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Perfusion studies in senology

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KEYWORDS
Breast; Functional MRI; Cancer; Microcirculation; Histological prognosis

Abstract  Microcirculation imaging in breast cancer involves studying tissue enhancement after contrast injection, which is used to calculate perfusion and permeability. The magnitude of enhancement reflects blood and interstitial volumes. This technique has benefitted from advances in MRI, which allow large volumes to be acquired with a good compromise between temporal and spatial resolution. Software has also advanced enabling microcirculation maps to be calculated and heterogeneity to be analyzed. If permeability is increased and interstitial volume is reduced, the microcirculation imaging suggests a suspicious aggressive lesion and can be used for early assessment of neoadjuvant therapies by demonstrating restoration of normal functional indices, which precede morphological changes.

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Aggressive tumors have a high angiogenic activity which helps to maintain adequate metabolic resources for the proliferating cells. Angiogenesis is a series of processes which leads to proliferation following simulation of quiescent endothelial cells. This stimulation leads to deterioration in the basal membrane followed by neovascularization.

Angiogenesis can be examined histologically by measuring the microvascular density (MVD). A high MVD is reported to be a positive prognostic indicator in patients treated with adjuvant chemotherapy [1]. However, the abnormalities seen in tumor angiogenesis are not restricted just to an increase in MVD, as morphological and functional changes are also seen with heterogeneous distribution of the microcirculation and hyperpermeable microvessels. Histologists also find arteriovenous shunts and disparities in vessel diameter. In addition, blood flows are unstable and reversible due to changes in interstitial pressure within the tumor.

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2211-5684/$ – see front matter © 2013 Published by Elsevier Masson SAS on behalf of the Éditions françaises de radiologie.
http://dx.doi.org/10.1016/j.diii.2013.06.011
The purposes of dynamic imaging are to measure functional abnormalities of the tumor microcirculation and to refine morphological findings in order to characterize a tumor and monitor treatments.

**Techniques**

**Indices measured**

The breast microcirculation can be characterized on imaging by measuring four conventional indices: tissue perfusion, capillary permeability, the plasma volume fraction and the interstitial volume fraction. Other indices described in the literature are calculated from these four basic parameters: permeability can be expressed as the transfer rate \( k_{\text{ep}} \) (min\(^{-1}\)) which is represented by the \( K_{\text{trans}} / V_p \) ratio. \( k_{\text{ep}} \) values generally range between 0.30 and 0.90 min\(^{-1}\). The mean transit time (MTT) which is the mean time taken by the tracer to cross a tumor is estimated from the \( V_p/F \) ratio. The MTT ranges between 20 and 60 seconds.

**Acquisition technique**

Functional imaging of tumor microcirculation is based on an analysis of enhancement of the MRI signal from the biological distribution of a contrast medium (gadolinium chelate) in the microvasculature and interstitial tissue of the lesion. To do this, the tissue enhancement kinetics are examined dynamically and measured for several minutes after contrast injection taking account of enhancement kinetics within the arteries (Dynamic Contrast Enhancement or DCE) (Fig. 1).

Perfusion studies require a very high image rate during the acquisition (temporal resolution in the region of one image per second) which is difficult to achieve practically in usual senology practice. Current recommendations are that spatial resolution should be optimized, working with pixels under a millimeter in size. On the other hand, permeability can be analyzed with temporal resolution of one image every 20 to 30 seconds and spatial resolution of under a millimeter and can therefore be incorporated into a clinical breast MRI protocol (Fig. 2).

Mathematical models such as those described by Tofts [3], Brasch [4] or Lawrence [5], can be used to measure the tumor angiogenesis indices from enhancement curves. The number of indices measured depends on the choice of mathematical model and the temporal resolution.

The Tofts model is adapted to the temporal resolution used routinely in breast MRI (1 image/30 seconds) and can be used to measure capillary permeability \( (K_{\text{trans}} \text{ and } k_{\text{ep}}) \) and the interstitial volume fraction \( (V_p) \). In this model, tumor enhancement is deemed only to depend on the content of the interstitial compartment, the contribution from the capillary compartment being ignored (Fig. 3). Conversely, the Tofts model to analyze data obtained under high temporal resolution (under 2 seconds) leads to an overestimate of permeability (over 50%). Enhancement due to contrast in the capillaries is clearly visible in the initial phase of high temporal resolution. The Tofts model assumes that capillary-related enhancement is part of the interstitial enhancement and includes a "perfusion dose" in the permeability calculation [2].

Analysis of tissue perfusion in the breast is complicated, firstly because of the necessary temporal resolution which is incompatible with the spatial resolution recommended for a reliable morphological analysis [6] and secondly by problems due to high concentrations of contrast in the blood vessels and tumors in the initial enhancement phase which is used to extract the perfusion data. High concentrations of

![Figure 1. Measurement of enhancement curves. Several acquisitions covering the whole breast are taken at different time after intravenous contrast injection. A region of interest is drawn on the lesions each time, allowing signal intensities to be measured and an enhancement-time curve to be constructed.](image)

![Figure 2. Enhancement curve within the tumor. The initial enhancement phase represents the arrival of contrast medium in the tumor capillaries and reflects perfusion (F). Amplitude reflects blood volume \((V_p)\), Observation of this first phase requires high temporal resolution (1 image/second) and is still a research tool. The second enhancement phase represents exchange of contrast between plasma and interstitium. The analysis of this second part of the curve does not require any major change to clinical MRI protocols with low temporal resolution (1 image/30 s).](image)
Figure 3. A 45-year-old patient treated with neoadjuvant chemotherapy for infiltrating ductal carcinoma of the right breast, with locally advanced disease. A, C and E before treatment; B, D and F at two cycles. Morphological analysis shows a partial response after two cycles with a 50% reduction in lesion size. Functional analysis using the Toft model shows insignificant progression of the mean Ve values (+16%). Mapping and histogram analysis show restoration of normal Ve, with a 75% fall in the number of abnormal pixels.

gadolinium chelate outside of blood cells which are impermeable to contrast create magnetic field heterogeneities within the capillary lumen, causing a fall in signal by the T2* effect [7]. On T1-weighted MR, the T2* effect is seen in blood vessels and tumors at the concentration peak immediately after contrast injection. The contrast then dilutes throughout the whole blood volume and is then excreted by the kidneys. The T2* effect is difficult to correct for and restricts
the capacity to calculate perfusion in the initial phase of T1-weighted enhancement kinetics [8]. Some authors therefore suggest that a second contrast injection be given and that enhancement be monitored on a T2*-weighted sequence. In T2*-weighting (and echo planar sequence), the arrival of contrast in the capillary lumens leads to a fall in signal. If we assume that the capillary wall is impermeable to the low molecular weight contrast, perfusion and blood volume can then be calculated, as the changes in signal are theoretically due to the contrast contained in blood vessels [9,10]. In breast tumors, however, the assumption that the capillary wall is impermeable is not valid and perfusion measurements from the intravascular component can be incorrect due to the T1 effect caused by contrast medium passing into the interstitium, which reduces the fall in signal.

Applications

Tumor characterization

Microcirculation studies appear to be useful to characterize the aggressive nature of a tumor. Koo et al. reported that capillary permeability (Ktrans and Kep) increases with Scarff and Bloom SBR grade (Fig. 4) [11]. The Kep index is reported to be higher in triple negative tumors than in hormone receptor and HER2 positive tumors and the interstitial volume fraction (Vi) is reported to be lower in high-grade tumors (SBR III), which do not express hormone or HER2 receptors. These histological features are seen in aggressive tumors in association with high cellular proliferation rates (with Ki67 overexpression). The fall in Vi is explained by the high cell density in these tumors.

Breast tumors have been identified from the level of messenger RNA expression and classified into several subgroups: such as luminal, HER2 and basal-like, etc. Immunohistochemical labeling provides a simplified approach to this classification for use in clinical practice from transcriptome data [12]. The proliferation index (Ki67), expression of estrogen (ER), progesterone (PR) and the HER2 proto-oncogene receptors are good practical clinical tools to make an approximation [13]. Clinically, therefore, the 'luminal A' (ER+ or PR+ and low Ki67 <14%) phenotype is defined and is associated with a good prognosis. The luminal B phenotype (ER+ or PR+ and high Ki67 and/or c-erb2+), the 'HER2' phenotype (ER−, PR− and overexpressed c-erb2) and the 'basal-like' phenotype (ER−, PR−, c-erb2−, high Ki67, CK5/6+ and EGFR+) can be defined. These latter three types are associated with a poor prognosis than the 'luminal A' type [13]. Microcirculatory indices appear to correlate with the different breast cancer subtypes (Fig. 5). Li et al. have shown that permeability (Kep) was increased and the interstitial volume fraction (Vi) was reduced in triple negative tumors compared to luminal tumors [10]. Levels of Vascular Endothelial Growth Factor (VEGF) are doubled or tripled in triple negative cancers compared to luminal cancers particularly because of P53 mutations which deregulate the VEGF promoter [14]. VEGF activity is therefore responsible for abnormal structural and functional hypervascularization. The fall in MTT is believed to reflect shunt effects in triple negative tumors and the high Kep to be due to a VEGF-induced increase in permeability. Huse et al. have also reported that Ktrans and Vi correlate with microvascular proliferation (endothelial cell Ki67 and CD-34 expression) which is increased in basal-like compared to luminal tumors [15].

MRI is the best technique to detect infiltrating lobular carcinomas (ILC), which are amongst the most difficult breast tumors to identify. We have shown that dynamic MRI can show differences in capillary permeability (Ktrans and Kep) and interstitial volume fraction (Vi) between ILC and infiltrating ductal carcinomas (IDC). The most useful indices are reported to be the Vi and Kep, with a higher Vi in ILC (47% compared to 30%, P = 0.0001) and a lower Kep (0.652 compared to 0.970, P < 0.0001). The histological appearances of ILC in our study were superimposable on those from Arpino’s study [16], with the majority of ILC being estrogen receptor positive and HER2 negative. These features show that the ILC mostly belong to the luminal subtype. The microcirculatory functional features of ILC are those seen in the luminal type of tumor (Fig. 6). On the other hand, the ILC behave far more aggressively than the luminal tumors and behave more like the triple negative cancers. The microcirculation therefore does not appear to explain the far poorer prognosis of the ILC [17]. These results could help us to understand the pathogenesis and treatment of ILC.

Monitoring treatments

 Anthracycline and taxane chemotherapies have antiangiogenic activity similar to the targeted therapies which reduce the number of microvessels in tumors and restore normal microvessel architecture. These microscopic changes are accompanied by functional changes, with a fall in capillary blood flow, which returns to being unidirectional, and a reduction in the permeability of the capillary wall. These microcirculatory changes have a cytostatic effect which stops tumor progression. Stabilization of tumor volume in response to angiogenesis inhibitors on morphological imaging can therefore be considered to be a good response. The RECIST criteria, which require a 30% or greater reduction in tumor volume to confirm a partial response, do not appear to apply well to monitoring these new treatments.

Functional microcirculatory MRI could have a role in optimizing the assessment of breast cancer treatments. It may also help to predict response after neoadjuvant chemotherapy in patients being followed up for locally advanced breast cancer. Changes in microcirculatory indices precede morphological changes and appear even to be more sensitive and specific [9,18]. Tumor microcirculation is reported to begin to return to normal from the sixth week of treatment in patients with a good histological response after surgery. An improvement of 50% or more in permeability, blood volume and perfusion appears to suggest a good response. Conversely, the indices tend to remain stable in non-responders. On the other hand, changes in size on MRI at 6 weeks do not distinguish responders from non-responders (Figs. 7 and 8). Permeability appears to be the best functional index to predict a poor response, achieving a sensitivity of 90—94% and a specificity of 80—83% [9,18]. These studies, however, were carried out on small patient numbers and larger studies are awaited.
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Figure 4. Comparison of microcirculation indices and histological SBR grade, A, C, E: a 55-year-old patient seen with low-grade (SBR I) infiltrating ductal carcinoma (IDC). B, D, F: a 58-year-old patient with high-grade IDC (SBR III). These examples illustrate that capillary permeability within the tumor increases and that the interstitial volume fraction is reduced in high-grade compared to low-grade lesions.

Discussion

Microcirculation imaging can add further supporting evidence for suspicious aggressive lesion. Increased permeability and reduced interstitial volume suggest that the lesion is high grade and does not overexpress hormone or HER2 receptors. Studying the microcirculation can also be used in the early assessment of neoadjuvant therapies by demonstrating restoration of normal functional indices, which precedes morphological changes.
Figure 5. Comparison of microcirculation indices and tumor phenotype. A, C and E: an 83-year-old patient with luminal A subtype infiltrating ductal carcinoma (IDC) (RE+, RP−, HER2− and negative Ki67). B, D and F: a 70-year-old patient with a triple negative subtype IDC (RE−, RP−, HER2−, Ki67+). These examples illustrate that capillary permeability within the tumor is increased whereas the interstitial volume fraction is reduced in triple negative lesions compared to the luminal A subtype.

The results published for some indices such as the $k^{\text{trans}}$ vary [11,19]. All contrast media used in routine clinical practice contain small gadolinium chelate molecules which circulate in the capillary lumen and cross their walls. Enhancement therefore reflects both permeability and perfusion. $k^{\text{trans}}$ therefore depends on changes in perfusion due to blood pressure and heart rate unrelated to the tumor microcirculation. On the other hand, $k_{\text{ep}}$ only...
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Figure 6. Comparison of its microcirculation indices and histological type. A, C and E: an 83-year-old patient with infiltrating lobular carcinoma (ILC). B, D and F: a 48-year-old patient with infiltrating ductal carcinoma (RE+, RP+, HER2− and Ki67−). Capillary permeability within the tumor and the interstitial volume of the ILC are similar to those in the luminal A IDC.

reflects capillary permeability and appears to be more robust [11].

Functional tissue heterogeneity can be displayed on histograms used to quantify the magnitude of abnormal areas and their change over time. The methods available to objectively compare these histograms however are limited and are generally not available in microcirculation analytical software. The available software also does not enable an analysis to be made of parametric images to assess spatial heterogeneity, whereas a heterogeneous microvascular
Figure 7. Early assessment (two cycles) of the effectiveness of neoadjuvant chemotherapy on an IDC of the left breast. A, C and E: before chemotherapy. B, D and F: after two cycles. Comparison of size and microcirculatory indices before and after two cycles shows an 80% fall in tumor size. Microcirculation analysis shows that capillary permeability has fallen by 99% and that the interstitial volume fraction has increased by 40%. Analysis of the surgical specimen at the end of chemotherapy shows a complete response (Sataloff A).

distribution is one of the criteria for identifying malignant tumors. Functional heterogeneity analytical modules for breast cancer incorporated into functional imaging software are therefore awaited with anticipation!

The performance of microcirculation analysis is generally compared to morphological findings. Numerous breast studies have shown that changes in microcirculatory indices precede changes in tumor size [9,20]. Whilst the
combined use of morphological and functional criteria could improve diagnostic performance [21], the examination is significantly prolonged in order to obtain morphological data (5 to 10 minutes), diffusion measurements (2 to 5 minutes) and acquisition of enhancement kinetics to analyze the microcirculation (10 to 15 minutes).

Various more or less sophisticated mathematical models are now available (such as Tofts, Brasch and Sir Laurence),

**Figure 8.** Early assessment (two cycles) of the effectiveness of neoadjuvant chemotherapy on an IDC of the right breast. A, C and E: before chemotherapy. B, D and F: after two cycles. A comparison of size and microcirculatory parameters before and after two cycles shows a 70% fall in tumor size. However, microcirculation analysis shows that capillary permeability was initially low and that it has not fallen on treatment. The interstitial volume fraction is also stable at two cycles. Analysis of the surgical specimen at the end of chemotherapy shows a histological response of under 50% (Sataloff C).
which have clearly defined application conditions. The main criteria used to decide on the choice of model are temporal resolution, type of tracer and anatomical conditions. The choice of temporal resolution used depends on the anatomical volume being examined: a staging assessment for breast cancer requires a large volume to be examined (a 3D study covering both breasts), in high spatial resolution in order to identify the morphological details suggestive of malignant disease such as spicules under a millimeter in size [6,22]. This type of analysis restricts temporal resolution (one acquisition per minute). A clearly identified target lesion can be monitored on treatment under high temporal resolution (1 image/second) by covering a small volume centered on the lesion (often a 2D study) [8].

Finally, acquisition and techniques and software to analyze the microcirculation have not been standardized [23]. The standardization limitations are delaying the large-scale validation of microcirculation analysis, which is required before these techniques pass widely into routine practice.

**Conclusion**

Angiogenesis imaging can improve breast MRI performance. Clinical applications are developing for the breast as in the colon, prostate and liver. Analysis of heterogeneity and the use of additional findings such as change in tumor volume should improve functional imaging performance. Simplifying the protocols is also an important stage. The emergence of new multichannel aerials should accelerate acquisitions allowing simultaneous investigation of morphology and the microcirculation with high temporal and spatial resolution in the same investigation.

**TAKE-HOME MESSAGES**

- Microcirculation analysis software is still expensive and not widely available in breast imaging departments.

**Main results**

- The microcirculation is related to the level of malignancy in breast cancers. Permeability increases and the interstitial volume fraction falls in high-grade compared to low-grade cancers.
- The microcirculation is related to the histological features of the tumor. Permeability increases and the volume fraction falls in tumors, which do not have hormone or HER2 receptors.
- The microcirculation also depends on tumor subtype defined from transcriptome data or more simply obtained from immunohistochemical findings. Permeability is increased and the interstitial volume fraction is reduced in the "basal-like" subtypes compared to the luminal subtypes. These findings are of prognostic use.
- Microcirculation can improve the reliability of MRI to assess the effectiveness of neoadjuvant chemotherapy by demonstrating restoration of normal permeability (lower) and volume fraction (higher) early, from the second cycle of treatment compared to pretreatment ("baseline") values.

**Clinical case**

This 51-year-old patient is being seen for a 53 mm infiltrating ductal carcinoma (IDC) in her left breast. The tumor is an irregular mass with circumscribed borders. It enhances early and intensely followed by an obvious washout.

**Questions**

1. Based on the findings in Fig. 9, the amplitude of the enhancement peak indicates:
   (a) Low capillary permeability.
   (b) High capillary permeability.
   (c) A high interstitial volume fraction.
   (d) A low interstitial volume fraction.

2. Fig. 10 shows that permeability is extremely high and that the interstitial volume fraction is normal overall. What can be concluded from this?
   (a) The tumor is probably a low histological grade (SBR I).
   (b) The tumor is probably luminal.
   (c) The tumor is progressing rapidly.
   (d) The tumor should respond well to neoadjuvant chemotherapy and mastectomy may be avoided.
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Ki67 marker was overexpressed indicating high tumor proliferation. Skin invasion is a contraindication to conservative treatment regardless of clinical or MRI response.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

References


Answers

1. Correct answer: b.

The amplitude of the enhancement peak depends mostly on perfusion, blood volume and permeability. An early intense enhancement peak indicates that one of these three microcirculatory indices is increased. It is not possible to calculate the interstitial volume fraction from the qualitative analysis of the enhancement peak.

2. Correct answer: c.

High capillary permeability and a normal interstitial volume fraction is more suggestive of a high-grade (SBR III) tumor and generally a triple negative tumor. In this case, the

Figure 9. Mass in the superior external quadrant of the left breast (left) and tumor enhancement curve (right).

Figure 10. Permeability mapping within the tumor (Ktrans) and tabulated summary of values (median, mean and standard deviation) of Ktrans, kep, Ve and area under the enhancement curve.


