Published in final edited form as:

Magn Reson Imaging. 2012 October ; 30(8): 1059–1067. doi:10.1016/j.mri.2012.04.026.

Microcirculatory fraction (MCF_I) as a potential imaging marker for tumor heterogeneity in breast cancer

Xiangyu Yang^{a,b}, Ewa Mrozek^c, Maryam Lustberg^c, Guang Jia^{a,b}, Steffen Sammet^{a,b}, Christina Sammet^{a,b}, Charles Shapiro^c, and Michael V. Knopp^{a,b,*}

^aDepartment of Radiology, Division of Imaging Science, The Ohio State University, Columbus, OH 43210, USA

^bWright Center of Innovation in Biomedical Imaging, The Ohio State University, Columbus, OH 43210, USA

^cDepartment of Internal Medicine, Division of Hematology and Oncology, The Ohio State University, Columbus, OH 43210, USA

Abstract

Cancer is a heterogeneous disease by nature. Current imaging studies usually ignore intratumor variability in imaging biomarkers. We postulate that quantifying tumor heterogeneity with imaging techniques can provide useful information about cancer biology and potentially serve as novel imaging biomarkers. In this retrospective study, we identify a potential imaging marker, the microcirculatory fraction (MCF_I), that quantifies tumor heterogeneity in normoxic/hypoxic cellular composition. We demonstrate its application on a test population of 22 women with stage II/III HER-2 negative breast cancer receiving antiangiogenic-cytotoxic combination neoadjuvant chemotherapy. Early change in MCF_I (Δ MCF_I) is assessed with dynamic contrast enhanced magnetic resonance imaging at the end of Cycle 2 and associated with pathologic response. Its performance is compared with other established volumetric imaging biomarkers (initial tumor volume and volume change) by statistical and graphic methods. We demonstrate that a significant ($P < .01$) difference in Δ MCF_I can be detected between good (median Δ MCF_I 0.27) and poor (median Δ MCF_I -0.12) responders, despite the limited population size. Differences in the volumetric biomarkers are not statistically significant. Receiver operating characteristic analysis also shows that Δ MCF_I is a good predictor for pathologic response (AUC=0.86, 95% CI 0.69–1.00, $P < .01$), while predictions made with the established volumetric biomarkers are not significantly better than random guesses. We conclude that Δ MCF_I has the potential of being a better predictive biomarker for therapeutic response assessment. Our findings support our postulation that quantifying tumor heterogeneity with imaging techniques can provide additional information that can serve as novel biomarkers.

Keywords

Tumor heterogeneity; Response assessment; Breast cancer; DCE-MRI

1. Introduction

In the past decade, cancer research and drug development have substantially benefited from better recognition and increasing use of biomarkers in clinical trials and patient care. Biomarkers are measurable biological indicators that quantitatively characterize certain processes or conditions in organisms. Prognostic and predictive biomarkers are useful tools for disease diagnosis and treatment planning. Surrogate biomarkers are widely used in clinical trials to replace traditional clinical end points in order to decrease costs and time in developing novel antitumor agents [1–12].

Among various types of biomarkers, imaging is particularly promising because of its non-invasive nature and spatial resolving capability that allows morphometric localization of biomarker expression in a 3D imaging volume [2,7–11]. However, these unique advantages are not fully exploited in current imaging studies. Recent advances in various fields of cancer research supported and developed the concept that a tumor can be highly heterogeneous. Substantial genotypical and phenotypical variability has been observed within almost all types of cancers. Tumor heterogeneity has been recognized as playing a key role in disease progression, metastasis and development of therapeutic resistance [13–15]. Although several groups have reported significant intratumor variability in various imaging biomarkers [16–18], the concept of tumor heterogeneity is still not widely used in the area of biomedical imaging. Currently, most imaging markers only report a morphometric [7,19] or averaged parameter [8–11]. The important information about intratumor heterogeneity is lost, and the powerful spatial resolving capability of advanced imaging techniques is, to a large extent, under-utilized. Therefore, we postulate that quantifying tumor heterogeneity with imaging techniques can provide information that is ignored in current imaging studies. This information may serve as potential prognostic and/or predictive biomarkers that can help improve patient care and drug development.

Tumor heterogeneity is a complex phenomenon that is expressed on almost all levels and aspects of cancer biology. In this retrospective study, we identified a potential image marker, the microcirculatory fraction (MCF_I , where the subscript I indicates that it is a quantity measured with an imaging technique), that is associated with tumor heterogeneity in normoxic/hypoxic cellular composition in a population of 22 human epidermal growth factor receptor 2 (HER-2) negative breast cancer patients receiving antiangiogenic-cytotoxic combination neoadjuvant chemotherapy (NCT). We monitored therapy-induced early changes in MCF_I (ΔMCF_I) with serial dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) studies prior to and during NCT and associated them with the histopathologic assessment of response. Previous literature has established the initial tumor volume and the relative volume change (δV) as predictive biomarkers to post-NCT survival or pathologic response in breast cancer [7]. These volumetric biomarkers were also evaluated for both gross tumor mass (V_g) and normoxic mass (V_n), and compared with ΔMCF_I . Receiver operating characteristic (ROC) analysis was used to assess the ability of the five imaging markers (ΔMCF_I , initial V_n , initial V_g , δV_n , and δV_g) as single-variable classifiers for predicting pathologic response, with the area under the ROC curve (AUC) reported as a summary statistic.

2. Materials and methods

2.1. Patient population

In this retrospective study, we reviewed MRI data from a patient population of 22 women (patient age range 35–67 years, median 45 years) with clinical stages II and III HER-2 negative breast cancer drawn from an institutional review board–approved and HIPAA-compliant clinical trial. Written informed consent was collected from all participants. All

participants received six 28-day cycles of antiangiogenic-cytotoxic combination NCT prior to definitive surgery. DCE-MRI was performed before NCT (baseline), after two cycles (F01) and prior to definitive surgery (F02). Five imaging markers (ΔMCF_I , initial V_n , initial V_g , δV_n and δV_g) were calculated from the Baseline and F01 MRI data. The F02 data set is not assessed in this study because our primary interest is in the predictive value of early quantitative changes in imaging markers. Pathologic response was assessed after definitive surgery by histopathological analysis of the dissected tumors.

2.2. Imaging markers

3D spatial distribution of normoxic cancer cells can be detected by imaging technique with sub-millimeter resolution. DCE-MRI contrast agents are small molecular gadolinium chelates that can penetrate the capillary wall, enter the interstitial space and enhance MR signal in regions with high microcirculation and/or perfusion. The signal enhancement is correlated with tumor oxygenation level [20]. Therefore, the normoxic tumor mass with sufficient oxygen supply (V_n) can be segmented by appropriate thresholding of DCE-MRI signal enhancement maps [21,22]. In this study, V_n was determined using a threshold of at least 50% signal enhancement at the first post-contrast time point. We define MCF_I as the volume fraction of V_n on the gross tumor volume (V_g):

$$\text{MCF}_I = V_n / V_g$$

Here V_g is estimated with the standard ellipsoid model [23–25]:

$$V_g = \pi \times d_1 \times d_2 \times d_3 / 6$$

where d_1 , d_2 and d_3 are the largest tumor diameters measured in three perpendicular directions.

In this study, we use V_n (baseline) and V_g (baseline) to evaluate the initial tumor volume. Volume change during therapy is evaluated by the relative decrease in tumor volume:

$$\begin{aligned} \delta V_n &= (V_n(\text{baseline}) - V_n(\text{follow-up})) / V_n(\text{baseline}) \\ \delta V_g &= (V_g(\text{baseline}) - V_g(\text{follow-up})) / V_g(\text{baseline}) \end{aligned}$$

Following the same tradition, change in MCF_I is evaluated by its decrease during NCT:

$$\Delta\text{MCF}_I = \text{MCF}_I(\text{baseline}) - \text{MCF}_I(\text{follow-up})$$

2.3. MRI data acquisition and clinical assessment

MR images for all patients were acquired with a 1.5-T whole body scanner (Signa HD, GE Medical Systems, Milwaukee, WI, USA) and a seven-channel phased-array breast coil. DCE-MRI data were acquired with a 3D sagittal bilateral dynamic VIBRANT (Volume Imaging for BREast Assessment) sequence using the following imaging parameters: repetition time 4.7 ms; echo time 2.2 ms; flip angle 10°; acquisition matrix 256×160 (reconstructed to 512×512); reconstructed in-plane voxel size 0.47×0.47 mm²; slice thickness 2.2 mm; 108 to 176 slices, depending on patient size; and six time points with temporal resolution of approximately 70 s. A standard dose (0.1 mmol/kg body weight) of gadopentetate dimeglumine (Magnevist, Bayer Healthcare, Wayne, NJ, USA) was administered intravenously as a contrast agent with a power injector (Medrad, Warrendale,

PA, USA) at an injection rate of 2 ml/s followed by a saline flush. The DCE-MRI images were clinically reviewed by an experienced radiologist with the assistance of a commercial computer-aided detection (CAD) system (CADstream; Confirma).

3. Results

3.1. Test population

At the time of surgery, six patients had achieved pathologic complete response (pCR), defined as no evidence of residual invasive cancer both in breast and lymph nodes. Three patients only had microscopic residual disease (MRD) remaining. All patients who had achieved a final outcome assessment of pCR or MRD are identified as the good responder group ($n=9$). One patient had both her MRI and histopathological results showing complete resolution of the tumor mass but was still classified as a non-responder due to the existence of multiple positive lymph nodes in the histopathological workup. Since DCE-MRI is not a lymphnode-sensitive technique, we decided that this patient should be considered an outlier and excluded from this initial analysis. Several groups have reported that some breast cancer patients may have false negative results in post-NCT MR examinations due to NCT-induced contrast agent uptake reduction [7,26]. In this study, we had identified three patients with false negative MR results according to the following criteria: (1) $V_g=0 \text{ cm}^3$ in at least one of the two follow-up MR studies; (2) the residual tumor size assessed by histopathologic analysis is larger than 1 cm; and (3). the cellularity of the residual tumor is not low. Residual tumor volumes are severely underestimated by DCE-MRI in these patients. As a result, the biased MCF_I is no longer a measure of tumor heterogeneity but rather reflects the limitation of a particular imaging technique. Therefore, these patients were also excluded from the group comparison. All other patients are identified as the poor responder group ($n=9$).

3.2. Correlation

Inspection of scatter plots does not reveal any substantially nonlinear (e.g. concave, convex, sigmoid, or periodic) relationship between any pair of the five imaging markers investigated. Therefore, Pearson's correlation coefficient (r) can be used to characterize the dependence of the information carried by those markers. It is known that V_n and V_g are highly dependent with each other. Their dependence is reflected by the strong correlation between the corresponding biomarkers (initial V_n and initial V_g : $r=0.94$; δV_n and δV_g : $r=0.94$). Conversely, tumor volume change and the initial volume are identified as independent imaging biomarkers that carry different information. The correlation between these biomarkers is much weaker (initial V_n and δV_n : $r=0.49$; initial V_g and δV_g : $r=0.54$). ΔMCF_I also has poor correlation with both the initial tumor volumes (initial V_n and ΔMCF_I : $r=0.28$; initial V_g and ΔMCF_I : $r=0.16$) and the relative volume changes (δV_n and ΔMCF_I : $r=0.24$; δV_g and ΔMCF_I : $r=0.01$), suggesting that it is not a replicate of the established volumetric imaging biomarkers; instead, it carries some unique information that is not conveyed by either the initial tumor volume or the relative volume change during therapy.

3.3. Group comparison

Descriptive statistics for the five imaging markers investigated are tabulated in Table 1. Substantial volume reduction is seen in every case after two cycles of antiangiogenic-cytotoxic combination NCT. Target tumor responses at the end of cycle two are assessed with the revised Response Evaluation Criteria in Solid Tumors (RECIST, version 1.1) [19]. Most patients are classified as having partial response (PR) at the end of cycle two (13 of the 18 patients, 72%). One patient (6%) is classified as having complete response (CR). Four patients (22%) are classified as having stable disease (SD). No progressive disease (PD) is observed in this patient population. The response status distribution in the good responder

group (CR/PR/SD/PD=1/6/2/0) is similar to that of the poor responders (CR/PR/SD/PD=0/7/2/0).

Due to the small sample size, the non-parametric Mann–Whitney U test (alternatively known as Wilcoxon rank sum test) is used to compare the five imaging markers obtained from the good and poor responder groups, with a preset overall significance level of 0.05. Bonferroni correction is applied for multiple comparisons. The test results are also summarized in Table 1. Among the five imaging markers investigated, ΔMCF_I is the only one that is significantly different between the good and poor responders ($P<.01$). Positive ΔMCF_I values are observed in eight of the nine patients in the good responder group (group median 0.27), while the poor responder group is mainly characterized by negative ΔMCF_I values (six of nine patients, with another two very close to zero; group median -0.12). Particularly, all six pCR patients have ΔMCF_I values greater than 0.2. Initial tumor sizes are similar in both groups. The good responders tend to have larger tumor reduction after two cycles of NCT, but the observed difference does not reach statistical significance due to large interpatient variability in δV_n and δV_g . These results are also supported by the percentile information summarized in the box-plot (Fig. 1), in which a well-defined separation is only observed between the “good” and “poor” boxes for ΔMCF_I .

3.4. ROC curve analysis

The predictive ability of the five imaging markers as single-variable classifiers for pathologic response is assessed by ROC curve analysis, with good/poor responder groups defined as positive/negative outputs (Table 2). Again, ΔMCF_I is the only good predictor for pathologic response ($P<.01$) among the five imaging markers. The AUC values of the four volumetric imaging biomarkers are not significantly larger than 0.5, suggesting that the corresponding classifiers are not substantially better than random guesses. The ROC curves for these imaging markers are illustrated in Fig. 3A–E. Particularly, the ROC curve for ΔMCF_I has high sensitivity over the whole range of specificity.

4. Discussions

4.1. ΔMCF_I : a pathophysiologic interpretation

In solid tumors, a significant fraction of tumor mass is exposed to prolonged oxygen shortage. Tumor cells in a hypoxic zone are substantially different from normoxic tumor cells because of hypoxia-induced alterations in gene expression, phenotype, and proliferation characteristics [27]. These differences are known to be associated with disease aggressiveness and therapeutic resistance [28,29]. Disease progression and anticancer therapy can induce substantial changes in tumor microvasculature and oxygenation level. The resulting change in tumor cellular composition has been demonstrated to have diagnostic or prognostic significance in various types of cancers [28,30,31].

Our result shows that most good responders have decreased fraction of normoxic cells in residual tumor after two cycles of NCT, while the poor responders tend to have better blood supply and oxygenation level in residual lesions. The link between this observation and the pathophysiologic basis is not straightforward, because improved microcirculation generally has a positive influence on the effectiveness of anticancer therapies. Hereby we propose a pathophysiologic interpretation for our observation that attributes the elevated MCF_I in poor responders to the existence of a non-responsive tumor cell subpopulation.

It is known that hypoxic tumor cells are resistant to chemotherapy and radiotherapy. Chronic exposure to nonlethal hypoxia leads to various changes in tumor cell gene expression, including down-regulation of apoptotic inhibitors and up-regulation of pro-resistance genes like the multidrug resistance gene. Cell cycle is slowed down or even arrested in hypoxic

tumor cells in response to inadequate supply of oxygen and nutrients. The low cell proliferation rate makes chemotherapeutic agents targeted at specific phases of cell cycle largely ineffective. The low oxygenation level in hypoxia zone also makes tumor cells less vulnerable to damages caused by oxygen free radicals [29,32]. Moreover, insufficient blood supply in the hypoxic zone poses a physical challenge for drug delivery. Inhomogeneous drug distribution has been reported for several chemotherapeutic agents in experimental tumors and human cancer in vivo [32,33]. All these factors contribute to the difference in therapeutic response between normoxic and hypoxic tumor cells. In the early stage of NCT, a larger proportion of more-sensitive normoxic tumor cells are killed by the higher local drug concentration, leading to a residual tumor with higher hypoxic/normoxic cell ratio. This change in cellular composition is captured by a decrease in MCF_I , i.e., the positive ΔMCF_I of the good responder group (Fig. 2A).

However, a different situation may appear when some tumor cells are non-responsive to the used NCT regimen. According to the contemporary theory of tumor heterogeneity, genetically instable cancer cells acquire mutations during disease progression, and generate progeny tumor cells with different genotypes and phenotypes that compete with each other [34,35]. In clonal evolution, administration of therapeutic agents acts as a strong selective force that favors non-responsive tumor cells. These cells gain an advantage in survival and proliferation by their capability of supporting a microvasculature network in the existence of therapeutic agents. This capability may come from the intrinsic non-responsiveness of tumor-associated endothelial cells, or from revascularization by the activation of alternative angiogenic pathways [36,37]. Growth of those nonresponsive tumor cells and associated microvasculature leads to an enhanced normoxia fraction in the residual tumor that is quantified by an increase in MCF_I (negative ΔMCF_I , Fig. 2B). Therapeutic resistant tumor cells also tend to be more invasive and metastatic. Therefore, a negative ΔMCF_I is a sign of poor prognosis and response, as demonstrated by the poor responder group in this study.

4.2. ROC curve analysis

The AUC calculated above represents the best performance that can be achieved by a ΔMCF_I predictor when DCE-MRI accurately measures residual tumor volume in all patients. In reality, the predictive ability of ΔMCF_I may be compromised by measurement biases like those observed in the three patients with false-negative MR results. When these patients are also included in the ROC analysis, both sensitivity and specificity of the ΔMCF_I predictor are decreased (Fig. 3F). However, the AUC values is still significantly larger than 0.5 ($P=.02$; AUC=0.75, with a 95% confidence interval of 0.53–0.97). Chen et al. have reported that lack of enhancement in post-NCT MRI causes less accurate residual tumor size measurement and response assessment in HER-2 negative patients and patients receiving bevacizumab (Avastin) [38]. Therefore, this AUC value gives an estimate for the worst case scenario. In real world applications, where both HER-2 positive patients and patients receiving other antiangiogenic agents like trastuzumab (Herceptin) will be examined, the true performance of the ΔMCF_I predictor is most likely to lie between those extremes.

4.3. Methodology

With our current approach, measurement error in MCF_I mainly comes from the uncertainty in V_g estimation. Although the ellipsoid formula is widely used in literature, it is certainly an oversimplified model and a major source of error. Moreover, the poorly enhanced region in large tumors may contain a significant fraction of necrotic tissue. DCE-MRI cannot effectively differentiate them from the truly hypoxic mass. Their inclusion is an important source of systematic error for V_g and MCF_I . Accuracy of MCF_I measurement might be improved by measuring both the normoxic and hypoxic volumes, and calculating V_g as their sum. Several molecular imaging tracers, such as ^{18}F -fluoromisonidazole [39], ^{18}F -

fluoroazomycin arabinoside [40] and ^{60}Cu -labeled diacetylbis (N^4 -methylthiosemicarbazone) [41], have been developed for in vivo hypoxia detection with positron emission tomography (PET). They might be used in combination with DCE-MRI to get a more accurate measurement of MCF_I . The emerging hybrid PET/MRI technology provides an ideal platform for such an application, on which complementary volumetric information can be simultaneously acquired with different imaging modalities in a single examination.

In longitudinal treatment response studies, occasionally a single primary tumor may regress to multifocal remnants after treatment. In this case, we propose taking the largest MCF_I among all residual lesions for calculation of ΔMCF_I . The RECIST summation approach for diameter measurements should not be used for ΔMCF_I because MCF_I of different tumors are non-additive.

From the practical perspective, ΔMCF_I has an advantage in the convenience of its implementation. Tumor diameter measurement is a routine radiologic procedure. V_n can be readily obtained from commercially available workstations or MRI CAD systems. Therefore, it can be readily incorporated into clinical research and practice without any need for additional imaging postprocessing or major protocol adjustment. Retrospective application of this approach to existing data is also easy and straightforward.

Moreover, our result suggests that the sample size needed by ΔMCF_I is much smaller than those for the established volumetric biomarkers. This observation has a strong implication on possible applications in clinical trials. The requirement for a large patient population has greatly contributed to the lengthy development cycle and the rocketing cost of developing new drugs. Therefore, this feature makes it attractive to explore the possibility of using ΔMCF_I as a surrogate end point in cancer trials. Another promising application is in personalized medicine, in which ΔMCF_I , by itself or in combination with other biomarkers, may be used to assess the efficiency of NCT regimens and facilitate personalized treatment planning.

4.4. Limitations

This study has some limitations. First, the patient population was pre-selected to be responsive enough to finish six cycles of NCT. Caution is needed for extrapolation to cases of non-responders. Secondly, we only considered the association between ΔMCF_I and post-NCT pathologic response. Further work, preferably in larger scale clinical studies with long-term follow up, is required to determine the relationship between ΔMCF_I and the ultimate target of cancer therapy, i.e., progression-free and/or overall survival. Thirdly, the limited sample size only allows us to assess the predictive ability of ΔMCF_I as a single-variable classifier. Model building techniques, such as stepwise logistic regression, may be used in a larger dataset to construct more powerful, multivariable predictors for pathologic response or patient survival. We expect to resolve these limitations in future prospective clinical studies with larger patient population and more dedicated design that are developed on the basis of the MCF_I concept and initial data presented in this article. A prospective study will also provide additional validation to our pathophysiologic interpretation by adding supplementary information on tumor genomic assay and immunohistochemical hypoxia biomarkers, which is not available in this patient population due to the retrospective nature of this study.

Although we chose to demonstrate the concept of MCF_I with a group of breast cancer patients in this article, its application can be generalized to other types of disease and treatment regimen. Current clinical practice sacrifices temporal resolution for higher spatial resolution in breast DCE-MRI, thus making it unsuitable for model-based pharmacokinetic

analysis. Applying the concept of MCF_I to high temporal resolution DCE-MRI data obtained from diseases in other organs will allow the comparison to other established imaging biomarkers of tumor perfusion and microvasculature permeability, and a more comprehensive evaluation of their values and limitations.

In conclusion, we have presented the concept of MCF_I and demonstrated that early change in MCF_I is associated with therapeutic response to NCT in breast cancer patients. A statistically significant difference between good and poor responders is demonstrated even in a pilot patient population with limited size. ROC curve analysis reveals that ΔMCF_I is a stronger predictive marker to therapeutic response than the established volumetric imaging biomarkers. These results suggest that MCF_I is a promising potential imaging marker for tumor heterogeneity that deserves further investigation. They also support our postulation that quantifying tumor heterogeneity with imaging techniques can provide additional information that can serve as novel prognostic or predictive biomarkers.

Acknowledgments

The authors thank the following physicians for their clinical services and/or academic support: Dr. Adele Lipari, Dr. Stephen Povoski, and Dr. Dimitrios Spigos. This work was supported by grant ODOT AGMT TECH 03-051, and the Wright Center of Innovation in Biomedical Imaging.

References

1. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther.* 2001; 69(3):89–95. [PubMed: 11240971]
2. Jain RK, Duda DG, Willett CG, Sahani DV, Zhu AX, Loeffler JS, et al. Biomarkers of response and resistance to antiangiogenic therapy. *Nat Rev Clin Oncol.* 2009; 6(6):327–38. [PubMed: 19483739]
3. Evelhoch J, Garwood M, Vigneron D, Knopp M, Sullivan D, Menkens A, et al. Expanding the use of magnetic resonance in the assessment of tumor response to therapy: workshop report. *Cancer Res.* 2005; 65(16):7041–4. [PubMed: 16103049]
4. Smith JJ, Sorensen AG, Thrall JH. Biomarkers in imaging: realizing radiology's future. *Radiology.* 2003; 227(3):633–8. [PubMed: 12663828]
5. Cianfrocca M, Goldstein LJ. Prognostic and predictive factors in early-stage breast cancer. *Oncologist.* 2004; 9(6):606–16. [PubMed: 15561805]
6. James CR, Quinn JE, Mullan PB, Johnston PG, Harkin DP. BRCA1, a potential predictive biomarker in the treatment of breast cancer. *Oncologist.* 2007; 12(2):142–50. [PubMed: 17296808]
7. Partridge SC, Gibbs JE, Lu Y, Esserman LJ, Tripathy D, Wolverton DS, et al. MRI measurements of breast tumor volume predict response to neoadjuvant chemotherapy and recurrence-free survival. *AJR Am J Roentgenol.* 2005; 184(6):1774–81. [PubMed: 15908529]
8. Martincich L, Montemurro F, De RG, Marra V, Ponzone R, Cirillo S, et al. Monitoring response to primary chemotherapy in breast cancer using dynamic contrast-enhanced magnetic resonance imaging. *Breast Cancer Res Treat.* 2004; 83(1):67–76. [PubMed: 14997056]
9. Manton DJ, Chaturvedi A, Hubbard A, Lind MJ, Lowry M, Maraveyas A, et al. Neoadjuvant chemotherapy in breast cancer: early response prediction with quantitative MR imaging and spectroscopy. *Br J Cancer.* 2006; 94(3):427–35. [PubMed: 16465174]
10. Smith IC, Welch AE, Hutcheon AW, Miller ID, Payne S, Chilcott F, et al. Positron emission tomography using [(18)F]-fluorodeoxy-D-glucose to predict the pathologic response of breast cancer to primary chemotherapy. *J Clin Oncol.* 2000; 18(8):1676–88. [PubMed: 10764428]
11. Ah-See ML, Makris A, Taylor NJ, Harrison M, Richman PI, Burcombe RJ, et al. Early changes in functional dynamic magnetic resonance imaging predict for pathologic response to neoadjuvant chemotherapy in primary breast cancer. *Clin Cancer Res.* 2008; 14(20):6580–9. [PubMed: 18927299]
12. Ayers M, Symmans WF, Stec J, Damokosh AI, Clark E, Hess K, et al. Gene expression profiles predict complete pathologic response to neoadjuvant paclitaxel and fluorouracil, doxorubicin, and

- cyclophosphamide chemotherapy in breast cancer. *J Clin Oncol.* 2004; 22(12):2284–93. [PubMed: 15136595]
13. Heppner GH. Tumor heterogeneity. *Cancer Res.* 1984; 44(6):2259–65. [PubMed: 6372991]
 14. Simpson-Herren L, Noker PE, Wagoner SD. Variability of tumor response to chemotherapy. II. Contribution of tumor heterogeneity. *Cancer Chemother Pharmacol.* 1988; 22(2):131–6. [PubMed: 3409443]
 15. Shipitsin M, Campbell LL, Argani P, Weremowicz S, Bloushtain-Qimron N, Yao J, et al. Molecular definition of breast tumor heterogeneity. *Cancer Cell.* 2007; 11(3):259–73. [PubMed: 17349583]
 16. Knopp MV, von Tengg-Kobligk H, Choyke PL. Functional magnetic resonance imaging in oncology for diagnosis and therapy monitoring. *Mol Cancer Ther.* 2003; 2(4):419–26. [PubMed: 12700286]
 17. Kidd EA, Grigsby PW. Intratumoral metabolic heterogeneity of cervical cancer. *Clin Cancer Res.* 2008; 14(16):5236–41. [PubMed: 18698042]
 18. Ross BD, Moffat BA, Lawrence TS, Mukherji SK, Gebarski SS, Quint DJ, et al. Evaluation of cancer therapy using diffusion magnetic resonance imaging. *Mol Cancer Ther.* 2003; 2(6):581–7. [PubMed: 12813138]
 19. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 11). *Eur J Cancer.* 2009; 45(2):228–47. [PubMed: 19097774]
 20. Cooper RA, Carrington BM, Lancaster JA, Todd SM, Davidson SE, Logue JP, et al. Tumour oxygenation levels correlate with dynamic contrast-enhanced magnetic resonance imaging parameters in carcinoma of the cervix. *Radiother Oncol.* 2000; 57(1):53–9. [PubMed: 11033189]
 21. Li KL, Partridge SC, Joe BN, Gibbs JE, Lu Y, Esserman LJ, et al. Invasive breast cancer: predicting disease recurrence by using high-spatial-resolution signal enhancement ratio imaging. *Radiology.* 2008; 248(1):79–87. [PubMed: 18566170]
 22. Meeuwis C, van V, Stapper G, Fernandez Gallardo AM, van den Bosch MA, Mali WP, et al. Computer-aided detection (CAD) for breast MRI: evaluation of efficacy at 3.0 T. *Eur Radiol.* 2010; 20(3):522–8. [PubMed: 19727750]
 23. Carlsson G, Gullberg B, Hafstrom L. Estimation of liver tumor volume using different formulas - an experimental study in rats. *J Cancer Res Clin Oncol.* 1983; 105(1):20–3. [PubMed: 6833336]
 24. Kim MS, Lee SY, Cho WH, Song WS, Koh JS, Lee JA, et al. Effect of increases in tumor volume after neoadjuvant chemotherapy on the outcome of stage II osteosarcoma regardless of histological response. *J Orthop Sci.* 2009; 14(3):292–7. [PubMed: 19499296]
 25. Wapnir IL, Wartenberg DE, Greco RS. Three dimensional staging of breast cancer. *Breast Cancer Res Treat.* 1996; 41(1):15–9. [PubMed: 8932872]
 26. Rieber A, Zeitler H, Rosenthal H, Gorich J, Kreienberg R, Brambs HJ, et al. MRI of breast cancer: influence of chemotherapy on sensitivity. *Br J Radiol.* 1997; 70(833):452–8. [PubMed: 9227225]
 27. Harris AL. Hypoxia—a key regulatory factor in tumour growth. *Nat Rev Cancer.* 2002; 2(1):38–47. [PubMed: 11902584]
 28. Axelsson H, Fredlund E, Ovenberger M, Landberg G, Pahlman S. Hypoxia-induced dedifferentiation of tumor cells—a mechanism behind heterogeneity and aggressiveness of solid tumors. *Semin Cell Dev Biol.* 2005; 16(4–5):554–63. [PubMed: 16144692]
 29. Shannon AM, Bouchier-Hayes DJ, Condrón CM, Toomey D. Tumour hypoxia, chemotherapeutic resistance and hypoxia-related therapies. *Cancer Treat Rev.* 2003; 29(4):297–307. [PubMed: 12927570]
 30. Brizel DM, Sibley GS, Prosnitz LR, Scher RL, Dewhirst MW. Tumor hypoxia adversely affects the prognosis of carcinoma of the head and neck. *Int J Radiat Oncol Biol Phys.* 1997; 38(2):285–9. [PubMed: 9226314]
 31. Vaupel P, Kelleher DK, Hockel M. Oxygen status of malignant tumors: pathogenesis of hypoxia and significance for tumor therapy. *Semin Oncol.* 2001; 28(2 Suppl 8):29–35. [PubMed: 11395850]
 32. Tredan O, Galmarini CM, Patel K, Tannock IF. Drug resistance and the solid tumor microenvironment. *J Natl Cancer Inst.* 2007; 99(19):1441–54. [PubMed: 17895480]

33. Lankelma J, Dekker H, Luque FR, Luykx S, Hoekman K, van d V, et al. Doxorubicin gradients in human breast cancer. *Clin Cancer Res.* 1999; 5(7):1703–7. [PubMed: 10430072]
34. Campbell LL, Polyak K. Breast tumor heterogeneity: cancer stem cells or clonal evolution? *Cell Cycle.* 2007; 6(19):2332–8. [PubMed: 17786053]
35. Chuthapisith S, Eremin J, El-Sheemey M, Eremin O. Breast cancer chemoresistance: emerging importance of cancer stem cells. *Surg Oncol.* 2010; 19(1):27–32. [PubMed: 19251410]
36. Bergers G, Hanahan D. Modes of resistance to anti-angiogenic therapy. *Nat Rev Cancer.* 2008; 8(8):592–603. [PubMed: 18650835]
37. Grepin R, Pages G. Molecular mechanisms of resistance to tumour anti-angiogenic strategies. *J Oncol.* 2010; 2010:835680. [PubMed: 20224655]
38. Chen JH, Feig B, Agrawal G, Yu H, Carpenter PM, Mehta RS, et al. MRI evaluation of pathologically complete response and residual tumors in breast cancer after neoadjuvant chemotherapy. *Cancer.* 2008; 112(1):17–26. [PubMed: 18000804]
39. Szeto MD, Chakraborty G, Hadley J, Rockne R, Muzi M, Alvord EC Jr, et al. Quantitative metrics of net proliferation and invasion link biological aggressiveness assessed by MRI with hypoxia assessed by FMISO-PET in newly diagnosed glioblastomas. *Cancer Res.* 2009; 69(10):4502–9. [PubMed: 19366800]
40. Sorger D, Patt M, Kumar P, Wiebe LI, Barthel H, Seese A, et al. [18F] Fluoroazomycinarabinofuranoside (18FAZA) and [18F]Fluoromisonidazole (18FMISO): a comparative study of their selective uptake in hypoxic cells and PET imaging in experimental rat tumors. *Nucl Med Biol.* 2003; 30(3):317–26. [PubMed: 12745023]
41. Lewis JS, McCarthy DW, McCarthy TJ, Fujibayashi Y, Welch MJ. Evaluation of ⁶⁴Cu-ATSM in vitro and in vivo in a hypoxic tumor model. *J Nucl Med.* 1999; 40(1):177–83. [PubMed: 9935074]

Fig. 1.

Box-plots summarizing percentile information (whisker: range; box: 25% and 75% percentiles; bold line: median; circle: outliers) for the five imaging markers investigated: initial V_g (A), initial V_n (B), δV_g (C), δV_n (D) and ΔMCFI (E). The “Good” and “Poor” boxes represent the corresponding responder groups as defined by their pathologic outcomes. Among the five imaging markers, only ΔMCFI shows distinctively different distributions between good and poor responders.

Fig. 2.

Cartoons and representative cases illustrating the proposed pathophysiologic interpretation for $\Delta MCFI$: (A) Hypoxic tumor cells (gray) are more resistant to radio- and chemotherapy than normoxic cells (red). If all the cells in a tumor, both hypoxic and normoxic, are responsive to therapy, a larger fraction of normoxic cells will be killed, leading to a more hypoxic residual tumor and a decrease in $MCFI$ ($\Delta MCFI > 0$). A representative case from a good responder is shown in parallel in subplot (C). The blue, yellow, and red color overlays on the sagittal images represent voxels that have persistent, plateau, and washout dynamic patterns. Their combined volume is V_n . In this patient, $MCFI$ decreased from the baseline value of 0.67 to 0.36 after two cycles of antiangiogenic-cytotoxic combination neoadjuvant chemotherapy. (B) If a subpopulation of cells in a tumor are non-responsive to a specific type of therapy (red cell with a lightning mark in the nucleus) and are able to support a functionally active microvasculature network in the existence of therapeutic agents, they will get an overwhelming advantage in survival and proliferation. Their progeny cells and the associated microvasculature will dominate the residual tumor, leading to an increase in $MCFI$ ($\Delta MCFI < 0$). A representative case from a poor responder with an $MCFI$ increase from 0.38 to 0.63 is shown in subplot (D). Note that substantial reduction in tumor volume is observed in both patients shown here (good responder: $\delta V_g = 98\%$, $\delta V_n = 99\%$; poor responder: $\delta V_g = 99\%$, $\delta V_n = 99\%$). The initial tumor volume of the poor responder (initial $V_g = 51.7 \text{ cm}^3$, initial $V_n = 19.9 \text{ cm}^3$) is even smaller than the good responder (initial $V_g = 256.9 \text{ cm}^3$, initial $V_n = 170.9 \text{ cm}^3$). Therefore, the established volumetric imaging biomarkers are unable to accurately predict the therapeutic outcome in these two particular cases.

Fig. 3.

The ROC curves display the relationship between sensitivity (or true-positive rate, TPR) and specificity (1 – specificity is also called false-positive rate, FPR) in prospectively classifying the patients with individual imaging markers according to their pathologic outcome. The AUC is a measure of classifier performance. A good classifier with both high sensitivity and high specificity should have an AUC close to 1.0, while a poor classifier is characterized by a low AUC that is close to the random guessing AUC of 0.5. Among the five imaging markers investigated, the performance of initial V_g (A, AUC=0.51, 95% CI 0.23–0.78), initial V_n (B, AUC=0.54, 95% CI 0.27–0.82), δV_g (C, AUC=0.60, 95% CI 0.34–0.87) and δV_n (D, AUC=0.68, 95% CI 0.43–0.93) are not significantly better than random guessing. ΔMCF_I (E) is the only good single-variable predictor with an AUC of 0.86 (95% CI 0.69–1.00). Even when the three patients with false-negative MR results are included, the predictive ability of the ΔMCF_I classifier is still good (F, AUC=0.75, 95% CI 0.53–0.97).

Descriptive statistics and Mann–Whitney U test (Wilcoxon rank sum test) results for the five imaging markers investigated

Table 1

Variable	Good responder ($n=9$)			Poor responder ($n=9$)			U test P
	Range	Median	Mean	Range	Median	Mean	
Initial V_g (cm^3)	5.1–256.9	67.8	78.0	5.0–187.0	46.2	68.7	1.00
Initial V_n (cm^3)	2.8–170.9	39.4	44.0	3.8–75.9	22.0	32.9	.80
δV_g	50%–100%	92%	87%	48%–99%	83%	83%	.49
δV_n	67%–100%	92%	90%	50%–99%	83%	82%	.21
ΔMCF_1	–0.25 to 0.70	0.27	0.24	–0.27 to 0.13	–0.12	–0.09	<.01 *

* Statistically significant results. A pre-set overall significance level of $\alpha=.05$ is used with Bonferroni correction for multiple comparisons.

Table 2

Receiver operating characteristic analysis results for the five imaging markers investigated

Variable	ROC curve analysis		
	AUC	95% CI	P
Initial V_g	0.51	0.23–0.78	.48
Initial V_n	0.54	0.27–0.82	.38
δV_g	0.60	0.34–0.87	.22
δV_n	0.68	0.43–0.93	.08
ΔMCF_I	0.86	0.69–1.00 *	<.01 *

CI, confidence interval.

* Statistically significant results. A pre-set overall significance level of $\alpha=.05$ is used with Bonferroni correction for multiple comparisons.