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Non-invasive vascular imaging: assessing tumour vascularity

S. Delorme, M. V. Knopp

Department of Oncologic Diagnostics and Therapy, German Cancer Research Centre, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany

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Abstract. Non-invasive assessment of vascularity is a new diagnostic approach to characterise tumours. Vascular assessment is based on the pathophysiology of tumour angiogenesis and its diagnostic implications for tumour biology, prognosis and therapy response. Two current techniques investigating vascular features in addition to morphology are Doppler ultrasonography and contrast-enhanced MRI. Diagnostic differentiation has been shown to be possible with Doppler, and a high degree of observed vascularity could be linked to an aggressive course of the disease. Dynamic MRI using gadolinium chelates is already used clinically to detect and differentiate tumours. The histological correlation shows that capillary permeability is increased in malignant tumours and is the best criterion for differentiation from benign processes. Permeability and perfusion factors seem to be more diagnostic than overall vessel density. New clinical applications are currently being established for therapy monitoring. Further instrumental developments will bring harmonic imaging in Doppler, and faster imaging techniques, higher spatial resolution and novel pharmacokinetic concepts in MRI. Upcoming contrast agents for both Doppler and MRI will further improve estimation of intratumoural blood volume and vascular permeability.

Key words: Doppler – US – MRI – Angiography – Contrast agents – Tumour vascularity

Why image tumour vascularisation?

Recent developments in colour Doppler sonography (CD) and MRI have enabled examinations related to tumour vascularity. Such examinations most frequently are designed to improve differential diagnosis. However, assessment of tumour blood flow for prognostic reasons, therapy monitoring or prediction of therapy success is increasingly gaining attention.

Thus far, no method allows the determination of true blood flow within a tissue volume. The only exception is in the brain, where the blood-brain barrier confines the currently available contrast media to the intravascular space. Therefore, we prefer to use the term "vascularity" rather than "blood flow". The use of the term "perfusion" in the literature appears to be entirely arbitrary and should be avoided in this context.

Basic facts: tumour angiogenesis, tumour blood flow and substrate exchange

During the avascular stage of a tumour, diffusion is the only mechanism of substrate transport to and from the lesion. Unless a vascular supply is established, a tumour will not grow beyond a few cubic millimetres size, where a balance between formation and loss of tumour cells is reached.

Tumours are not capable of generating vessels [1]. Initially, there are only minor, pre-existing vessels encased by the tumour growth, until de novo formation of new vessels by the host is stimulated by production of angiogenic factors. Angiogenesis probably begins in venules where endothelial sprouts develop, which in later steps form a new lumen and gain access to the arterial branch of the capillary bed. Already during this stage, arteriovenous shunts as well as veno-venous connections may develop from interconnected, lower-order branches [2].

Structural abnormalities characteristic for tumour vessels have been reported [3]:

1. Calibre variations with dilated and narrowed segments in a single branch.
2. Elongation and coiling.
3. Non-hierarchical vascular networks, vascular rings and sinusoids.

Correspondence to: S. Delorme

4. The normal, precapillary architecture with dichotomous branching and decreasing size and diameter of the higher-order branches is disturbed.
5. Finally, the vascular wall is incomplete. Almost invariably, there is no muscular layer (except for pre-existing vessels encased by the tumour). But there may also be gaps in the endothelium, an incomplete basal membrane, and thereby a direct tumour-to-blood contact.

The degree of abnormality of the vascular anatomy probably depends on whether structural maturation can keep pace with angiogenesis. An almost normal architecture can be found in highly differentiated tumours, whereas anaplastic tumours may only show a chaotic network of irregular vessel-like spaces without recognisable, mature elements.

Such structural abnormalities cause numerous functional impairments [2]:

1. The transcapillary permeability is increased (up to eight times normal). Mural defects may even be large enough to allow extravasation of red blood cells. Generally, increased permeability causes haemoconcentration and increased viscous flow resistance. A different mechanism appears to be related to vesico-vacuolar organelles. They make substances pass across the endothelial cell itself and may, in addition to mural defects, be involved, for example, in the transcapillary exchange of MRI contrast agents. Vascular endothelial growth factor (VEGF) has been shown in increase vascular permeability [4].
2. In highly vascularised tumours the total vascular cross-sectional area is increased. This will lower the peripheral flow resistance. However, lumen irregularities may regionally increase flow resistance. Along with haemoconcentration and increased interstitial pressure (see below), this may even lead to local stasis.
3. Interstitial pressure is near atmospheric values in normal tissue, whereas in tumours it may reach 50 mm Hg or even more. The main factors attributed to interstitial hypertension are increased vascular permeability and lack of lymphatic drainage. Furthermore, the interstitial space is usually three to five times larger than normal. High interstitial pressure leads to compression of vessels inside the tumour.
4. Where arteriovenous shunts develop, a fraction of blood will bypass the capillary bed. Consequently, despite a low global flow resistance and a high global blood flow, a variable proportion of the tumour may be deprived of its blood supply. Shunt fractions are estimated to range between 8 and 43 %. Tissue with only venovenous flow will be hypoxic due to slow flow velocity and low oxygen saturation of the supplied blood.

The effect of such functional mechanisms is not constant throughout the tumour but highly inconstant, spatially and temporally. There may be stasis in one part and maintained flow in another part of the lesion. It is estimated that only between 20 and 80 % of the tumour vessels are actually patent. Considering also local factors, such as invasion by tumour cells, or variations in intercapillary distance, it is not surprising that vessel density and blood flow can differ between tumours by a factor of 100, and even inside a tumour by a factor of 55 [2]. Even information on global blood flow and flow resistance may be of limited relevance regarding local oxygen and nutrient supply.

Numerous angiogenic factors have been identified. They appear not only to influence growth of the primary tumour but (systemically distributed) also of distant metastases, by means of a complex regulatory systems of angiogenic and anti-angiogenic factors. Angiogenic factors may be either released by the tumour cells themselves, or by host cells recruited by the tumour. They may also possibly be mobilized from the extracellular matrix [1, 5]. Factors known to stimulate the release of angiogenic factors are, for example, hypoxia (from macrophages) [6], or a p53 tumour suppressor gene defect (via a deficiency of the antiangiogenic factor thrombospondin-1) [7]. Increased levels of the angiogenic basic fibroblast growth factor (bFGF) have been found in the serum of children with brain tumours or men with prostate carcinoma [8, 9].

Prediction of therapy response

Despite unpredictable variations in the local supply, blood flow is essential for tissue oxygenation and local delivery of cytotoxic agents. The radiosensitising effect of oxygen is mainly attributed to free radical formation induced by radiation. The role of oxygen for radiosensitivity has been challenged, objecting that hypoxic states like those used in the related studies are rarely found in vivo. According to Vaupel, the radiosensitivity with 100 mm Hg is only 2.8 times the sensitivity with 0.1 mm Hg. Median partial pressures measured in tumours range between 5 and 20 mmHg [2]. However, these are "macroscopic" measurements, commonly using an Eppendorf probe. Values found in cellular dimensions are not known thus far.

Differential diagnosis

Many researchers may not realize that they already apply vascularisation-dependent criteria in daily routine for differential diagnostic purposes. Enhancement patterns or kinetics are diagnostic criteria, e. g. in CT of liver lesions. They permit diagnosis of haemangioma, focal nodal hyperplasia, as well as primary and secondary malignant lesions with high confidence. With angiography, arteriovenous shunts may be pathognomonic, as in, for example, glioblastomas. Unlike other imaging methods, angiography permits excellent assessment of vessel morphology, showing calibre variations, coiling or sinusoids. However, the clinical use of catheter angiography has been restricted mainly to operation planning and interventional procedures, owing to the good performance of CT, MRI or US, and the availability of imaging-directed biopsy.

Current efforts are mainly directed to extend vascularity-related diagnostics towards methods which are

less invasive, and either more potent or flexible (e. g. MRI), or cheaper, more widely available and versatile (e. g. colour Doppler sonography).

Prognosis

According to histomorphometrical studies of tumour vasculature, the microvessel density currently appears to be a major factor determining the aggressiveness of the disease. With highly vascular tumours, distant metastases occur significantly earlier and more frequently than in poorly vascularised ones, independent of other risk factors [10–20]. Not only is neovascularisation necessary to enable tumour cells to be shed into the blood stream, but also distant metastases need angiogenesis if they are to grow beyond a clinically undetectable size [17]. The most supported hypothesis is that both the primary tumour and distant metastases are involved in a complex regulation by angiogenic and antiangiogenic factors. In the most simple case, metastases inherit the angiogenic properties of the primary tumour. They may, however, also lose the capability of angiogenesis and entirely depend on systemically distributed angiogenic factors produced by the primary tumour. Conversely, they may remain dormant under the *anti*angiogenic control by the primary tumour. These are models to explain surprising clinical courses after removal of the primary tumour, like the disappearance of overt metastatic disease, or rapidly progressive distant metastases of a primary lesion which itself did not appear to be particularly aggressive [5]. Only a subfraction of the tumour cells appears to be angiogenic. It is thus far unknown what triggers a cell to switch to the angiogenic state.

Therapy monitoring

Changes of tumour vascularity under chemotherapy are not entirely understood. All that is known is that they are gone once the tumour has been successfully treated. The effects of radiotherapy on vessels and circulation are much better known. In the acute stage, ionising radiation causes an increase in transcapillary permeability by damaging the mucopolysaccharide cement substance and by histamine and serotonin liberation [21]. Later effects are obliteration of capillaries due to progressive damage to the endothelium and the basal membrane [22, 23], resulting in an overall reduction in vascular density [24]. Whether this constitutes an epiphenomenon or plays an important role in tumour response is still under discussion [25, 26].

Aspects of tumour vascularity

There are different aspects of tumour vascularity which are best imaged with one or the other modality:

1. The *overall volume flow* in a tumour is the most difficult to measure. Such measurements would have to be performed over the lesion itself, unless it is supplied over a single vascular pedicle (which is only rarely the case). Thus far, no method has been proven to measure overall blood flow in vivo and non-invasively inside a grossly amorphous volume.
2. The *presence of blood flow and its velocity* are registered with Doppler sonography, if the related vessels are large enough. Doppler also permits estimation of the intratumoural flow resistance, which may reflect interstitial pressure.
3. *Microvascular permeability* is a major factor affecting the distribution of contrast media in the extracellular space. Together with the arrival via supplying vessels, extravasation is probably a major factor contributing to contrast enhancement with CT or MRI. Owing to radiation dose restrictions, *dynamic* enhancement measurements are limited to MRI. Pharmacokinetic models have been developed to extract the components of intravascular delivery and transcapillary permeation from a complex signal-time curve. However, pathophysiological basis of these models remains to be confirmed.
4. Finally, there are *architectural criteria,* best depicted with angiography, but increasingly well depicted with MR angiography. With colour Doppler, architectural features can be assessed only by the highly experienced examiner, but they are difficult to reproduce and hard to communicate. There are attempts to improve documentation of tumour vessel architecture using three-dimensional Doppler reconstruction programs, but they are still in a prototype stage.

Methods available for assessing tumour vascularity in the patient

Doppler sonography

Methodological considerations

Despite some reports that the presence or absence of detectable flow could help to reliably differentiate benign from malignant lesions [27], it is now clear that not only malignant, but also some benign, tumours show detectable Doppler signals [28–37]. Consequently, some method of quantification needs to be applied.

Early studies have been carried out using continuous wave (CW) Doppler [28, 38, 39]. The use of colour Doppler (CD) in contemporary examinations is mainly directed at detecting vessels and determining their spatial orientation. The ultimately evaluated measurements are obtained with pulsed spectral Doppler to measure flow velocities. Approaches at a quantification of the CD information itself are only possible using computer-assisted image analysis methods [32, 33, 40–42]. Still, such quantification of the findings is subject to potential flaws owing to the Doppler angle, aliasing, interpolation algorithms as well as spatial and temporal irregularities of tumour blood flow. The advantage of quantitative colour Doppler methods is that the influence of the examiner is limited to slice and frame selection. Also,

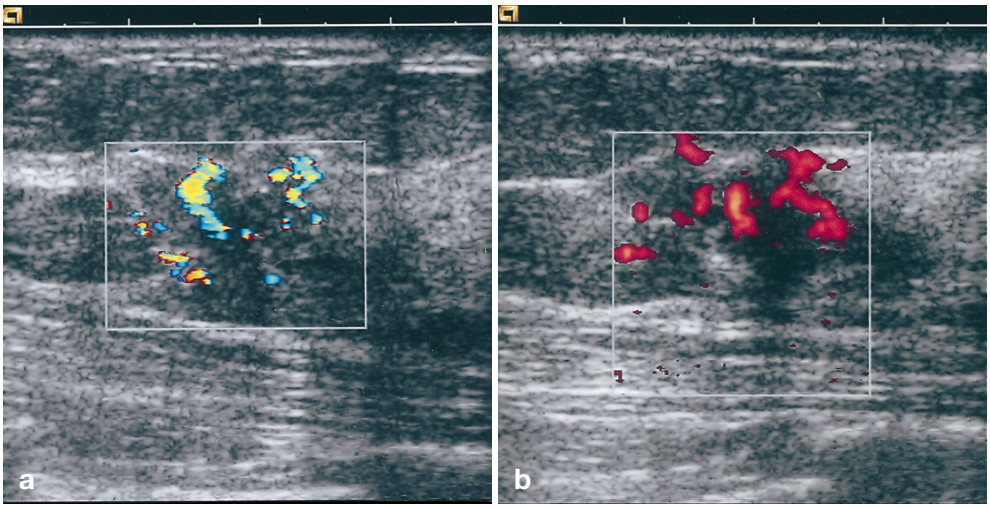

Fig. 1 a, b. Invasive ductal breast carcinoma. Colour Doppler shows irregularly arranged vessels. **a** Colours encode flow velocities and flow directions but may be flawed by aliasing if sensitive settings are used. **b** With amplitude encoding Doppler, flow velocities are neglected, the image only showing local Doppler signal amplitudes, independent of flow velocity or vessel orientation. Whether conventional or amplitude encoding colour Doppler is more sensitive appears to be different from platform to platform

quantitative Doppler enables a "global" assessment of vascularity (i. e. flow velocities and vessel density) for an entire tumour cross section. In comparison, spectral Doppler quantification is physically more precise but is limited to a few intratumoural vessels, the selection of which is arbitrary.

Despite recent technological advances, Doppler sonography has limited sensitivity for recognition of slow flow. This is not entirely a technical problem. Under routine conditions, flow velocities under 1 cm/s are difficult to detect, owing to artefacts ("clutter") caused by tissue movement, which only to some degree are suppressed by motion discrimination algorithms. Therefore, capillary blood flow thus far remains undetectable with available Doppler methods.

Results

Most studies have been carried out in breast tumours or suspicious lymph nodes, since these lesions are easily accessible and can be examined using higher transmit frequencies. They almost entirely address differential diagnostic issues.

There is general agreement that Doppler signals are found in the majority of malignant tumours and are only seen in a few benign breast lesions (Fig. 1). Furthermore, flow velocities (or Doppler shifts, respectively) are higher in cancer than in benign lesions (fibroadenomas, mastopathy, inflammatory lesions, papillomas, etc.). Analysis of colour Doppler images reveals that carcinomas have more colour, indicative of higher vessel density, and that the colour hues of cancer encode for higher flow velocities [32, 33]. However, the sensitivity and specificity reported in the literature diverge considerably (Table 1), probably owing to the wide variety of the methods used, or to the evaluation of results. Criteria commonly used to evaluate results include presence or absence of Doppler signals, flow velocities, Doppler shifts, acoustical properties of the CW Doppler signal, resistance indices, as well as parameters from image analysis. As a rule, up to 10 % of malignant breast tumours show no or only weak Doppler signals. Practically, the lack of detectable flow does not necessarily mean that the lesion is benign. Also, since weak signals may appear in benign lesions, blood flow shown with Doppler is not always indicative of cancer.

The role of peripheral flow resistance in the diagnosis of cancer is controversial and depends on the organ examined. With regard to breast tumours, some report a decreased resistance due to arteriovenous shunts and lack of a muscular layer in the tumour vasculature [43, 44], whereas others report increased resistance due to increased interstitial pressure [35, 45]. The results reported by Burns et al. [28] and Huber et al. [32] indicate that the measurement of resistance or pulsatility indices of tumour vessels fails to be helpful in diagnosis of breast cancer, and should be abandoned. For lymph nodes there is agreement that increased pulsatility and resistive indices (probably owing to interstitial hypertension) may be observed far beyond values found in reactive nodes [46–48]. In our experience, however, particularly small metastatic nodes may well show normal values with Doppler sonography, whereas such with abnormal measurements often uppear unambiguously malignant according to their B-mode features (diameter, shape, internal echostructure). Thus far, no study has shown that Doppler sonography might valuably add to B-mode sonography. To summarize, Doppler sonography may indeed increase diagnostic confidence but will never obviate biopsy. All available results rely on retrospective studies. Prospective evaluations will be necessary to ultimately define the diagnostic value of the procedure.

One recent study was related to *therapy monitoring.* Kedar et al. [49] showed that during preoperative chemotherapy, a reduction in Doppler signals occurred in 77 % of patients who had a partial or complete remission. In 40 % of these, the Doppler changes appeared 4 weeks before a size reduction was detectable using B-mode ultrasonography [49]. At our centre, patients with head and neck cancer have been monitored during radiochemotherapy using quantitative CD image analy-

Table 1. Available studies on Doppler sonography in breast tumours

Reference	Year	Method	Transducer frequency (MHz)	Criteria	*N*	Sensitivity (%)	Specificity (%)
[28]	1982	CW Doppler	10	Acoustical properties	404	73–96	73–96
[39]	1988	CW Doppler	10	Maximal systolic frequency shift	105	Overlap precluding any diagnostic use	
[27]	1988	Duplex Doppler	5	Presence or absence of signals	38	100	100
[83]	1990	Duplex Doppler	7.5	Presence or absence of signals	50	91	89
[30]	1990	Colour Doppler	5	Presence or absence of signals	59	95	97
[84]	1992	Colour Doppler	5	Presence or absence of signals	53	78	100
[43]	1992	CW, Duplex and colour Doppler	Not stated	Resistance index (RI) in the affected breast, less than 80% of the RI in the unaffected breast	83	96	100
[29]	1993	Colour Doppler	5	Presence or absence of signals	210	98	96
[85]	1993	Colour Doppler	5	Presence or absence of signals	37 (carcinomas only)	89	
[32]	1994	Colour Doppler	5	Image analysis parameters	57	64–92	78–91
		Duplex Doppler	5	Maximal systolic flow velocity	55	60	70
[33]	1995	Colour Doppler	7	Image analysis parameters	74	100	100

sis (see above). We found that colour features related to flow velocities (i.e. the colour hues) shifted towards lower velocities, but that the spatial density of detected vessels did not change (unpublished results). Thus far, this remains an isolated observation which deserves to be further pursued in order to become interpretable.

Prognostic implications remain nearly uninvestigated. One small study showed a possible association with other risk factors [50]. However, this study lacked quantitation of Doppler findings, using only descriptive criteria, and did not assess actual patient survival. Studies from this institution in patients with head and neck cancer treated with combined, accelerated radiochemotherapy showed that high vascularity in lymph node metastases, as determined with CD image analysis, is indeed associated with a significantly shorter survival [51]: in the patient group with a colour pixel density below median, the median survival was 958 days; in the group with a higher amount of colour, it was 423 days. The time to detection of distant metastases was 18 months with a small, and 6 months with a high, colour pixel density. However, the time to local or nodal progression was not different between the groups. Interestingly, the Doppler findings did not predict the initial treatment response.

Magnetic resonance imaging

Methodological considerations

Magnetic resonance imaging has evolved tremendously in the past 5 years with respect to non-invasive vascular imaging. There are two main areas of development, contrast-enhanced three-dimensional magnetic resonance angiography (3D MRA) [52] of the vascular system and contrast-enhanced dynamic studies (dMRI) of tumours to assess their microcirculation [53].

Magnetic resonance angiography

While non-enhanced MR angiography (MRA) is a truly non-invasive imaging technique, its clinical application has been hampered by long acquisition times, sensitivity to motion artefacts and limited spatial resolution. The introduction of contrast-enhanced rapid 3D MRA has made this technique applicable for the macroscopic assessment of the vascular system supplying a tumour. Although the spatial resolution is still limited compared with digital subtraction X-ray angiography (DSA), it is already sufficient for the majority of clinical situations. Vessel diameters up to 4 mm can be reliably imaged. With the introduction of ultrafast gradient systems, rapid sequential imaging within a single breathhold can be achieved. This multiphasic angiography allows a sequential imaging of the arterial and venous system [54].

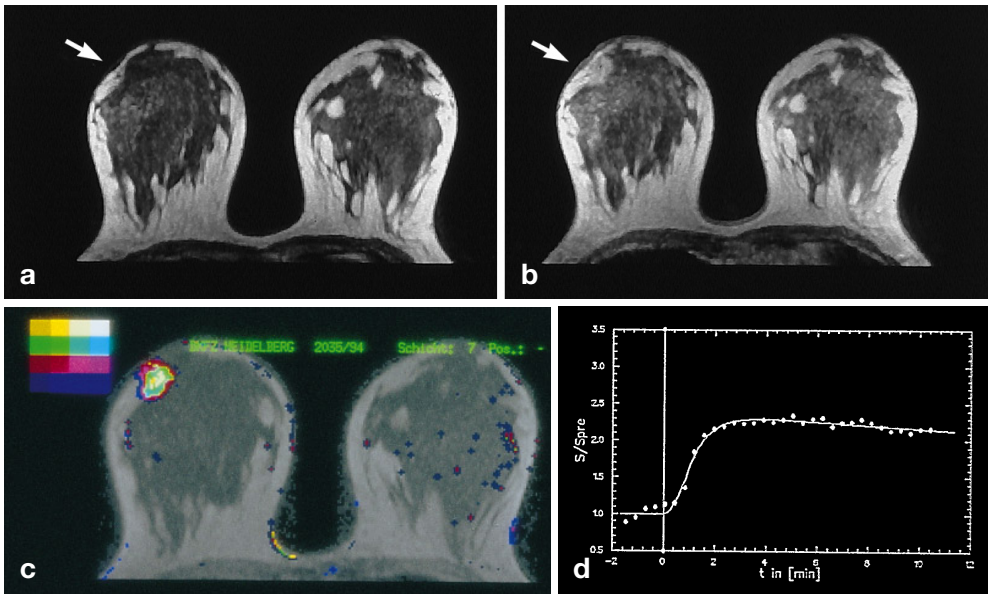

Fig. 2 a–d. Invasive ductal carcinoma in the right upper outer quadrant *(arrow)*. The tumour cannot be detected in **a** the precontrast image and shows an intense enhancement on **b** the postcontrast image. **c** The pharmacokinetic, colour-coded map visualises the features of the contrast enhancement. **d** The time-intensity curve reveals the characteristic finding of intense enhancement, rapid rise and subsequent washout

These recent developments are currently integrated into imaging procedures designed for tumour staging and preoperative evaluation. In addition to morphological features, functional flow parameters can be determined by phase contrast angiography [55, 56]. Although this method is highly accurate, its clinical application is currently limited to vessels of 3 mm or larger.

Dynamic studies with MRI

Current MRI contrast agents are based on gadolinium chelates and pass from the intravascular space to the interstitium, thereby causing parenchymal enhancement. Malignant tumours frequently reveal higher enhancement with these extracellular contrast agents than does the normal surrounding parenchyma. Whereas this feature has been the diagnostic basis for the use of contrast agents, the involved pathophysiological processes and their potential diagnostic information have been largely neglected. With the introduction of fast sequential imaging techniques in MRI, the necessary tools to assess the kinetic enhancement patterns in tumours have become available [57]. For reliable assessment by MRI, an optimised sequence is required in order to use the relative changes of signal intensity as a parameter of contrast enhancement [58]. Furthermore, the temporal resolution of a dynamic sequence as well as the speed of contrast media infusion is relevant for detecting characteristic changes in the enhancement pattern. If those aspects are addressed, tumours with an active neoangiogenesis enhance rapidly, with an early peak followed by a subsequent washout (Fig. 2). Dynamic multislice studies allow a detailed analysis of tumour enhancement but generate a vast number of images. To facilitate diagnostic assessment, data reduction techniques should be implemented for visualisation such as the use of pharmacokinetic parametrisation.

Dynamic imaging based on $T2^*$ effects can also be used for assessment of microcirculation. Analysing the changes in signal intensity with respect to an input function obtained from a major supplying vessel enables a quantitative assessment of absolute blood flow [59]. Compared with T1-based sequences, this method *directly* measures relative blood volume. However, its use is restricted to the brain with its intact blood-brain barrier until purely intravascular contrast agents become available.

Results

Non-enhanced MR angiographic techniques have been established as valid diagnostic methods to assess vascular changes caused by tumours in the brain or extremities, for example. A more faster and better visualisation of the vasculature itself can be achieved using contrast-enhanced 3D angiography. Its application for tumour assessment is currently under evaluation and is promising (Fig. 3).

Already in clinical use is the dynamic assessment of contrast enhancement [60]. Evaluation of breast cancer and other lesions have greatly improved our understanding. Correlative MRI studies and histopathological analysis (e. g. breast tumours, brain tumours, cervical cancer) [53, 61–64] have established that the main factors determining the enhancement pattern of tumours are:

1. The vessel density within a tumour
2. The permeability for micro- and macromolecules
3. The extent of the extracellular space.

Analysing those features has led to a new pathophysiological concept of enhancement in tumours demonstrated in breast lesions. The vascular density in malignant tumours is higher than in normal parenchyma, but there

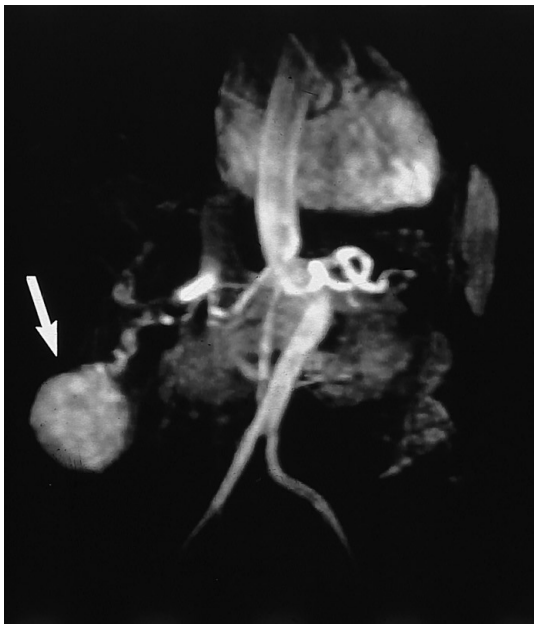

Fig. 3. Contrast-enhanced 3D magnetic resonance angiography of the abdomen in late arterial phase shows the vascular system and intense nodular enhancement in a hepatocellular carcinoma *(arrow)*. Note the clear visualisation of the intrahepatic feeding artery

is a great overlap with benign lesions. Inflammatory and proliferative processes as well as epithelial hyperplasia also lead to increased vascular density. Although vascular density is the major factor contributing to the overall intensity of enhancement, the latter does not help for differential diagnosis. However, significant differences with regard to the transcapillary exchange rate of the contrast agent (according to pharmacokinetic models) was noted not only between malignant and benign lesions, but also between invasive ductal and lobular carcinomas, or subtypes of fibroadenomas, respectively (Fig. 4) [65].

Unfortunately, in situ carcinoma (DCIS) frequently escapes detection by MR mammography. This is not surprising considering that detectable neoangiogenesis begins when in situ carcinomas progress to invasive cancers.

Thus far, dynamic MRI allows a non-invasive assessment of microcirculation. The detectable enhancement patterns are directly influenced by the vascular density and permeability. These features can be used to assess changes in microcirculation during therapy [66]. Therapy monitoring with MRI has been done during chemotherapy in breast cancer, osteosarcomas and brain tumours. Changes in contrast enhancement were observed prior to changes in tumour volume (Fig. 5). Currently, no data are available as to whether changes during therapy occur first in the metabolism (detectable by FDG PET or MR spectroscopy), in the microcirculation, or whether they are both interdependent. According to the available data, alterations of the transcapillary exchange rate seem to be more indicative of therapy response than are changes in absolute intensity [67]. Under therapy, a decline in vascular permeability appears to occur before regression of vessel density. These findings are important to understand potential differences between the accessible diagnostic information from Doppler sonography compared with dynamic MRI.

Prognostic implications of dynamic MRI are already actively investigated in different tumour entities such as cervical cancer, where pharmacokinetic parameters relating to the exchange rate seem to be better diagnostic criteria with respect to patient survival than the histopathological assessment of vascular density [68]. However, these preliminary observations still need to be prospectively evaluated in larger populations.

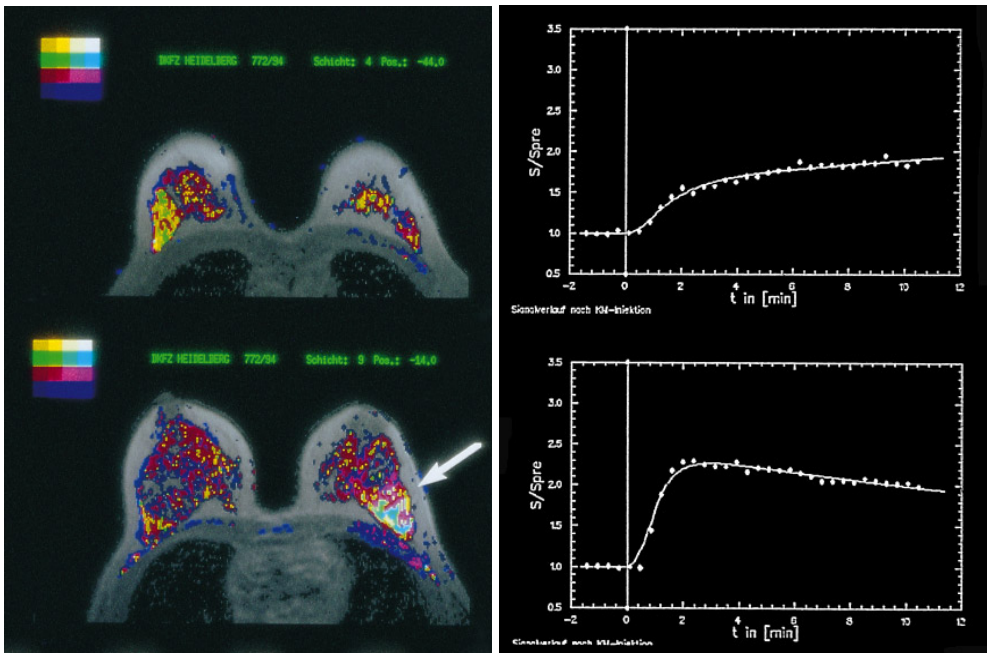

Fig. 4. Patient with proliferative mastopathy and a large, invasive ductal carcinoma in the left upper outer quadrant *(arrow)*. The time-intensity curves reveal characteristic features, a continuous, moderate rise in intensity over time in the proliferative mastopathy, whereas the carcinoma present a rapid, intense rise with a maximum and a subsequent decrease

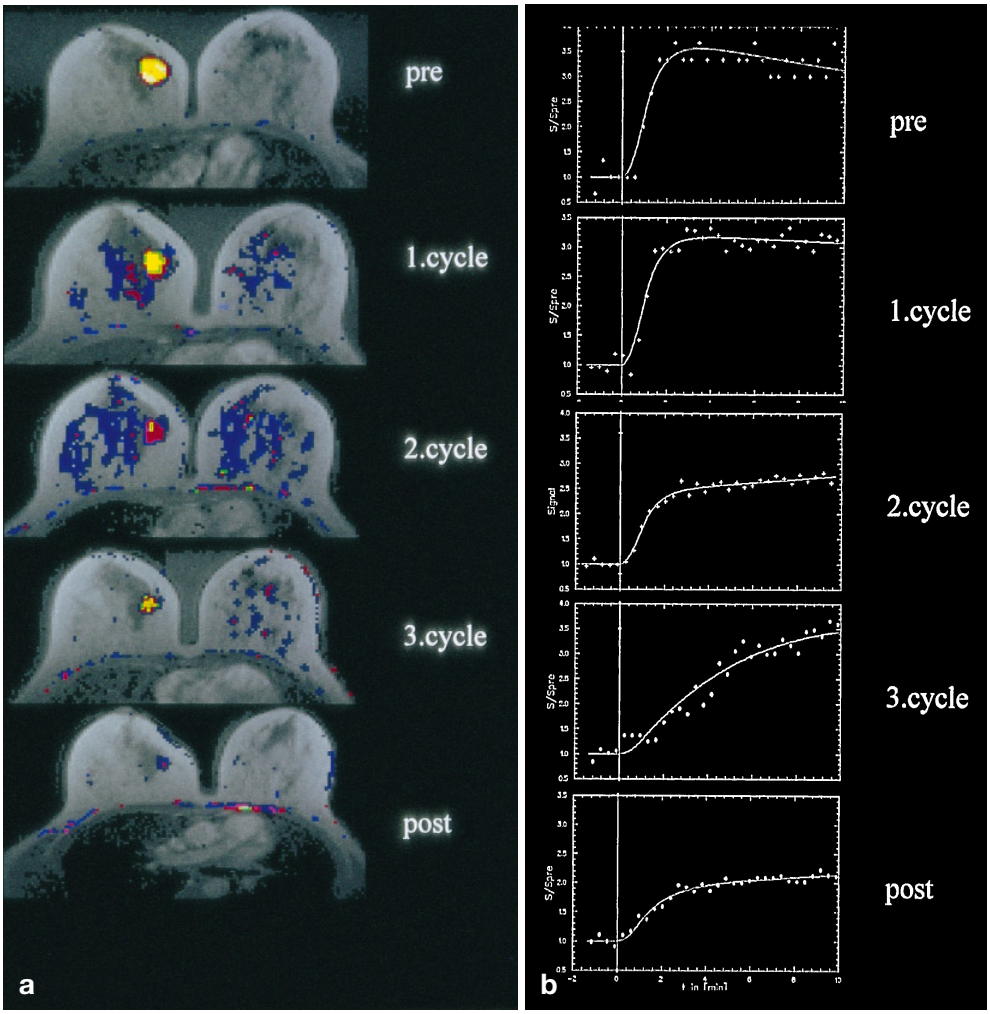

Fig. 5 a, b. Patient with invasive ductal carcinoma receiving neoadjuvant chemotherapy monitored by functional MR mammography. **a** During therapy, a decrease in volume can be readily recognised. **b** The pharmacokinetic maps and the time-intensity curves reveal a slower enhancement coinciding with response to therapy

Perspectives

Upcoming Doppler techniques

Due to the low amplitude of the Doppler signal, examinations of tumour vascularisation are thus far limited to superficial lesions only. Recent technical improvements of instrumentation with higher signal-to-noise ratio as well as the advent of echo-enhancing substances ("ultrasound contrast agents") do certainly extend the applicability of Doppler [69–72]. Whether this will result in real diagnostic improvement remains to be determined [73, 86]. However, with regard to quantitative aspects, Doppler is neither yet a tool to estimate tumour blood flow, nor does it appear to reflect microvessel density [74]. With available techniques, only blood flow in comparably large vessels is depicted, but not in the capillaries. Echo-enhancing substances, even in combination with harmonic imaging [75], will at least partially shift the detection threshold towards slower and lower volume flow. Under experimental conditions this might get close to microvascular flow [76], but under clinical conditions, as far as we can extract from personal communications, capillary flow will remain unexplorable terrain. Justified hopes are placed upon newer-generation echo enhancers which upon insonation produce "stimulated acoustic emissions" which can be recorded with any conventional CD system. This now is a signal which is solely dependent on the *presence* of the substance, but no more on its velocity or on vessel calibre. Shortly after injection, it acts as a blood pool agent, whereas later it is stored in the reticulo-endothelial system and may thereby serve as a liver-specific contrast agent [77].

Magnetic resonance imaging

Technical improvements with more rapid acquisition and higher spatial resolution continue to open new frontiers in MRI, e. g. leading to diagnostic information comparable to X-ray angiography. Additionally, we expect phase-contrast flow measurements to be available not only for macroscopic vessels, but also for major tumour vessels. However, the main diagnostic potential of MRI will rely on its ability to detect differences in vascular permeability. Improved quantitative concepts using pharmacokinetic models, parameterising the dynamic information, will allow standardised visualisation and facilitate clinical communication. Novel contrast

agents with different pharmacokinetic properties (e. g. reduced transcapillary permeability) will further potentiate the diagnostic applications of MRI.

As our understanding of neoangiogenesis for diagnostic and prognostic characterisation continues to grow, MRI with its inherent quantitative potential will provide clinically relevant findings. The applications outlined above mark the beginning of new concepts in imaging, not limited to morphology but also dedicated to biological properties for tumour characterisation, assessment of prognosis and therapy response.

Monitoring of antiangiogenic therapy

Considering the role of angiogenesis in tumour growth and spread, agents directed either against de novo formed vessels ("antineovascular therapy" or against the angiogenic process ifself ("antiangiogenic therapy") are potentially powerful strategies to eradicate or at least control a tumour [78, 79]. In adults, constant neoangiogenesis is peculiar to tumours or (to a lesser degree) chronic inflammatory processes; temporary angiogenesis mainly occurs with wound healing and is of limited character. Therefore, antiangiogenic therapy can be expected to cause only few side effects. Successful trials have been conducted using interferon-alpha for life-threatening haemangiomas in children [80, 81]. Suramine is evaluated in patients with prostate cancer [82]. In human tumour xenografts transplanted to mice, a remission to microscopic dimensions has been achieved with angiostatin, a potent angiogenesis inhibitor [9]. Monitoring such therapy approaches is an upcoming application field for both Doppler sonography and MRI.

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