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Functional ultrasound methods in oncological imaging

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Abstract

The real time nature of ultrasound and functional methods such as Doppler ultrasound mean that ultrasound can claim to have always been a functional imaging method, but recent developments in quantitation, dramatic improvement in Doppler performance and now microbubbles have created many exciting new applications. These include methods for assessing the neovascularity of tumours, for following the effects of therapy and for predicting the likelihood of development of metastatic disease at the staging of primary tumours.

Keywords: Neovascularity; Doppler ultrasound; Functional methods; Microbubbles; Metastatic disease

1. Introduction

Ultrasound is both one of the most widely used imaging modalities (one in three imaging tests worldwide are ultrasound scans) [1] and arguably the most rapidly evolving technologically. Whilst the real time nature of ultrasound has always lent itself to functional assessments, recent developments have dramatically improved the quality of conventional and Doppler ultrasound. In addition, new quantitative approaches, three-dimensional (3-D) scanning methods and the increasing availability of microbubble contrast agents, all open exciting new avenues for functional ultrasound imaging. There is emerging evidence that these new methods can give both direct and indirect information on the state of the tumour neovascularity and its response to therapy. This review will provide a brief overview of current technology and discuss some current and emerging clinical applications.

2. Technology

2.1. Doppler ultrasound

Ultrasound, like all sound, can be regarded as a form of energy, transmitted in a medium by periodic longitudinal waves of compression and rarefaction, with a frequency measured in cycles per second or hertz (Hz). Thus, sound cannot be transmitted in a vacuum, and the behaviour of the sound wave depends heavily on the medium. The human ear can hear sounds at frequencies up to 20 kHz: ultrasound refers to any sound at higher frequencies than this, although diagnostic ultrasound is generally performed at frequencies of 1–20 MHz. Pulses of ultrasound are sent out in trains from arrays of transducers ('probes'), and the returning echoes analysed to build up a two-dimensional (2-D) image of the plane being scanned, and displayed using a grey-scale display to code the intensity of the returning echoes. This produces the familiar 'conventional' ultrasound image, usually called B-mode (for brightness), 2-D, or grey-scale ultrasound (Fig. 1).

The power, or intensity, of ultrasound is markedly reduced as it travels through tissue and the rate of this attenuation increases with frequency. Typically, a 10-MHz probe can only be used for superficial structures, a 5-MHz probe penetrates approximately 8 cm, and a 2.5-MHz probe approximately 15 cm, although new methods are improving these figures. Conversely, spatial resolution increases with frequency: in general, therefore, the choice of probe frequency is a balance between detail resolution and depth penetration, with higher frequencies used for more superficial structures.

If sound strikes a moving structure, such as a red blood cell, it will be reflected at a slightly different frequency due to the additive effect of the velocity of the

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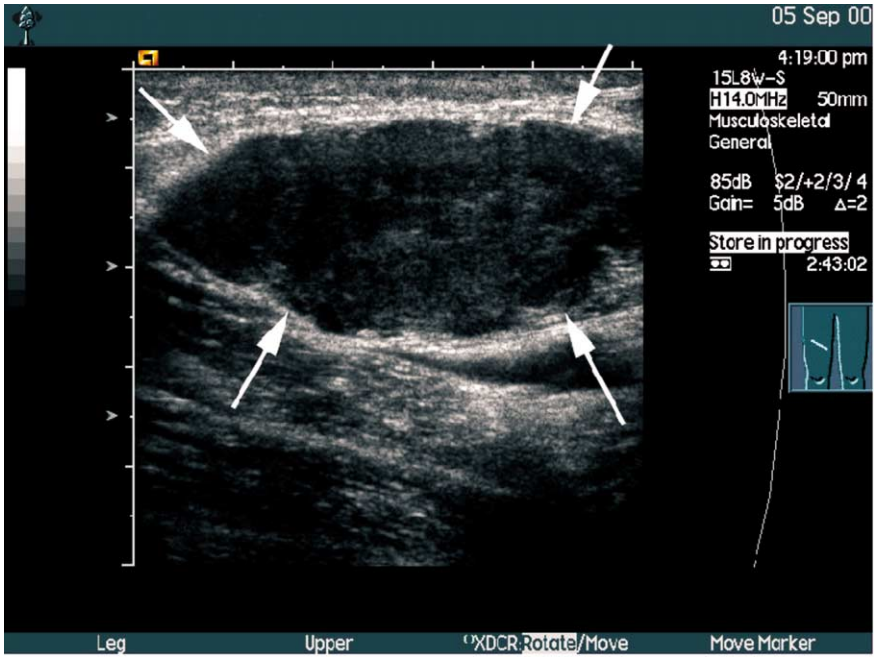


Fig. 1. Grey-scale ultrasound image of a leg sarcoma, indicated by arrows.

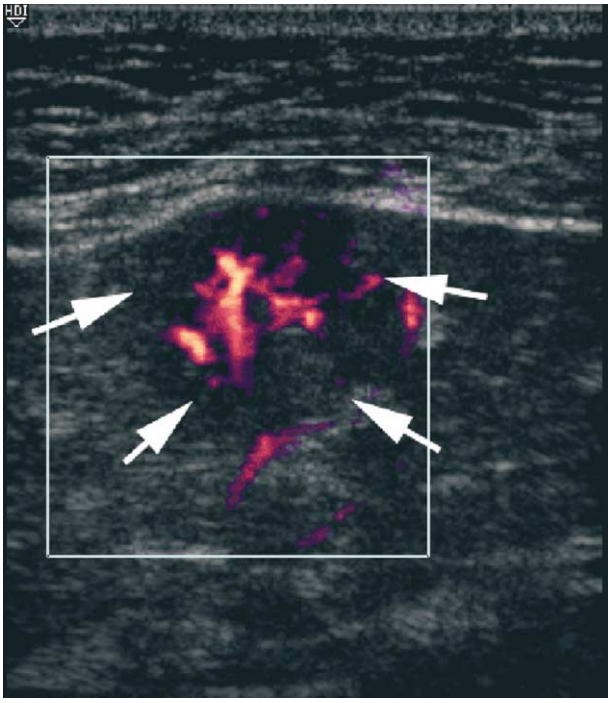


Fig. 2. Power Doppler study showing hypervascularity in a superficial hepatocellular carcinoma in a cirrhotic liver.

reflector along the line of the sound wave. This phenomenon is the same Doppler effect that causes the sound of a whistling train to change pitch as it moves past an observer. The frequency shift can be displayed as a spectral trace, or colour image representing the movement of erythrocytes.

In colour Doppler imaging, frequency shifts from many areas of the image are analysed and displayed as a colour overlay. Typically positive Doppler shift signals, corresponding to flow towards the transducer are displayed at the red end of the spectrum, and flow away from the transducer at the blue end. The amount of vascularity in a lesion and the arrangement of blood vessels may help distinguish some tumours from benign masses [2] (Fig. 2). Power Doppler imaging (also called Energy, Intensity or Amplitude imaging) shows the intensity or 'loudness' of the Doppler signal—as opposed to the frequency shift or 'pitch'—displayed as a colour overlay on the grey-scale image. This is less dependent on flow direction and more sensitive to low flow signals, but gives no information on flow direction or velocity.

2.2. Microbubbles

Ultrasound contrast agents are a relatively recent development in which materials are given to improve imaging [3]. The commonest approach is the use of intravenous injections of small (typically 3 μ) air or gas bubbles ('microbubbles') that boost the Doppler signal from blood vessels. Although developed mainly to improve an undiagnostic Doppler examination or show flow in otherwise undetectable vessels, they have many more promising 'microbubble-specific' applications which are likely to be much more important.

Microbubbles work not only because they provide a strongly reflective blood/gas interface, but also because they resonate in the ultrasound beam, rapidly contracting and expanding in response to the pressure changes

of a sound wave. By a fortunate co-incidence, they vibrate particularly strongly at the high frequencies used for diagnostic ultrasound imaging. This makes them several thousand times more reflective than normal blood. In this way, they enhance both normal grey-scale, and flow-mediated Doppler signals. Although this effect is very useful in itself, the resonance that microbubbles produce has several special properties that can be exploited to improve diagnoses. Just as with a musical instrument, multiple harmonic signals—or overtones—are produced. Ultrasound scanners can be tuned to 'listen' to these harmonics, producing strong preferential imaging of the microbubbles in an image. At higher acoustic powers, although still well within the recommended limits, ultrasound can be used to destroy microbubbles relatively easily. Some microbubbles are tissue-specific (for example, some show tropism for the reticuloendothelial system) and this can also be exploited in a number of emerging applications. For example, many malignant liver lesions stand out as defects when liver specific microbubbles are imaged, and their visibility can be greatly increased.

Microbubbles can help in visualising flow in smaller vessels, but newer microbubbles in particular can be detected at the capillary level, especially when harmonic and other non-linear methods are used to image them.

As with conventional Doppler signals, the changes produced by microbubbles can also be quantified for functional imaging purposes. Quantitation of ultrasound data can be performed offline from the external video and audio output signals produced by the machines, or increasingly from proprietary software provided by the manufacturers. In all cases, corrections need to be made for non-linear machine processing, notably the log compression widely employed in the display of ultrasound data [4,5].

Microbubbles are unique amongst contrast agents in that they can be destroyed by the very act of imaging them, even at low acoustic power settings. This consideration leads to two opposing approaches to functional imaging [5]. The first is to scan with low acoustic powers, and passively observe the enhancement effects produced. A particularly promising method is to quantify the intensity changes in spectral Doppler or power Doppler signals. It has been shown that regional microbubble concentration can indeed be measured by quantifying the intensity of these signals [6,7]. Grey-scale signals can also be quantified, although usually newer harmonic imaging modes are needed to observe useful enhancement.

The alternative to this passive approach is a more recent active approach that seeks to destroy microbubbles with a transient pulse of high acoustic power and then observe refilling into an area of interest during an infusion. One method is to repeatedly pulse ultrasound at varying triggering intervals, waiting for a steady state, and study the relationship between steady state enhancement levels and triggering delay settings [8,9]. The second is to momentarily scan with high acoustic power and then scan using very low power settings, watching the microbubble refilling rates [10]. These reperfusion, or kinetic, methods have the advantage of measuring novel indices which cannot be measured in any other way. In addition, by allowing microbubbles to completely refill a tissue volume without disruption by the ultrasound, they are more sensitive to flow at the level of the microcirculation. In principle, both methods can give information on microcirculatory flow speeds and fractional vascular volume of a tissue. Quantitative flow data can be extracted from regions within the image plane and these results displayed as colour overlays on the original image data (Fig. 3).

A number of microbubbles have been engineered which show specificity for various tissues or targets although at present these are still at the preclinical stage and are mainly focused on vascular as opposed to oncological application. In addition, microbubbles can be used to facilitate drug delivery, both by potentiating the production of transient cell membrane pores by ultrasound ('sonoporation') and by acting as drug delivery vehicles [11,12]. Although this field is an area of increasing academic and commercial interest there is currently little information in the public domain on applications in oncology. It seems likely that it will become an important clinical application of microbubbles in the future.

3. Current and emerging applications

3.1. Doppler methods

Conventional Doppler imaging is able to directly image flow in vessels down to approximately the millimetre level in size. It is thus best seen as a tool for imaging the macrocirculation, rather than the microcirculation. Despite this, it is of considerable utility in tumour imaging, with the simplest approach being a qualitative visual assessment of the Doppler blood flow. Many benign lesions are hypovascular compared with the adjacent tissues, while some (but not all) malignant lesions show hypervascularity. For example, the presence of significant blood flow within a suspicious lesion in tissues such as the breast, prostate or ovary is a useful, although non-specific, marker for malignancy and may heighten concern. The presence of arterial (as opposed to venous) flow is another 'soft', but useful sign in tissues such as the liver [13]. Abnormal morphology of blood vessels, with calibre irregularity or marked tortuosity might also give clues to the malignant nature of a lesion. 3-D imaging may offer advantages here, and this is an area of active research [14].

Another Doppler approach that has been explored extensively in some areas, notably the ovary, breast, uterus and liver, is to study the arterial spectral Doppler waveforms of vessels within or feeding a lesion. The hypothesis underlying this approach is that characteristic features of malignant neovascularity can be detected by the changes produced in feeding vessels. For example, arteriovenous shunting could produce low impedance flow (i.e. a low difference between the systolic peak and diastolic trough) and/or raised peak systolic velocity. Raised microcirculatory resistance might conversely produce a high impedance pattern. Unfortunately, both types of derangement may be present simultaneously in a tumour, which may be one explanation for the sometimes disappointing results seen with this approach. Overall the literature on this subject is conflicting and there has been some disillusionment with this approach, although many authorities still find it useful in defined areas such as ovarian imaging [2].

A third approach is to use higher frequency ultrasound allowing the imaging of progressively smaller vessels. Recently, several manufacturers have developed commercial real time ultrasound systems, using ingenious signal processing methods, which can scan in Doppler modes at between 10 and 20 MHz with good tissue penetration. Such systems can detect flow in submillimetre vessels with relative ease, but it seems unlikely that they will be able to image vessels much smaller than approximately 0.1 mm. The principle has been extended to experimental Doppler systems; able to perform at frequencies in the 20–100 MHz range and these have been shown to be able to detect flow in vessels as small as 15–20 μ [15]. Although of potential value in experimental systems (e.g. superficial murine tumour

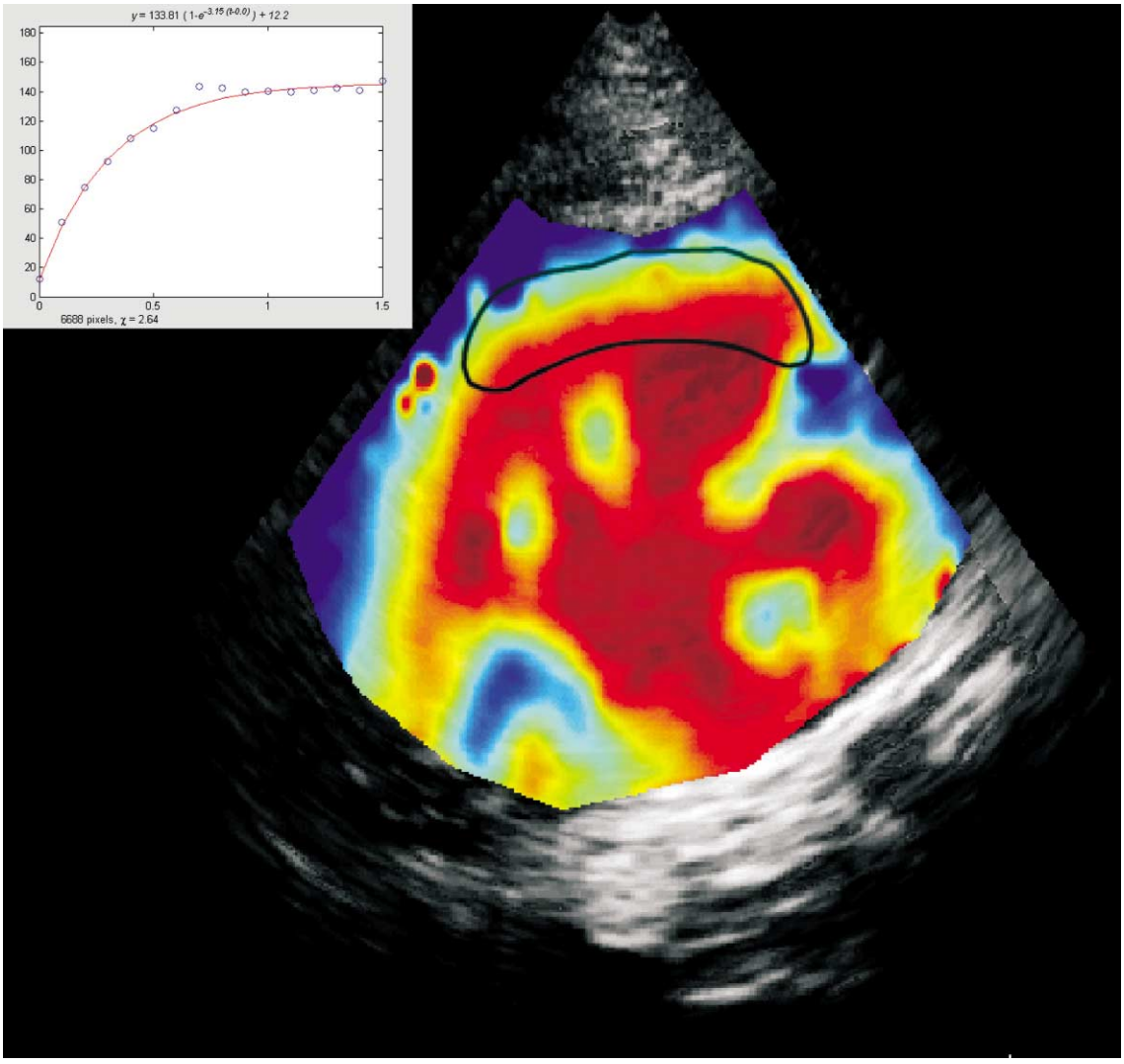
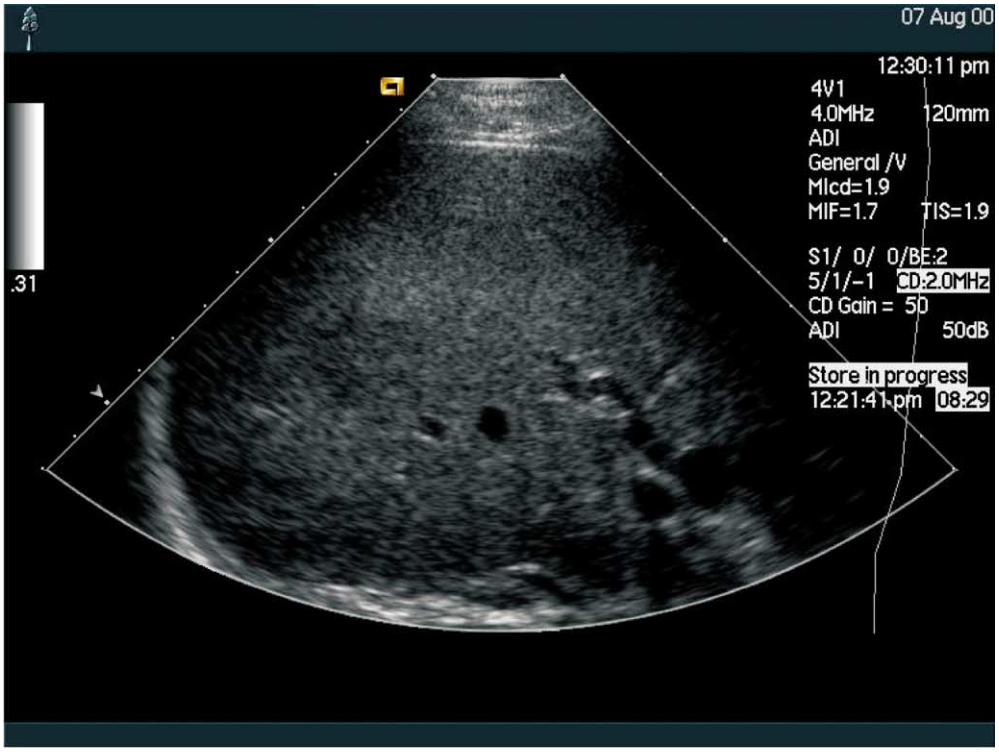
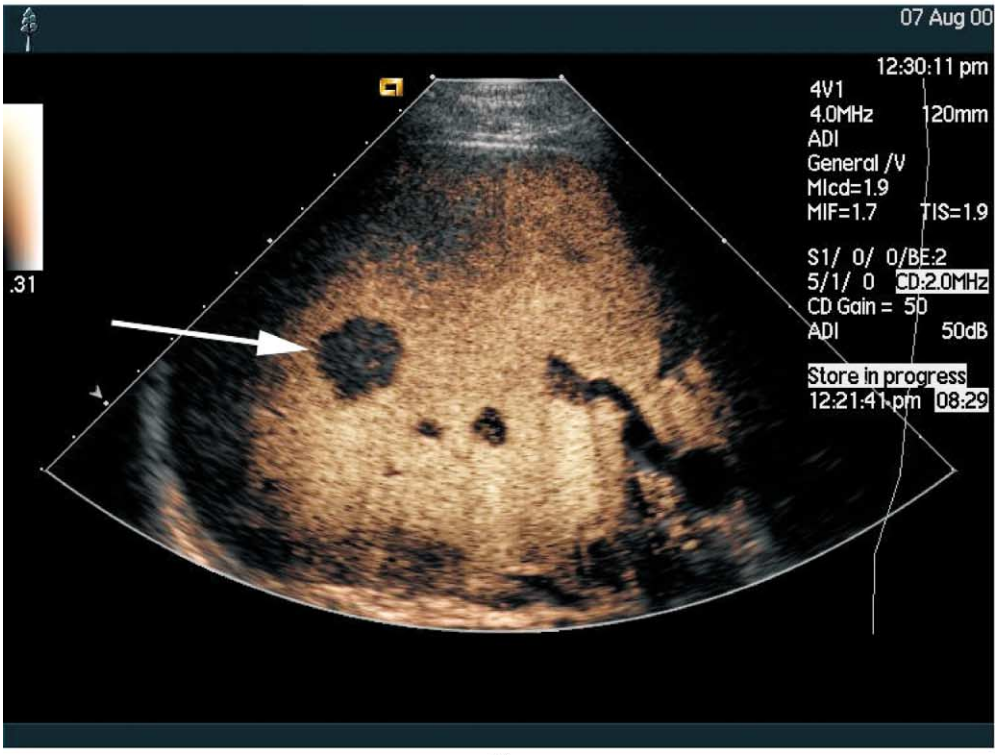


Fig. 3. Parametric image of a transplant kidney showing the relative vascular volume of the tissue over the image plane. This image was obtained using experimental inhouse software that fits a mono-exponential curve to the refill data [8]. Quantitative results from regional analysis can also be extracted (see inset).



(a)



(b)

Fig. 4. (a) Ultrasound of the liver in a case of suspected metastatic disease showed no definite lesion. When microbubble-specific data is overlain (b) using a microbubble which has a reticuloendothelial phase, a metastasis is seen as a defect (arrow). Many benign lesions show uptake and this can be used to distinguish between benign and malignant liver masses.

models), the poor penetration of such biomicroscopic systems, however, means they are unlikely to be of value in human scanning, apart from possibly in skin or ocular applications.

Whatever approach is used, quantification of the Doppler signals obtained is a prerequisite for quantitative functional imaging applications. Although a large number of velocity based measurements (such as peak systolic velocity or pulsatility indices based on relative systolic/diastolic flow) are employed, the most direct measurement should be the Doppler intensity, which is a measure of the relative number of moving red cells and thus of relative fractional vascular volume. Various manufacturer and offline measurement packages have been described or are available, and the interested reader is referred elsewhere for a fuller description [5].

Increased understanding of the role of angiogenesis in malignancy and its treatment has heightened interest in understanding the relationship between Doppler and the microcirculation. Several researchers have studied the relationship between histological indices of angiogenesis (notably microvessel density count (MVD)) and indices derived from Doppler ultrasound. In general, it would appear that tumours showing increasing MVD show higher Doppler-derived indices, but current Doppler methods often perform relatively poorly when directly correlated with measures of tumour angiogenesis indices such as MVD [16]. This is not surprising, given the problem of sampling errors in heterogeneous tumours and the fact that, as described above, conventional Doppler is essentially a tool for imaging the macrocirculation rather than the microcirculation. A much more promising clinical application at the moment appears to be imaging the response of a tumour blood supply to cancer therapy. Estimates based on the fractional vascular volume, a surrogate of quantitative power Doppler signals show particular promise. An encouraging recent study by Gee and colleagues using a murine model evaluated the effect of angiogenesis inhibition with interleukin-12 on a tumour strain, K1735, known to be sensitive and a variant, K1735.N23, engineered to be unresponsive [17]. All K1735 tumours showed declines in power Doppler scores by 3 weeks of therapy, and this was associated with a reduction in vessel density assessed by confocal microscopy. The therapeutically unresponsive K1735.N23 variants showed, by contrast, a consistent increase in Doppler signals. The relative simplicity and ease of use of ultrasound would potentially make this a very appealing method of following cancer therapy in patients. One early study showed that conventional (frequency based) Doppler indices in breast cancers in patients were altered by adjuvant chemotherapy [18]. A more recent multicentre study led by our group evaluated the effect on power Doppler intensitometry on transrectal scanning after therapy with androgen ablation in men with prostate cancer. Marked falls in Doppler scores (both with and without microbubbles) were seen within 1 week, paralleling falls in prostate serum antigen (PSA) [19,20].

Another exciting application, which is showing considerable promise, is predicting distant metastases and survival, especially in colonic cancer. A test which could help to stratify risk of distant disease could inform decisions on the nature of adjuvant chemotherapy and how intensively follow-up monitoring is performed in this common tumour. One approach is to assess the Doppler vascularity of the primary tumour, on the hypothesis that the more vascular tumours are, the more they are likely to metastasise. A recent study by Chen and colleagues showed the colour Doppler vascularity index (CDVI) of the primary tumour helped to predict the outcome to curative resection. Recurrence was significantly more likely in patients with a high CDVI. Surprisingly, the same was not seen with the histological MVD score [21]. An alternative approach to detecting early metastatic disease is to try and detect vascular changes in the liver blood supply, which may indicate a propensity to develop visible metastases at a later date. It has been known since the 1980s that abnormal liver blood flow on staging isotope liver scans ('hepatic perfusion index') helps identify patients at a higher risk of developing metastases [22]. The mechanism is unclear. Although the obvious explanation would be the presence of microscopic liver metastases ('micrometastases'), some researchers claim evidence of an undetermined humoral factor, which both alters liver blood flow and increases the propensity to metastases [23]. Similar results have been shown using a Doppler ultrasound test developed by Edward Leen from Glasgow. This is based on measurement of the relative fraction of liver blood flow which is arterial ('Doppler perfusion index' (DPI) and requires measurement of total flow in the portal vein and hepatic artery. In a large series of patients, Leen and his colleagues have provided impressive data to show that the presence of a raised DPI at preoperative staging predicts which patients will develop metastases with a high accuracy, and is a better predictor of outcome than the Dukes staging or other scores [24]. Unfortunately, the test is hard to perform and, so far, data from other groups on the reproducibility and practical utility of this test is conflicting. It may be that an alternative microbubble-based technique, described in the next section, offers advantages.

3.2. Microbubble methods

A number of investigators have hypothesised that there may be alterations between the way microbubbles traverse the circulation of malignant and benign lesions. Most of the earlier research in this area was been based on studying enhancement in larger vessels, for example,

by scanning in power Doppler mode after an injection of microbubbles. Whilst some studies [25] showed encouraging evidence that longer enhancement times were seen in malignant as opposed to benign breast lesions, this has not, in general, been borne out by other follow-up studies to date, once allowance has been made for the confounding effect of the overall vascularity of a lesion. It is very likely, however, that the use of newer 'active' approaches, such as the reperfusion kinetic method will offer considerable advantages, as they allow capillary imaging and thus the ability to assess tumour neovascularity directly. An encouraging recent study by Halpern and colleagues using transrectal scanning of the prostate showed that the visibility of cancers could be greatly improved by the abnormal enhancement seen using a reperfusion kinetic method [26].

One area, however, where functional methods are moving beyond the research arena and becoming established tools is in the liver. Haemangiomas, which are common incidental 'nuisance' findings on ultrasound, show a characteristic pattern of peripheral globular enhancement with progressive centripetal infilling on angiography, Computed Tomography (CT) and Magnetic Resonance (MR). Similar findings can be seen using microbubble ultrasound, particularly if care is taken to avoid microbubble disruption by using either intermittent/low power scanning or newer more robust microbubbles [27,28]. The use of microbubble ultrasound should produce cost benefits and be more convenient for the patient as well as reduce the need for ionising radiation. Another emerging area is the use of liver-specific microbubbles. These agents (notably the commercial agent Levovist, which has been shown to have a liver-specific phase) can be used not only to improve the detection of liver malignancies, but also to characterise them. If a liver lesion is scanned during the liver phase of Levovist, marked differences can be seen between malignant lesions, which usually appear as a defect (Fig. 4) and most benign lesions (focal fat or fatty sparing, focal nodular hyperplasia and many haemangiomas), which show uptake, presumably because they contain Kupffer cells or show delayed vascular retention in the case of haemangiomas [29].

An analysis of the first-pass kinetics of microbubbles through the liver circulation is also showing great promise as a functional imaging tool. Changes in the intensity (or loudness) of a spectral Doppler signal, known to be proportional to microbubble concentration, can be used as a measure of relative time concentration. If a hepatic vein is scanned after a bolus injection, analysis of the Doppler time–intensity changes gives much useful information. Much earlier enhancement is seen in cirrhosis and malignancy because of vascular shunting and arterialisation of the liver's blood supply [30,31]. This simple technique may be particularly useful in the distinction between diffuse liver disease and cirrhosis, where the imaging findings can be notoriously unhelpful and biopsy is otherwise required. It has been shown that differences are seen between the arrival times in livers with and without metastases, and at our institution a major long-term study led by Chris Harvey is nearing completion to see if this can predict the development of micrometastases. This may provide an alternative to the Doppler Perfusion Index (DPI) approach of Leen and colleagues. Current data, based on a follow-up of over 90 patients, suggests that the presence of 'normal' late arrival times in an apparently normal liver is an extremely reassuring finding (all such patients followed-up remain disease-free). Conversely, all patients to date who have developed metastases on follow-up had abnormal 'early' arrival times at presentation [32].

4. Summary

Ultrasound, despite its popularity, wide availability and low cost has lagged behind other modalities such as MR and Positron Emission Tomography (PET) in the functional assessment of cancer. This is rapidly changing with a dramatic improvement in scanner technology, the availability of quantitative methods and now microbubbles, which can be used as tracers for functional studies. A number of useful functional methods are already starting to find increasing application.

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