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Imaging tumour angiogenesis

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Abstract

The development of neovasculature via angiogenesis is a vital component of many normal physiological processes and a number of disease states. Neovascularisation is critical for the growth of malignant tumours and for the development and survival of metastases. Recently, the potential of non-invasive imaging for the functional characterisation of neovasculature has become realised. In this review we describe the process of tumour angiogenesis for radiologists and present a summary of the most available computed tomography/magnetic resonance imaging techniques that can depict the functional vascular status of human tumours.

Keywords: *Angiogenesis; neovascularisation; imaging.*

Introduction

Angiogenesis is a vital component of both normal physiological processes and a number of disease states. It is critical for the growth of primary malignant tumours and for the development of metastases. The importance of tumour angiogenesis is well known to clinical oncologists but until recently has been less familiar to radiologists. This review describes the process of tumour angiogenesis and features unique to tumour microvasculature. The potential of imaging to non-invasively characterise neovasculature is demonstrated with an emphasis on the techniques of perfusion computed tomography (CT) and dynamic magnetic resonance imaging (MRI)[1,2].

Tumour angiogenesis

Angiogenesis involves a cascade of events in which host endothelial cell are stimulated to form new blood vessels. The angiogenic process is a complex multistep phenomenon with interactions between a variety of cell types and cytokines. As tumours grow, an initial avascular phase is followed by neovascularisation which permits further tumour expansion. It is clear that tumour growth beyond 1–2 mm requires vascular in-growth[3]. The primary stimulus for new vessel formation is presumed to be hypoxia. It is well established that for soluble oxygen within tissues, diffusion distances are of the order of 100–150 μm[4]. Tissue angiogenesis is invoked by the expression of pro-angiogenic growth factors (cytokines) and by suppression of anti-angiogenic factors. Expression of angiogenic cytokines can be induced as a response to hypoxic stress, by hormonal stimulation and can also result from the activation of oncogenes.

The factors involved in angiogenesis can be classified according to the role they play in the process[5]. Many tumours secrete high levels of proangiogenic cytokines including vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF). Many more pro-angiogenic cytokines are known about and importantly, pro-angiogenic factors serve as survival factors for proliferating endothelial cells. Tumours also produce anti-angiogenic factors, many of which suppress angiogenesis at metastatic sites but not the primary tumour[6,7]. Anti-angiogenic factors are proapoptotic for proliferating endothelial cells. It is the net balance of

positive and negative regulators of angiogenesis that determine the state of angiogenesis at a local level.

Angiogenesis within malignant tumours is a disorganised and chaotic process. Many different features of vascularity permit the distinction between malignant and benign processes, some of which can be interrogated by imaging techniques. Structural and functional characteristics of malignant tumour vasculature include:

(1) Spatial heterogeneity and chaotic structure

(2) Poorly formed, fragile vessels with high permeability to macromolecules

(3) Arterio-venous shunting, high vascular tortuosity and vasodilatation[8]

(4) Intermittent or unstable blood flow due to transient rises in already raised interstitial pressure[9]

(5) Extreme heterogeneity of vascular density with areas of low vascular density mixed with regions of high angiogenic activity[8].

There are a number of clinical examples where neo-vascularisation has been related to tumour progression, e.g. the change from breast ductal carcinoma *in situ* to invasive cancer[10]. Prognosis is related to the state of angiogenesis and elevated tumour levels of VEGF in breast cancer patients[11]. Immuno-histochemical staining measurements of angiogenic activity, known as microvessel density (MVD), have been shown to be an important prognostic factor for overall survival that is independent of other known prognostic variables including stage, grade and lymph node status in a number of cancer types[12].

MVD provides direct assessment of angiogenesis and requires tumour tissue, generally from operative specimens. This process is, however, limited by the inability to provide information about vascular functionality, particularly in response to treatment. Indirect methods of assessing angiogenesis include serum estimates of angiogenic cytokines and circulating endothelial cells as well as imaging. These methods have the advantages of being non-invasive and can be performed on tumours *in situ*. Indirect techniques are quantitative and in the case of imaging, the functional status of the vasculature can be assessed.

Imaging angiogenesis: comparison of methods

Several commonly available imaging techniques are able to assess human tumours with respect to their angiogenic status. Both CT and MRI have the advantage of good spatial resolution, which is often equal to that of corresponding morphological images. They are minimally invasive, involve little patient risk and data acquisition is quick thus allowing their incorporation into routine patient studies. MRI techniques are also sensitive to a variety of contrast mechanisms including blood flow, microvessel permeability and diameter, water diffusion, tissue oxygenation and metabolism. MRI techniques are discussed in more detail below.

Perfusion CT, also called functional multi-detector row CT (f-MDCT), can be performed with contrast medium to measure vascular characteristics including blood flow, blood volume, mean fluid transit time and capillary permeability in a variety of organs and tumours[13–15]. f-MDCT can show increases in tissue perfusion that may reflect underlying malignancy even in the absence of gross anatomical abnormality[16]. There are differences between f-MDCT and dynamic MRI that will influence the choice of technique in a given clinical situation. Both CT and MRI techniques can provide qualitative and quantitative assessments of tumour vascularity. However, quantification by dynamic MRI is technically more challenging than f-MDCT as there is a lack of a direct relationship between MRI signal intensity and contrast agent concentration, particularly in large vessels. This is related to the fact that tissue signal intensity on MRI is derived from the effect of contrast medium on water in the surrounding microenvironment which changes tissue relaxivity in complex ways that can be unpredictable at times (the contrast medium per se is not detected). The relationship between contrast concentration and CT enhancement is straightforward as there is a direct linear relationship between enhancement change and iodine concentration. For example, at 120 kV, an enhancement change of 25 HU is equivalent to 1 mg/ml of iodine[17]. However, compared with MRI there has so far been little validation of f-MDCT with accepted surrogates of angiogenesis and sensitivity to physiological motion and radiation exposure remain potential drawbacks. In addition, the signal to noise ratio remains poor for f-MDCT when compared to MRI (Fig. 1).

Ultrasound imaging can identify vascular features in tumours at different levels of resolution depending upon the technique employed[18,19]. Doppler ultrasound is used to identify flowing blood within vessels with a resolution in the order of 1 mm although this range is extended power Doppler. Spectral Doppler is able to quantify blood flow, informing on its direction and to determine whether flow is presence in relation to the phase of the cardiac cycle. The detection of cancer using Doppler techniques is dependent on increased, asymmetric flow to a region due to an increased number and size of vessels. A recent development has been the introduction of ultrasound contrast agents which are gas-encapsulated microbubbles of less than 10 μm in diameter. Since microbubbles are confined to the vascular space, this makes them ideal for perfusion imaging techniques. Microbubble-specific techniques allow imaging of vessels down to 50–100 μm in diameter. In addition, since microbubbles are vascular tracers, following a bolus injection, their passage through a tissue of interest can be quantified to generate time intensity curves from which many functional indices can be derived, including bolus arrival
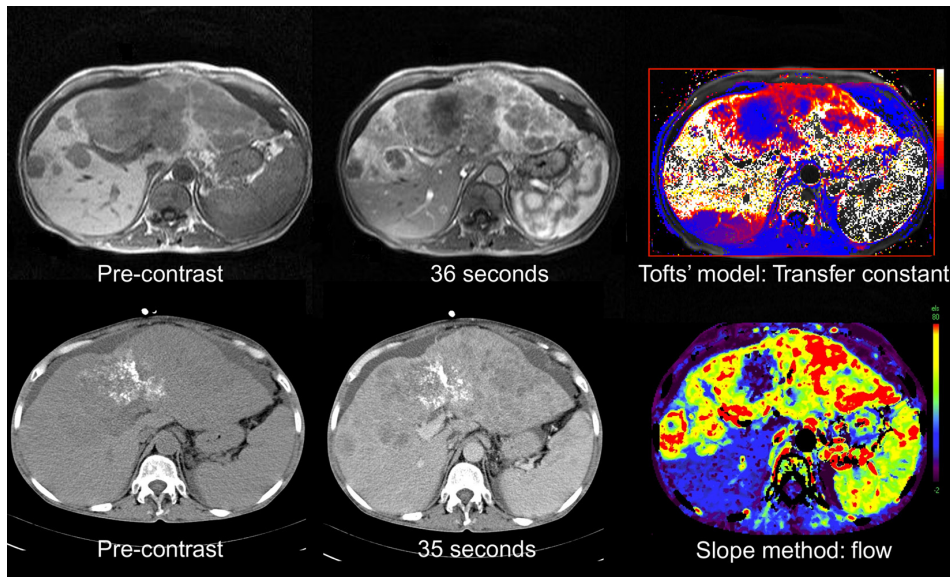
Figure 1 **CT and T1-weighted images of the liver in a patient with metastatic colorectal cancer. The MRI scans (top row) were acquired before and 36 s after 0.1 mol/kg body weight of Gd-DTPA contrast medium. The CT images (bottom row) were acquired before and 35 s after 50 ml of iodinated contrast medium (300 mg I/ml). These represent the standard doses of contrast medium used for T1-weighted DCE-MRI and functional CT. The CT images are acquired at 80 kV, which is optimal for demonstrating contrast medium uptake. Note that, even when these technical factors are taken into account, the signal to noise ratio for MRI is better than for CT. Corresponding parametric maps derived from the dynamic data are shown together with the quantification method that has been used.**

time, time to peak intensity, area under the curve, wash in/out curves, as well as more complex deconvolution indices. Contrast-enhanced ultrasound generated indices of blood flow, blood volume, or vascularity within malignant tissue correlate well with intravascular red blood cell velocities[20,21]. To date, however, there has been little validation of ultrasound with accepted histological surrogates of angiogenesis[19]. Poor accessibility to certain anatomic regions (e.g. lung and brain) and operator dependence are other outstanding issues. Ultrasound cannot of course provide information on microvessel permeability.

Positron emission tomography (PET) can also be used to evaluate tumour metabolism, oxygenation as well as blood flow and volume depending on the tracer used. A number of radiotracers are available to characterise tissue vascularity, e.g. ^{15}O labelled water and carbon monoxide, which can quantify tissue perfusion and blood volume, respectively[22]. PET is considered by many to be the gold standard for the non-invasive measurement of tissue perfusion but there is little tumour data or independent validation outside the brain. PET methods are limited by high cost, limited availability of equipment and poor anatomic resolution. Furthermore, the short lives of radioisotopes require that a cyclotron and onsite radiochemist be present, all of which preclude PET's inclusion into routine clinical assessment of angiogenesis.

Low molecular weight contrast agent kinetics used for CT/MRI

Currently the most commonly used contrast agents are 'low molecular weight' agents (typically <1 kDa) which diffuse freely between the intravascular and extravascular, extracellular space (EES) but never cross cell membranes. These contrast agents have a high first pass extraction in most normal (with the exception of the brain, testes and retina) and tumour tissues. Three major factors determine the behaviour of low molecular weight contrast media in tissues during the first few minutes after injection: blood perfusion, transport of contrast agent across vessel walls and diffusion of contrast medium in the interstitial space. If the delivery of the contrast medium to a tissue is insufficient (flow-limited situations or where vascular permeability is greater than inflow) then blood perfusion will be the dominant factor determining contrast agent kinetics; this situation is commonly found in tumours. If tissue perfusion is sufficient and transport out of the vasculature does not deplete intravascular contrast medium concentration (non-flow limited situations, e.g. in areas of fibrosis or after treatment) then transport across the vessel wall (i.e. permeability) is the major factor that determines contrast medium kinetics. As low molecular weight contrast media do not cross cell membranes, their volume of distribution is effectively the interstitial space. It is

Table 1   Comparison of dynamic-MRI with functional-MDCT

	Dynamic susceptibility contrast enhanced MRI (DSC-MRI)	Dynamic relaxivity contrast enhanced MRI (DCE-MRI)	Functional multi-detector CT (f-MDCT)
Mechanism of tissue enhancement	Susceptibility effects of contrast agent on magnetic field	Relaxivity effects of contrast agent on tissue water	Contrast medium attenuation of X-rays
Tissue compartment being interrogated	Vascular space	Vascular and extravascular space	Vascular and extravascular space
Tissue signal intensity change	Darkening	Enhancement	Enhancement
Duration of effect and optimal data acquisition	Seconds; every 1–2 s	Minutes; 2–25 s	Minutes; 2–5 s
Magnitude of effect	Small	Larger	Small
Signal to noise ratio of technique	Low	Very high	Relatively low
Quantification method used	Central volume theorem	General multi-compartment pharmacokinetic model	Deconvolution distributed parameter model and Patlak analysis
Kinetic parameters measured	Relative blood flow, relative blood volume, mean transit time	Transfer constants, leakage space, blood volume and flow	Blood flow, blood volume, mean transit time, permeability

the differences in these contrast agent kinetics between normal tissue and tumour that are exploited by both f-MDCT and dynamic MRI to provide lesion/tissue specific information.

Dynamic MRI

When injected as a paramagnetic bolus, gadolinium containing MRI contrast agents are transiently confined within the vascular space. While in that vascular space they produce magnetic field (B_o) inhomogeneities that result in a decrease in the signal intensity of surrounding tissues. MR sequences can be designed to be sensitive to the vascular phase of contrast medium delivery (so-called T2* or susceptibility-based methods which reflect on tissue perfusion and blood volume). Similarly, sequences sensitive to the presence of contrast medium in the EES reflect on microvessel perfusion, permeability and extracellular leakage space (so-called T1 or relaxivity-based methods). These methods are compared with f-MDCT in Table 1.

Dynamic susceptibility contrast enhanced MRI (DSC-MRI)

Perfusion-weighted images can be obtained with 'bolus-tracking techniques' that monitor the passage of contrast material through a capillary bed[23,24]. The decrease in signal intensity of tissues can be observed with susceptibility-weighted T1 or T2*-weighted sequences, the latter providing greater sensitivity and contrast to perfusion effects. In this context, spin-echo sequences are more sensitive to capillary blood flow compared with gradient-echo sequences, which incorporate signals from larger vessels[25]. The degree of signal loss observed is

dependent on the vascular concentration of the contrast agent and microvessel size[26] and density. The signal to noise ratio (SNR) of images can be improved by using high doses of contrast medium (i.e. 0.2 mmol/kg body weight)[27]. High specification echo-planar capable MRI systems, which allow rapid image acquisition, are required to adequately characterise these effects. Such studies are possible on conventional MRI systems using standard gradient-echo sequences but are limited to fewer slices.

Tracer kinetic principles can be used to provide estimates of relative blood volume (rBV), relative blood flow (rBF) and mean transit time (MTT) derived from the first-pass of contrast agent through the microcirculation[23,24,28] (Fig. 2). These variables are related by the central volume theorem equation (BF = BV/MTT). The most robust parameter that can be derived from the first pass is rBV; this is obtained from the integral of the time series data of the first pass[29]. For extracranial tumours, the time series data are usually fitted to a gamma variate function from which kinetic parameters are obtained. Absolute quantification is not currently possible for the evaluation of visceral tissues and tumours. From a practical perspective, it is not always necessary to quantify T2*-weighted DSC-MRI data in order to obtain insights into the relative distribution of tissue perfusion. Simple subtraction images can be calculated to demonstrate the maximal signal drop, which in turn has been strongly correlated with relative blood flow and volume in tumours[30,31].

Quantitative DSC-MRI is currently most reliable in brain applications as the contrast medium is largely retained within the intravascular space[32]. There is very little data in the literature regarding the use of DSC-MRI outside the brain. Qualitative observations of signal loss observed on DSC-MRI have been reported in preliminary clinical studies to characterise liver, breast and brain
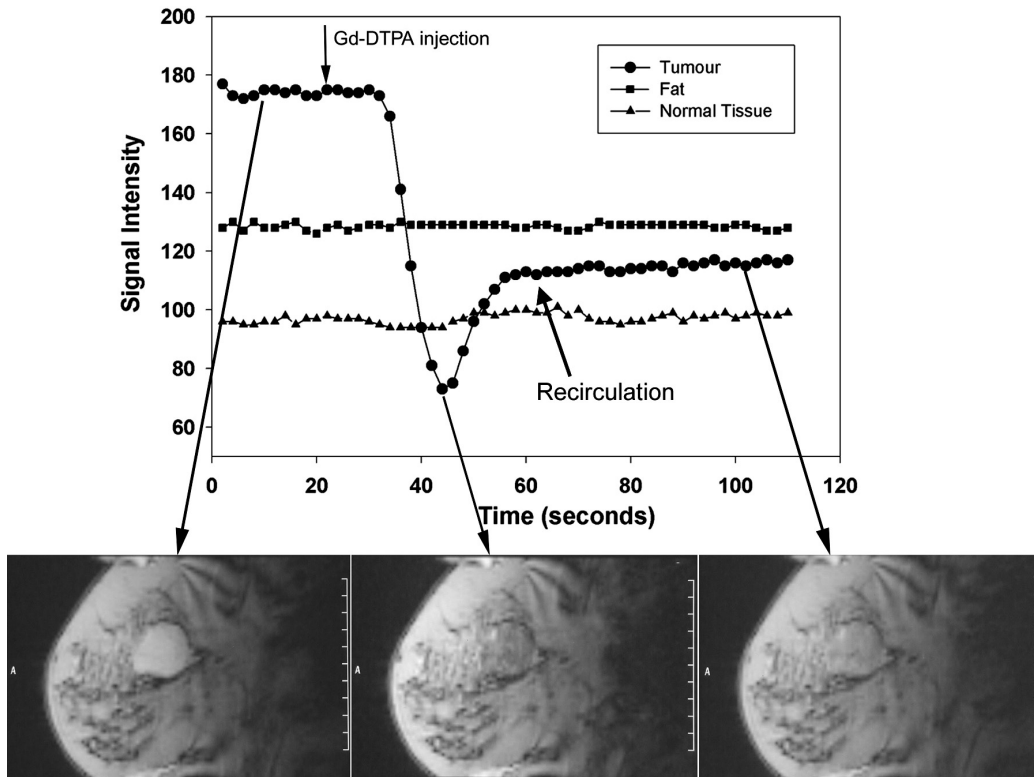
Figure 2   **Typical DSC-MRI study in a patient with breast cancer. Gd-DTPA contrast medium (0.2 mmol/kg body weight) was administered after the 10th baseline data point acquisition. Images were acquired every 2 s. The first pass susceptibility effects cause marked darkening of the tumour with little alteration of the fibro-glandular breast parenchymal tissue. The recirculation phase is not well appreciated. The signal intensity fails to return to baseline because of marked leakage of contrast medium out of the vascular space.**

lesions. For example, Ichikawa *et al.* were able to discriminate between liver metastases, hemangiomas and hepatomas on the basis of characteristic signal intensity changes on echo-planar MRI[33]. Both Kuhl *et al.* and Kvistad *et al.* have qualitatively evaluated the value of DSC-MRI for characterising breast lesions[34,35]. Both studies showed strong signal intensity decreases in malignant tissues with only minor susceptibility effects in fibroadenomas.

T1-weighted dynamic MRI (DCE-MRI)

Most dynamic relaxivity enhanced DCE-MRI studies employ T1-weighted gradient-echo sequences to monitor the tissue enhancing effects of contrast media. This is because gradient-echo sequences have good contrast medium sensitivity, high signal to noise ratio and the data acquisition can be performed rapidly. Unlike f-MDCT, the degree of signal enhancement seen on T1-weighted MRI is dependent on a number of physiological and physical factors. These include tissue perfusion, capillary permeability to contrast agent, extracellular leakage space volume, native T1-relaxation rates of the tissue, contrast agent dose (and its protein binding),

imaging sequence used, imaging parameters utilised and on machine scaling factors.

Signal enhancement seen on a dynamic acquisition of T1-weighted images can be assessed either by analysing signal intensity changes (semi-quantitative) and/or by quantifying contrast agent concentration changes using pharmacokinetic modelling techniques (Fig. 3). Semi-quantitative parameters have the advantage of being relatively straightforward to calculate but have a number of limitations including an inability to accurately reflect contrast medium concentration in the tissue of interest. They can also be influenced by scanner settings. Quantitative methods use pharmacokinetic modelling techniques that are applied to tissue contrast agent concentration changes. Signal intensity changes during dynamic acquisition are used to estimate contrast agent concentration *in vivo*[36,37]. Quantitative parameters are more complicated to derive which deters their use at the workbench. The main advantage of quantification is the ability to directly compare examinations acquired serially in a given patient and in different patients imaged at the same or different scanning sites.

Many studies have attempted to correlated tissue MR enhancement with immuno-histochemical microvessel density (MVD) measurements. Some MRI studies have
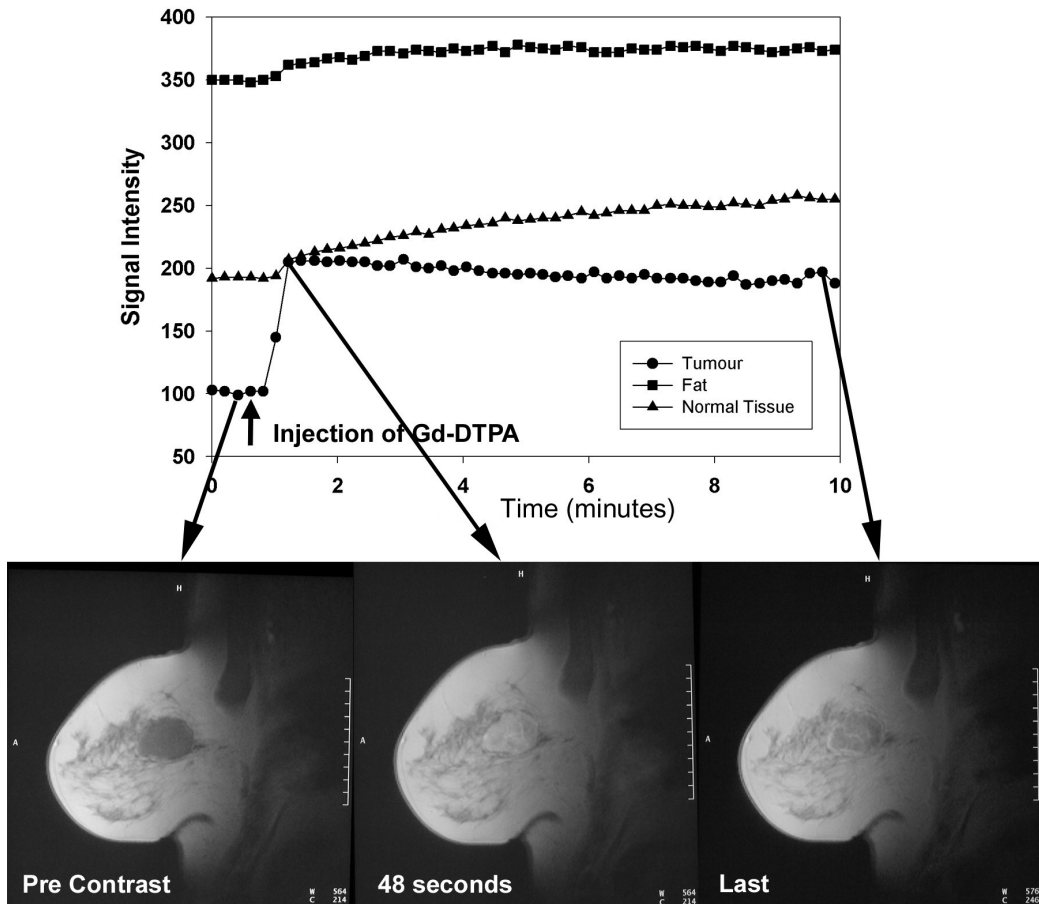
Figure 3   **Typical T1-weighted DCE-MRI study in the same patient shown in Fig. 2. Gd-DTPA contrast medium (0.1 mmol/kg body weight) was administered after the 4th baseline data point acquisition. Images were acquired every 12 s. Marked early enhancement of the breast tumour is seen with washout. The pattern of enhancement is in marked contrast to that observed in Fig. 2.**

shown broad correlations between T1 kinetic parameter estimates and MVD, whereas others have not. Recently, VEGF, which, as noted above, is a potent vascular permeability and angiogenic factor, has been implicated as an additional explanatory factor that determines MR signal enhancement although the relationship between MRI enhancement and tissue VEGF expression is not straightforward. Other characteristics that have been correlated with enhancement patterns include the degree of stromal cellularity and fibrosis and tissue oxygenation (see Padhani and Dzik-Jurasz[2] for a comprehensive review).

Enhancement seen on T1-weighted DCE-MRI is a valuable tool in a number of clinical situations. The most established role is in lesion characterisation where it has found a role in distinguishing benign from malignant breast and musculoskeletal lesions[38,39]. Dynamic T1-weighted MRI studies have also been found to be of value in staging gynaecological malignancies, bladder and prostate cancers[40–43]. DCE-MRI studies have also been found to be of value in detecting tumour relapse in the presence of fibrosis within treated tissues of

the breast and pelvis[44–51]. Recently, DCE-MRI has been shown to be of value for screening women at high genetic risk of breast cancer[52]. DCE-MRI is also able to predict response or monitor the effects of a variety of treatments. These include neoadjuvant chemotherapy in bladder and breast cancers and bone sarcomas[53–57]. Other treatments that can be monitored include radiotherapy in rectal and cervix cancers[58–61] and androgen deprivation in prostate cancer[56]. A number of studies have recently reported on the use of T1-weighted DCE-MRI for monitoring the effects of antiangiogenic/antivascular treatments[62,63]. These response assessment studies show that successful treatment results in a decrease in the rate and magnitude of enhancement and that poor response results in persistent abnormal enhancement.

Conclusions

There is a definite clinical need for non-invasive tumour angiogenesis imaging assays. Ultrasound, f-MDCT,

DSC-MRI and DCE-MRI are currently the favoured techniques for evaluating tumours with respect to their functional microcirculation. Diffusion MRI is another but less validated technique. The choice between CT and MRI is determined by several key factors including local availability and expertise, tumour site, desired perfusion parameter and the need to reduce radiation burden. The widespread availability of MDCT may be a major determinant in future use. To date there have been no comprehensive studies that have compared the performance of functional CT and dynamic MRI in tumour assessment. There are anatomical regions where functional CT is preferable to dynamic MRI, mainly due to the presence of artefacts that would interfere with MRI evaluations. These include the upper abdomen, in particular the root of the visceral vessels, the mediastinum and at the pulmonary hila. On the other hand, for brain examinations, dynamic MRI should be the preferred technique, as the radiation burden from functional CT, particularly for serial examinations, may become unacceptable.

A number of challenges must be met if quantitative imaging of angiogenesis is to enter wider clinical practice. These include the need for commercial equipment manufacturers to provide robust methods for rapidly measuring time-varying changes in tissue contrast agent concentration and robust analysis software with validated statistical tools for the evaluation of heterogeneity. Such developments will be essential for multicentre trials where it will be necessary to establish effective cross-site standardisation of measurements and evaluation. As imaging scientists and clinicians, the radiological community will need to become enthusiastic key players if there is to be successful clinical implementation of angiogenesis imaging.

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