and disruption–replenishment analysis

In this technique, UCA is administered as a continuous infusion and a disruption–replenishment analysis is applied. An infusion pump or bag is used for a continuous UCA supply over 5–20 min. The UCA microbubbles can be disrupted at acoustic pressure amplitude higher than that required for imaging, but which is still within the range of amplitudes accepted in diagnostic ultrasound. Microbubbles are first imaged without being disrupted at a low acoustic amplitude pressure or mechanical index (MI), then the MI is increased for a few frames causing microbubble disruption; immediately after, imaging is returned to the non-disrupting level to observe the replenishment of the microbubbles into the ROI (Fig. 4). The initial rate of replenishment and steady state are related to flow and vascular volume, respectively. Models have been developed to describe the echo signal dynamics during the replenishment phase that account for the vessel network morphology (e.g. fractal) and beam characteristics [13, 15, 17].

Table 2 outlines a practical clinical protocol of the infusion technique. Its advantage is that multiple planes can be evaluated allowing measurement over an entire tumour volume [19]. Also, the local input function of the negative bolus is known, so that analysis yields relative flow and vascular volume, as well as velocity and transit time distribution. It is limited, however, by the need for a UCA infusion pump (continuous mixing is required) and to in-plane-only motion compensation.

Table 1 Sample clinical protocol for the evaluation of tumour vascular response using bolus injection of contrast material and wash-in/wash-out analysis

Acquisition	Analysis
<p>Ultrasound system preparation</p> <ul style="list-style-type: none"> • Abdominal probe; contrast imaging preset; side-by-side contrast/B-mode; MI=0.06; temporal compounding off; prospective acquisition duration to 90 s • Optimise dynamic range (high), image depth (large to include diaphragm), focal depth (about two thirds of depth), single focal zone at baseline and record; replicate precisely at follow-up • Optimise gain at baseline for good SNR and avoid image saturation <p>Contrast agent bolus (1.2/2.4 mL)</p> <ul style="list-style-type: none"> • Insert IV line into cubital vein of patient using 20 or 22 G catheter and add a stop valve (no extra line should be used) • Activate contrast agent according to manufacturer's instructions; draw bolus amount from vented vial • Open stop valve, inject contrast material directly to IV line and close stop valve; start contrast timer • Repeat above procedure for subsequent boluses if needed <p>Clinical tumour imaging</p> <ul style="list-style-type: none"> • Acquire and save a loop by taking care to maintain the same plane; minimise out-of-plane motion; avoid rib shadowing; it may be better to observe the tissue image during acquisition • Hold probe steady during acquisition • Record and replicate precisely at follow-up 	<p>ROI analysis</p> <ul style="list-style-type: none"> • Draw two ROIs, one encompassing entire tumour, and one at same depth with normal parenchyma • Apply consistent standards in determination of tumour margins • Exclude saturation artefacts from image if possible • Exclude large non-tumour vessels if possible <p>Respiratory motion compensation</p> <ul style="list-style-type: none"> • Perform rigid or deformable registration <p>Estimation of vascular properties</p> <ul style="list-style-type: none"> • Calculate ROI TICs using linearised image data to remove log-compression • Fit perfusion model (log-normal or similar) to TICs • Record MTT, time to peak, and AUC parameters for both tumour and parenchyma; they are related to relative blood flow and volume changes <p>Longitudinal analysis</p> <ul style="list-style-type: none"> • For baseline and follow-up, calculate median tumour vascular parameters • Calculate percentage change in median at follow-up relative to baseline

MI mechanical index; TICs time–intensity curves; MTT mean transit time; AUC area under the receiver operating characteristic curve

Application of DCE ultrasound to clinical trials

There are limited published international clinical studies evaluating DCE ultrasound in assessing tumour vascular support [19, 21–26]. These are summarised in Table 3. Whilst earlier studies were useful for proof-of-concept,

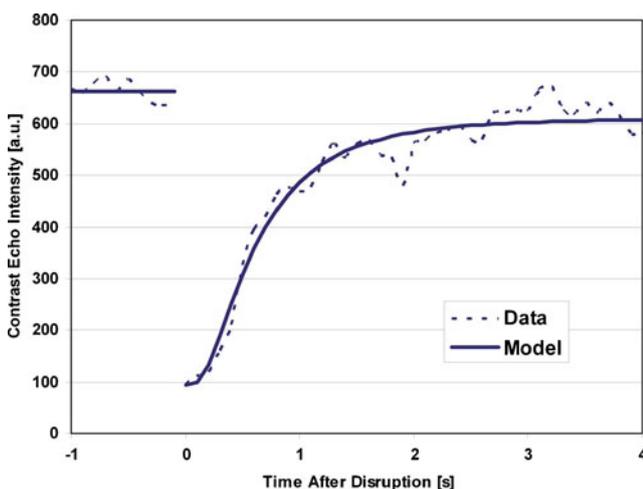


Fig. 4 Illustration of the disruption–replenishment technique applied in normal kidney cortex, where a cumulative log-normal model is used to fit the replenishment phase, following disruption frames (0.1 s duration in this case)

their methodology was questionable with the use of different UCAs, unusually high UCA doses and slow injection rates. Data analysis was also purely subjective, quasi-quantitative or without any normalisation scheme and none had been validated or shown to be reproducible. Recent studies provide estimates of reproducibility with a coefficient of variation of about 9% [18, 19]. Variability is reduced by the selection of time-based parameters, which are less susceptible to extrinsic effects than intensity-based parameters, including the use of an appropriate normalisation scheme and respiratory gating. DCE ultrasound measurements of time-based parameters had been shown to identify non-responders at an earlier stage than conventional methods, following treatment with biological agents.

Areas of consensus

Tumour vascularity quantification with DCE ultrasound can be performed for solid tumours amenable to ultrasound. The portability/bedside availability of DCE ultrasound, the repeatability of multiple injections and good reproducibility are important advantages in therapy monitoring. UCAs are also safe and well-tolerated even in those with impaired renal function.

Table 2 Sample clinical protocol for the evaluation of tumour vascular response using infusion of contrast material and disruption–replenishment analysis [15]

Acquisition	Analysis
Ultrasound system preparation <ul style="list-style-type: none"> • Abdominal (e.g. Philips iU22: C5-1) probe; contrast general preset; side-by-side contrast/B-mode; MI=0.06; CGen/CPen-RS pulse sequence; persistence off; XRES off; SonoCT off; TGC aligned to the centre; flash power to maximum; prospective acquisition duration to 30 s; native DICOM output • Parameters optimised at baseline: dynamic range, image depth, focal depth, focal zone size. Settings to be replicated precisely at all future imaging sessions 	ROI analysis <ul style="list-style-type: none"> Draw ROIs encompassing the entire tumour
Contrast agent infusion (12 min) <ul style="list-style-type: none"> • Insert an IV line into the cubital vein of the patient using an 18 G needle • Activate Definity according to manufacturer's instructions; vent vial, invert and withdraw 0.9 mL using the 18 G needle • Dilute and mix well in 54 mL of saline in a 60-mL syringe • Begin infusion using the infusion pump (Medfusion 3500); start contrast timer • Occasionally rotate the pump to ensure agent remains well mixed 	<ul style="list-style-type: none"> Apply consistent standards in the determination of tumour margins Exclude saturation artefacts from the image if possible Exclude large non-tumour vessels if possible
Clinical tumour imaging <p>Acquire view; rehearse imaging before contrast medium infusion; minimise out-of-plane motion; avoid rib shadowing; record details at baseline and reproduce at follow-up</p> <p>Wait 1–2 min for the enhancement steady state; encourage the patient to breathe gently</p> <p>Optimise the gain at baseline for good SNR and avoid image saturation; record and replicate precisely at follow-up</p> <p>Perform disruption–replenishment measurements over seven contiguous planes in the tumour: Acquire view; press Record; press Flash; repeat</p> <p>Hold the probe steady during acquisition</p>	Respiratory motion compensation <ul style="list-style-type: none"> Perform rigid or deformable registration using custom software Estimation of vascular properties <ul style="list-style-type: none"> Calculate the ROI TIC using linearised image data Begin with first frame after the flash; treat as the background and subtract from all subsequent TIC values Fit the log-normal perfusion model to TIC Record vascular volume and velocity parameters; their product is a measure of perfusion Longitudinal analysis <ul style="list-style-type: none"> For baseline and follow-up, calculate the median tumour vascular parameters Calculate the percentage change in medians at follow-up relative to baseline

UCA administration

In Europe, SonoVue (Bracco spa, Milan, Italy) is the only commercially available UCA for radiology. Definity (Lantheus Medical Imaging, Boston, MA, USA) is approved for radiology indications in Canada and other countries. Sonazoid (Daichi Pharma, Tokyo, Japan) is licensed for radiology indications in Japan. The recommended dose for SonoVue is 2.4 mL for bolus and 1 mL/min for infusion administration; for Definity it is 0.2–0.4 mL as a bolus. The dose used should ensure adequate uniform enhancement, while avoiding shadowing effects; hence high doses of UCAs should be avoided. Low MI is recommended to minimise bubble destruction and consequent bias in estimates of blood perfusion.

Patients undergoing DCE ultrasound for abdominal tumour monitoring should be instructed to fast for at least 4 h before the examination, as well as in the importance of keeping still and maintaining a gentle and regular respiratory pattern.

For UCA delivery, an extension line and saline flush are not advised; only an angio-catheter of 20–22 gauge (no longer than 33 mm) should be used, ensuring a single, well-formed bolus. The endpoints of quantification studies performed with either of the two analysis protocols are: relative vascular volume, relative vascular flow rate and perfused fraction. Both approaches are valid and produce relative measures of vascular change.

Ultrasound equipment

Systems must have contrast-specific multi-pulse and non-linear imaging methods (e.g. pulse inversion, power modulation and their combinations), which effectively detect the non-linear responses of UCAs while suppressing tissue echoes and producing an image of the vascular compartment. The choice should be one that has the most appropriate balance between sensitivity and resolution for the chosen application; usually set as a default by the manufacturer. Low frequency curvilinear and phased array transducers

Table 3 Summary of clinical trials using dynamic contrast enhanced (DCE) ultrasound to demonstrate drug effects

Authors	Tumour type	Drug	Patient number	Technique	Contrast agent	Analysis	Standardisation	Results	Reproducibility study
De Giorgi et al. [22]	GIST (liver metastasis)	Imatinib	10	Angiosonography	SonoVue (1.2 mL bolus)	Subjective visual assessment	No	Decrease in signal correlated with CT	No
Lassau et al. [26]	GIST	Imatinib	30	Contrast enhanced colour Doppler	Levovist SonoVue	Subjective visual assessment+% of enhancing pixels assessed with Photoshop analysis	No	Initial contrast medium uptake and decrease in contrast medium uptake at D7 and D14 correlated with T Response	No
Lamuraglia et al. [23]	Renal cell carcinoma	Sorafenib			SonoVue	Subjective visual assessment+% of enhancing pixels assessed with Photoshop analysis	No	>20% decrease in contrast medium uptake with stability or decrease in T volume correlated with increased PFS and OS examinations at baseline and day 15 significantly correlated with response in 5 of the 7 DCE ultrasound parameters. Two DCE ultrasound parameters (time to peak intensity and slope of the wash-in) were significantly associated with DFS; time to peak intensity was also significantly associated with OS	No
Lassau et al. [24]	Renal cell carcinoma	Sunitinib	38	DCE ultrasound	SonoVue (4.8-mL bolus)	Descriptive analysis of the time-intensity curve Seven parameters: peak intensity, area under the curve, area under the wash-in, area under the wash-out, time to peak intensity, slope of the wash-in and MTT	No	Ratio between DCE ultrasound examinations at baseline and day 15 significantly correlated with response in 5 of the 7 DCE ultrasound parameters. Two DCE ultrasound parameters (time to peak intensity and slope of the wash-in) were significantly associated with DFS; time to peak intensity was also significantly associated with OS	No
Averkiou et al. [18]	Colorectal cancer (liver metastasis)	Cytotoxic + anti-angiogenic	7	DCE ultrasound respiratory gating	SonoVue (2.4-mL bolus)	WTR	Yes, internal reference	Increase in the WTR for patients responding to treatment	The average deviation of WTR was found to be 9%
Bertolotto et al. [21]	HCC	Thalidomide		DCE ultrasound	SonoVue	Disruption-replenishment	No	A and A*beta reduced significantly after 15 days, 3 and 6 months of treatment earlier than the morphological changes Increase AUC	No
Lassau et al. [25]	HCC	Bevacizumab	42	DCE ultrasound	SonoVue	Area under the curve, area under the wash-in, area under the wash-out, time to peak intensity	No		
Williams et al. [19]	Renal cell carcinoma	Sunitinib	30	DCE ultrasound, CT	Definity	Bolus injection and disruption-replenishment	Yes	Relative vascular volume decreased in responding patients before RECIST or modified Choi criteria	Coefficient variation 9% for the infusion method

GIST gastrointestinal stromal tumour; HCC hepatocellular carcinoma; PFS progression-free survival; OS overall survival; DFS disease-free survival; WTR ratio of wash-in time of the lesion to that of the normal parenchyma; RECIST Response Evaluation Criteria In Solid Tumours

should be used for abdominal imaging and high-frequency linear probes for imaging superficial tissue.

The imaging parameters should be set as a preferred default setting, requiring manufacturers to introduce specific “quantification settings”. The MI should be set low, typically below 0.20. Gain, which is applied in the ultrasound signal path to the electrical signals generated by the ultrasound transducer receiving backscattered acoustic signals, should be high enough to enable adequate signal visualisation and the dynamic range should be higher than 40 decibels to avoid saturation. The depth and width chosen for the ultrasound examination should provide a large enough field of view to accommodate for possible motion compensation and the focus zone should be placed deep enough to ensure a relatively uniform acoustic image.

The dual-image display format, where a contrast enhanced and a tissue image are displayed next to each other, is strongly recommended, so that the target ROI can be tracked in-plane. Acquisition of the image loop should start from the time of the bolus injection and last 1–2 min, and at a moderate frame rate, avoiding over-sampling (5–10 fps). Loops must be saved in Digital Imaging and Communications in Medicine (DICOM) format (rather than Audio Video Interleave (AVI)) which will allow access to linear or linearised data (with logarithmic compression removed) for analysis.

It is mandatory that once the acquisition settings for a patient have been defined at baseline, these will remain unchanged for all the subsequent images as should the position of the probe with respect to the lesion imaging plane. Currently 2D acquisition is performed, whilst 3D is in development and is expected to be available in the near future. As ultrasound is operator-dependent, trained and disciplined operators are required.

Analysis

Post-processing software for analysis is available from various manufacturers and academic researchers. Only packages operating on linear data or having a way of linearising log-compressed data should be considered. Most ultrasound systems export and display the data logarithmically compressed. Once the data are linearised, curve-fitting with perfusion models is used to extract relative blood flow measures.

As quantification must reflect UCA concentration independently of the equipment and settings used, the user settings should be such as to avoid signal processing that interferes with objective quantification (persistence, compounding and spatial equalisation, etc.). Only quantitative estimates relative to a reference area are meaningful as the ultrasound attenuation, arbitrary gain, time gain compensation and probe sensitivity interfere with absolute quantification of perfusion with UCA. A normalisation scheme uses a reference tissue ROI, which is at the same depth as the lesion and allows estimates

of relative perfusion in a reliable way. The multitude of ultrasound systems and image analysis software provides great options, but also some inherent difficulty in comparing DCE ultrasound techniques within multi-centre clinical trials. Whenever possible, a validation study of the technique should be incorporated for any clinical trial.

For accurate reporting, all imaging information should be recorded to allow assessment of the therapeutic efficacy in an unambiguous way. It should include the type of ultrasound equipment, analysis software and version used to derive the parameters, the method and dose of contrast medium administration, the curve fit model (degree of fit), the processing parameters (smoothing \pm motion compensation) and the quantitative parameters (relative vascular volume, flow and flow velocity).

Reproducibility

The reproducibility of the flow parameters measured is dependent upon many factors including device settings, UCA type and delivery technique, quantification analysis software, image plane motion and pathophysiological factors. Some may be controlled by selecting identical conditions or accounting for their change.

Administration of UCA should follow the same standardised protocol during all sessions. The device settings should remain identical between imaging procedures (defaulted preset). Baseline study is the best time to establish and fix equipment and UCA administration settings. Maintaining the same imaging plane is key in 2D DCE ultrasound, with minimal changes potentially resulting in significant differences in measured parameters. As it is inherently difficult to use the same plane in subsequent examinations, especially with lesional evolution during treatment, the measurement and averaging of measurements over multiple planes and volumes are preferred. Multi-slice analysis is easier to perform using replenishment analysis than using bolus injection and it would benefit from UCA with longer blood half life. Anatomical markers (in B-mode) or the use of navigation imaging techniques for selecting the image plane offer possible solutions. Out-of-plane motion during clip recording must be minimised, although difficult when imaging obliquely. This underlines the need for 3D and 4D imaging development. Patients should be instructed to breathe normally. Respiratory gating techniques should be used where available, and data volume and the time–intensity points should be reduced for easier and robust processing.

Areas for further research

Studies monitoring treatment response of liver and renal tumours have been published. A number of quantification

tools currently available were developed for this specific application. Clinical validation and reproducibility studies must also be extended to other clinical areas such as breast, prostate and kidney etc. [27].

Further instrumentation development is needed, especially in the area of 3D/4D acquisition and analysis. This would enable DCE ultrasound to quantify multiple planes in tumours simultaneously and address tumour heterogeneity. With 3D DCE ultrasound, motion compensation can be performed in 3D, resolving the “out-of-plane” motion issue.

For improved reproducibility of results across centres (with different systems), standardised data formats, protocols and common analysis software are required. Different curve-fitting models and algorithms are being used; there is as yet no clear consensus on the specific elements and steps for the quantification process. This will emerge from future studies. However, the tools available today should be used to acquire knowledge and improve our understanding of disease management. Presently, these operate either on-line (on the ultrasound system) or off-line, after DCE ultrasound is performed, as post-processing on the collected image data. In the future, real-time analysis (on-board quantification) should be made available to match the specific needs of the therapy protocol, ensuring that the data collected are capable of providing reliable quantification parameters, thus enabling comparisons in longitudinal studies in a fast, convenient and efficient way.

Challenges in implementation

The main challenges for widespread use and integration of these techniques into clinical trials are:

- (1) Lack of trained personnel, equipment and expertise in most international cancer centres: these facilities tend to focus on their own imaging expertise and few have expertise or interest in DCE ultrasound;
- (2) Most pharmaceutical industry representatives and oncologists are unaware of the true value of DCE ultrasound: as a result assessment of novel therapies in most clinical trials is still primarily based on CT/PET and MRI;
- (3) Few reproducibility data are available;
- (4) DCE ultrasound cannot be used to assess bone or lung malignancies.

There is no real solution with regard to the latter challenge; however with respect to the first two challenges, education and training are clearly needed, as are published data demonstrating the efficacy of DCE ultrasound. Reproducibility can be improved underlining the need for the development of 3D and 4D data acquisition and analysis.

Conclusion

Dynamic contrast enhanced ultrasound is safe, well-tolerated, available at the bedside and reproducible. A number of clinical trials using DCE ultrasound are already complete with encouraging results. Imaging biomarkers correlating with clinical outcomes have also been established, suggesting that DCE ultrasound should be recommended for investigating the pharmacokinetics and pharmacodynamics of experimental cancer drugs. UCAs provide blood pool information that may complement outputs from other imaging techniques, and it is advised that DCE ultrasound should be used in conjunction with other methods in future clinical trials to compare their reproducibility and predictive efficacy.

Acknowledgements The authors would like to acknowledge the Experimental Cancer Medicine Centre Imaging Steering Committee and Secretariat for organising the two-day workshop in May 2010 and all the speakers and delegates who contributed to this initiative. The Experimental Cancer Medicine Centre Initiative is jointly funded by Cancer Research UK, the National Institute for Health Research in England and the Departments of Health for Scotland, Wales and Northern Ireland.

References

1. Ferrara N, Kerbel RS (2005) Angiogenesis as a therapeutic target. *Nature* 438:967–974
2. Folkman J (2007) Angiogenesis: an organizing principle for drug discovery? *Nat Rev Drug Discov* 6:273–286
3. Kerbel R, Folkman J (2002) Clinical translation of angiogenesis inhibitors. *Nat Rev Cancer* 2:727–739
4. Therasse P, Arbuck SG, Eisenhauer EA et al (2000) New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 92:205–216
5. Choi H, Charnsangavej C, Faria SC et al (2007) Correlation of computed tomography and positron emission tomography in patients with metastatic gastrointestinal stromal tumor treated at a single institution with imatinib mesylate: proposal of new computed tomography response criteria. *J Clin Oncol* 25:1753–1759
6. Nathan PD, Vinayan A, Stott D et al (2010) CT response assessment combining reduction in both size and arterial phase density correlates with time to progression in metastatic renal cancer patients treated with targeted therapies. *Cancer Biol Ther* 9:15–19
7. Rini BI, Atkins MB (2009) Resistance to targeted therapy in renal-cell carcinoma. *Lancet Oncol* 10:992–1000
8. Levashova Z, Backer M, Hamby CV et al (2010) Molecular imaging of changes in the prevalence of vascular endothelial growth factor receptor in sunitinib-treated murine mammary tumors. *J Nucl Med* 51:959–966
9. Hahn OM, Yang C, Medved M et al (2008) Dynamic contrast-enhanced magnetic resonance imaging pharmacodynamic biomarker study of sorafenib in metastatic renal carcinoma. *J Clin Oncol* 26:4572–4578
10. Wilson SR, Burns PN (2010) Microbubble-enhanced US in body imaging: what role? *Radiology* 257:24–39

11. Quaia E (2011) The real capabilities of contrast-enhanced ultrasound in the characterization of solid focal lesions. *Eur Radiol* 21(3):457–462
12. Quaia E (2011) Assessment of tissue perfusion by contrast-enhanced ultrasound. *Eur Radiol* 21:604–615
13. Wei K, Jayaweera AR, Firoozan S, Linka A, Skyba DM, Kaul S (1998) Quantification of myocardial blood flow with ultrasound-induced destruction of microbubbles administered as a constant venous infusion. *Circulation* 97:473–483
14. Krix M, Kiessling F, Vosseler S, Farhan N, Mueller MM, Bohlen P, Fusenig NE, Delorme S (2003) Sensitive noninvasive monitoring of tumor perfusion during antiangiogenic therapy by intermittent bolus-contrast power Doppler sonography. *Cancer Res* 63:8264–8270
15. Arditi M, Frinking PJA, Zhou X et al (2006) A new formalism for the quantification of tissue perfusion by the destruction-replenishment method in contrast ultrasound imaging. *IEEE Trans Ultrason Ferroelectr Freq Control* 53:1118–1129
16. Palmowski M, Lederle W, Gaetjens J, Socher M, Hauff P, Bzyl J, Semmler W, Günther RW, Kiessling F (2010) Comparison of conventional time-intensity curves vs. maximum intensity over time for post-processing of dynamic contrast-enhanced ultrasound. *Eur J Radiol* 75:e149–e153
17. Hudson JM, Karshafian R, Burns PN (2009) Quantification of flow using ultrasound and microbubbles: a disruption replenishment model based on physical principles. *Ultrasound Med Biol* 35:2007–2020
18. Averkiou M, Lampaskis M, Kyriakopoulou K et al (2010) Quantification of tumor microvasculature with respiratory gated contrast enhanced ultrasound for monitoring therapy. *Ultrasound Med Biol* 36:68–77
19. Williams R, Hudson JM, Lloyd B et al (2011) Dynamic microbubble contrast-enhanced ultrasound (DCE ultrasound) to measure tumor response to targeted therapy: a proposed clinical protocol with results from renal cell carcinoma patients receiving anti-angiogenic therapy. *Radiology* 260:581–590
20. Strouthos C, Lampaskis M, Sboros V et al (2010) Indicator dilution models for the quantification of microvascular blood flow with bolus administration of ultrasound contrast agents. *IEEE Trans Ultrason Ferroelectr Freq Control* 57:1296–1310
21. Bertolotto M, Pozzato G, Croce LS et al (2006) Blood flow changes in hepatocellular carcinoma after the administration of thalidomide assessed by reperfusion kinetics during microbubble infusion: preliminary results. *Invest Radiol* 41:15–21
22. De Giorgi U, Aliberti C, Benea G et al (2005) Effect of angiography to monitor response during imatinib treatment in patients with metastatic gastrointestinal stromal tumors. *Clin Cancer Res* 11:6171–6176
23. Lamuraglia M, Escudier B, Chami L et al (2006) To predict progression-free survival and overall survival in metastatic renal cancer treated with sorafenib: pilot study using dynamic contrast-enhanced Doppler ultrasound. *Eur J Cancer* 42:2472–2479
24. Lassau N, Koscielny S, Albiges L et al (2010) Metastatic renal cell carcinoma treated with sunitinib: early evaluation of treatment response using dynamic contrast-enhanced ultrasonography. *Clin Cancer Res* 16:1216–1225
25. Lassau N, Koscielny S, Chami L et al (2011) Advanced hepatocellular carcinoma: early evaluation of response to bevacizumab therapy at dynamic contrast-enhanced US with quantification – preliminary results. *Radiology* 258:291–300
26. Lassau N, Lamuraglia M, Chami L et al (2006) Gastrointestinal stromal tumors treated with imatinib: monitoring response with contrast-enhanced sonography. *AJR Am J Roentgenol* 187:1267–1273
27. Caproni N, Marchisio F, Pecchi A, Canossi B, Battista R, D’Alimonte P, Torricelli P (2010) Contrast-enhanced ultrasound in the characterisation of breast masses: utility of quantitative analysis in comparison with MRI. *Eur Radiol* 20(6):1384–1395