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A Noninvasive Tumor Oxygenation Imaging Strategy using Magnetic Resonance Imaging of Endogenous Blood and Tissue Water

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Abstract

Purpose—To present a novel imaging strategy for noninvasive measurement of tumor oxygenation using MR imaging of endogenous blood and tissue water.

Theory and Methods—The proposed approach for pO₂ estimation is based on intravoxel incoherent motion (IVIM) diffusion MRI and the dependence of the blood R₂ relaxation rate on the inter-echo spacing measured using a multiple spin-echo CPMG sequence and weak-field diffusion model. The accuracy of the approach was validated by comparison with ¹⁹F MRI oximetry.

Results—The results in eight rats at 4.7 T showed that tumors have longer T₁ (1980 ± 186 ms) and T₂ (59 ± 9 ms) relaxation times, heterogeneous blood volume fraction (0.23 ± 0.1), Y (0.53 ± 0.12), and pO₂ (36 ± 15 mm Hg) distributions compared with normal muscle (T₁, 1480 ± 86 ms, T₂, 29 ± 2 ms, blood volume fraction, 0.22 ± 0.03, oxygen saturation level, 0.49 ± 0.06 and pO₂ 39 ± 5 mm Hg). pO₂ estimates based on the novel ¹H approach were essentially identical with ¹⁹F observations.

Conclusion—The study indicates that non-invasive measurement of tumor pO₂ using ¹H MRI derived multi-parametric maps is feasible and could become a valuable tool to evaluate tumor hypoxia.

Keywords

tumor; oxygenation; R₁; R₂; diffusion; IVIM

Introduction

Tissue oxygenation is an important physiological parameter related to perfusion and metabolism. Oxygen partial pressure (pO_2) is a crucial factor in the response of tumors to irradiation and other cytotoxic treatments (1,2). Tissue pO_2 depends upon oxygen delivery and utilization, which are governed by blood flow, dissociation of oxygen from hemoglobin and tissue metabolism (3,4). Therefore, a physiologically-based method is needed to evaluate the factors contributing to tumor oxygenation. Various pO_2 measurement techniques currently exist, but many are invasive and others provide qualitative measurements only (1). The invasive techniques are often limited by the restricted tissue sampling of needle electrodes or the necessity for biopsy and associated logistics of the histological techniques. Several noninvasive techniques such as near-infrared spectroscopy, electron paramagnetic resonance oximetry, and positron emission tomography (PET) with hypoxia markers(5) are available. However, to date there is still no non-invasive method that is clinically accepted to directly monitor tissue oxygenation with high spatial resolution and sensitivity. An ideal noninvasive tumor oxygenation imaging strategy would provide precise accurate reproducible measurement with spatial and temporal resolution allowing dynamic measurements.

Although 1H MRI cannot directly image molecular oxygen, the paramagnetic properties of deoxyhemoglobin and molecular oxygen can be revealed by relaxation time (i.e. T_1 , T_2 , T_2^*) measurements (6-8) or a phase difference-based method such as quantitative susceptibility mapping (9). Deoxyhemoglobin is compartmentalized in red blood cells and tissue water does not have ready access to coordination sites thereby influencing T_2^* relaxation with little T_1 effect. Blood oxygen level-dependent (BOLD) (10-12) or T_2^* contrast, is sensitive to changes in magnetic susceptibility generated by deoxyhemoglobin so that different levels of blood oxygenation can be potentially used as a non-invasive biomarker for studying tumor oxygenation. However, the results have been mixed because the changes in tumor oxygenation may result from changes in the vessel oxygen wall permeability, rheological properties of blood, the geometry of the blood vessels, oxygen content of blood and the shape of the oxygen dissociation curve (13). Furthermore, vascular blood volume and flow can influence BOLD measurements as appreciated in the FLOOD (Flow and Oxygen Level Dependent Contrast) approach (14).

At the capillary-tissue interface, it has been previously shown that the perfused vasculature is considered to be the source of oxygen supply and thus gives the initial spatial distribution of oxygen concentration (15). The changes of biophysical parameters such as the microcirculation in capillaries and water diffusion in tissue can be detected by quantitative T_1 and T_2 imaging as well as diffusion MRI. Therefore, the tumor pathophysiological aspects determined by combination of water diffusion and quantitative MRI imaging of endogenous blood and tissue water may provide a potential imaging biomarker that can distinguish tumor hypoxia non-invasively.

In this study, we present a new approach that attempts to non-invasively determine tumor pO_2 using a multi-parametric 1H MRI-based OXYgen Imaging (MOXI) strategy. Since the approach does not require exogenous contrast agents, it can be incorporated into a standard

clinical MRI. The successful clinical translation of this approach would allow hypoxic tumor identification to predict radiation therapy response. The proposed strategy uses quantitative multi-parametric maps including T_1 , T_2 , and tissue blood volume fraction based on intravoxel incoherent motion (IVIM) diffusion-weighted MR. Oxygen saturation level Y , and hence pO_2 , are estimated based on the dependence of blood R_2 relaxation rate on the inter-echo spacing measured using a multiple spin-echo Carr-Purcell-Meiboom-Gill (CPMG) sequence and weak-field diffusion model proposed by Jensen and Chandra (16). Following the 1H MRI, the oxygen reporter molecule hexafluorobenzene (HFB) was injected directly into each tumor followed by ^{19}F MRI for pO_2 measurements for comparative validation. The site-to-site 1H - and ^{19}F -based pO_2 measurements were directly compared.

Theory

The normalized MR signal from a voxel is a volume-weighted sum of blood and non-blood components. Contributions from the two components combine linearly with a slow exchange across the capillary wall (17,18), though the water exchange between plasma and erythrocytes is fast. The transverse relaxation rate due to magnetic susceptibility variations in tissue reflects the changes of tissue blood volume and the susceptibility difference between the blood and surrounding tissue (19,20). Therefore, noting the dependence of blood R_2 relaxation rate on the inter-echo spacing of the CPMG sequence (21,22), the signal intensity can be described as:

$$\frac{S}{S_0} = f_p \exp[-TE \cdot (R_{20} + R_{2ex})] + (1 - f_p) \exp(-TE \cdot R_{2nonbl}), \quad [1]$$

where f_p is the blood volume fraction, R_{20} is the R_2 of fully oxygenated blood, R_{2ex} is the enhanced exchange/diffusion contribution to the measured relaxation rate and R_{2nonbl} is the relaxation rate of non-blood components. R_{20} has been described previously (22) and can be expressed as:

$$R_{20} = R_{2plas} + Hct \cdot (R_{2oxy} + R_{2dia}), \quad [2]$$

where R_{2plas} is the R_2 relaxation rate from plasma contributions inside the erythrocytes (4.6 s^{-1} at 4.7 T) and $R_{2oxy} + R_{2dia}$ is the relaxation rate from oxygenated hemoglobin and diamagnetic contributions inside the erythrocytes (9.3 s^{-1} at 4.7T) (22,23). Hct is the volume fraction of erythrocytes (Hct=0.43 for rat (24)), and an additional vascular factor ($f_{vas} = 0.85$) was applied to account for Hct differences between large and small vessels (23). For tissue blood Hct, the weighting factor blood volume fraction f_p should be also included. Therefore, the effective Hct was introduced to this study as: $Hct \cdot f_{vas} \cdot f_p$.

According to the weak-field diffusion model proposed by Jensen and Chandra (16), R_{2ex} is described by:

$$R_{2ex} = G_0 \frac{\gamma^2 r_c^2}{2D} F\left(\frac{2D\tau_{cp}}{r_c^2}\right), \quad [3]$$

where γ is the proton gyromagnetic ratio (2.67502×10^8 rad/s/T), r_c is the characteristic length scale for the spatial variations of the field inhomogeneities (rat erythrocyte has a typical volume of $56 \mu\text{m}^3$ (25), which this leads to an effective radius R of about $2.4 \mu\text{m}$, $r_c \approx 0.91 \times R = 2.2 \mu\text{m}$), D is the diffusion constant ($2.0 \mu\text{m}^2/\text{ms}$), τ_{cp} is the inter-echo spacing (10 ms). Based on the properties of the function $F: F\left(\frac{2D\tau_{cp}}{r_c^2}\right), F\left(\frac{2D\tau_{cp}}{r_c^2}\right) \approx 1$ (16). G_0 relates to the magnitude of the field inhomogeneities and hence to the concentration of deoxyhemoglobin in the blood as described previously (26):

$$G_0 \approx \frac{64}{45} \pi^2 B_0^2 Hct [(1 - Y) \chi_{deo}]^2, \quad [4]$$

where χ_{deo} is the susceptibility difference between fully oxygenated and deoxygenated blood (0.264 ppm (27)). Therefore, Y can be determined using Eqs.1- Eq.4.

If only intravascular pO_2 is considered, then pO_2 (mm Hg) can be calculated by using the hemoglobin- oxygen dissociation curve via the Hill equation:

$$pO_2 = \frac{Y \cdot p_{50}^n}{(1 - Y)^{1/n}}, \quad [5]$$

where Y is the fraction of oxygenated hemoglobin in the capillary, P_{50} is the pO_2 corresponding to half-maximal hemoglobin saturation, and n is the Hill coefficient (for rat, $p_{50} = 36.9$ mm Hg and $n = 2.26$ (28)).

Methods

Tumor Models

This study was approved by the Institutional Animal Care and Use Committee. Two well-characterized Dunning R3327 rat prostate cancer lines: AT1 and MAT-Lu were chosen. The AT1 subline is anaplastic with a tumor volume doubling time of 5 days, while the MAT-Lu is a highly metastatic carcinoma, which arose spontaneously from the AT1 and has less organized filamentous structures and grows more rapidly (29). Small tumor pieces from donor rats were implanted subcutaneously in the thigh of eight male Copenhagen rats (~200 g; 4 AT1 and 4 MAT-Lu) by making a small incision. MRI was performed when tumors reached approximately 0.5-2.5 cm in diameter, typically, 8-9 days after implantation for MAT-Lu and 14-20 days for AT1 tumors.

^1H MR Imaging

MRI measurements were performed using a horizontal bore 4.7-T system (Varian, Palo Alto, CA). Anesthesia was induced in the rats with isoflurane (5%) and maintained as an isoflurane/air mixture (1.5% isoflurane at 2 L/min air) during MRI. Anesthetized animals were placed on a plastic bed with a thermal blanket and a flow of warm water to maintain body temperature at 37°C . The tumors were placed inside a 35 mm home-built single-turn solenoid volume coil, tunable to ^1H or ^{19}F . A fast spin-echo (FSE) sequence provided sagittal anatomic images of the entire tumor, $\text{TR}/\text{TE} = 3000/56$ ms, Echo Train Length

(ETL)= 4, FOV= 40 × 40 mm with 128 × 128 acquisition matrix, and 15-25 slices 1-mm thick without gap. A single slice encompassing the largest cross section of tumor was selected for pO₂ measurements.

An Inversion Recovery (IR) turbo Fast Low-Angle SHot (FLASH) pulse sequence with magnitude reconstruction was performed for quantitative T₁ measurement. It comprised a single inversion pulse and acquisition of a series of 16 rapid gradient echo FLASH images. At each inversion pulse 16 k-space lines were sampled and this was repeated with subsequent inversion pulses until all lines of k-space were sampled. TR/TE= 2700/5 ms, flip angle= 10°, FOV= 40mm × 40 mm with 128 × 64 acquisition matrix, and single slice with 2-mm thickness. Optimized TI with five different TI values, 50, 200, 500, 1100, 2300 ms was used. The total acquisition time for 3 averages was 2 min 50 sec. The T₁ mapping was performed using Eq.:

$$S = P_1 + P_2 e^{-TI/T_1}, \quad [6]$$

where S is signal intensity, TI is the inversion time and p₁ (= M₀) and p₂ (= M₀*(1-cosθ)) are model parameters determined by the equilibrium magnetization M₀, the effective flip angle θ, and pulse sequence structure and timing. The magnitude of p₂ represents the maximum dynamic signal range that can be sampled during T₁ recovery (30). Curve-fitting to estimate T₁ from the inversion-recovery images was based on the robust rapid reduced dimension non-linear least squares (RD-NLS) method of Barral *et al.* (31).

A multiple spin-echo CPMG sequence was performed for quantitative T₂ measurement using TR = 2000 ms, τ_{cp} = 10 ms, TE = n × τ_{cp}, n is the echo train length (ETL) of 12, image matrix 128 × 64, and one slice with 2 mm thickness and FOV of 40mm×40mm. The total measurement time was therefore 4 min 22 sec for 2 averages. T₂ maps were generated by fitting the following two parameter equation to corresponding pixels from each of the echo images:

$$S = S_0 e^{-TE/T_2}, \quad [7]$$

where S is signal intensity, S₀ is initial transverse magnetization.

Diffusion weighted (DW) images were obtained by using a multi-shot FSE-based DWI sequence (FSE-DWI). The FSE-DWI sequence comprised a 90° excitation pulse, a pair of pulsed gradient spin echo diffusion gradients about the first 180° refocusing pulse, and an FSE readout. Diffusion gradients were applied in three orthogonal directions and 10 b-values were acquired with TR = 2000 ms, effective TE = 56 ms, ETL = 8, image matrix 128 × 64, and a single slice 2 mm thick slice with FOV of 40×40mm. On the basis of IVIM theory, diffusion-weighted MRI (DWI) using low b-values (<300 s/mm²) permits imaging of IVIM, which combines diffusion of water molecules and microcirculation in capillaries (32). The bi-compartmental IVIM model used for the diffusion-weighted magnetization S is defined by four parameters(33): total magnetization S₀, perfusion fraction f_p, pseudodiffusivity D_p, and tissue diffusivity D_t. Previous reports indicate that the TE dependence of f_p is most important for tissues with transverse relaxation times significantly

shorter than that of blood (34). To account for the TE dependence of f_p , the signal intensities of the dataset were fitted to a modified IVIM-equation as (34):

$$\frac{S}{S_0} = \frac{(1 - f_p) (1 - \exp(-TR/T_{1tiss}) \exp(-TE/T_{2tiss} - bD_t)) + f_p (1 - \exp(-TR/T_{1blood}) \exp(-TE/T_{2blood} - bD_p))}{(1 - f_p) (1 - \exp(-TR/T_{1tiss}) \exp(-TE/T_{2tiss})) + f_p (1 - \exp(-TR/T_{1blood}) \exp(-TE/T_{2blood}))}, \quad [8]$$

where S_0 and S are signal intensities at b of 0 and b of 25, 50, 100, 150, 200, 300, 500, 1000 and 1500 s/mm², respectively. T_{1tiss} (from Eq. 6), T_{2tiss} (from Eq. 7) and T_{1blood} (1773 ms (35)) and T_{2blood} (from Eq. 2) are the longitudinal and transverse relaxation times of tissue and blood, respectively.

After the blood volume fraction f_p was determined (Eq. 8), a biexponential fit on the CPMG sequence was applied to Eq. 1 to determine R_{2ex} . All sequential scans including T_1 , T_2 maps and IVIM DWI were repeated five times to examine the spatial and temporal variations of tumor oxygenation over a time period of about 70 minutes.

pO₂ Mapping Based on ¹⁹F MRI

For direct comparison, the tumor oxygenation was also measured using the FREDOM (Fluorocarbon Relaxometry using Echo planar imaging for Dynamic Oxygen Mapping) method, which has been validated previously using oxygen electrodes, fiber optic oximetry and histological correlation (36). Following ¹H MRI the rat was removed from the magnet and hexafluorobenzene (HFB, 50 µl; Lancaster, Gainesville, FL) was injected directly into a plane of the tumor closely matching that for ¹H using a Hamilton syringe (Reno, NV) with a custommade fine sharp needle (32G). Conventional spin echo scout images were obtained for both ¹H (200.1 MHz) and following retuning of the RF coil ¹⁹F (188.3 MHz) to reveal HFB distribution within the tumor. The FREDOM sequence has been described in detail previously (36), briefly, it consists of a train of saturation pulses followed by a recovery time τ ranging from 0.2 to 90 ms ($n = 14$ delay times) and a spin-echo echo planar readout. The acquisition parameters were used: FOV=40×40 mm, matrix size= 32×32, slice thickness =10 mm, in-plane voxel size = 1.25×1.25 mm, and acquisition time 6.5 min. FREDOM scans were also repeated 5 times. The ¹⁹F R_1 was estimated using a three-parameter mono-exponential fitting and pO₂ (mm Hg) was estimated using the relationship (36):

$$pO_2 = (R_1 - 0.0835) / 0.001876, \quad [9]$$

Data Analysis and Processing

All ¹H MRI multi-parametric and ¹⁹F MRI data were fitted using the Levenberg-Marquardt nonlinear least squares algorithm and the adjusted R^2 statistic and root mean square error (RMSE) were used to assess goodness-of-fit. The quantitative results obtained for all ¹H MRI-derived parameters are shown as mean values for the group of eight tumor and muscle ROIs (Table 1). Tumor regions of interest (ROIs) were manually delineated on the anatomic T_2 -weighted images. ROIs were also selected for the muscle on the same slice. The mean ROI size across animals was $0.93 \text{ cm}^3 \pm 0.72$ (standard deviation). Each ROI was transferred on T_1 , T_2 , f_p , Y and pO₂ maps. For site-to-site comparison between ¹H and ¹⁹F MRI pO₂ estimates, all ¹H MR images were downsampled to match the matrix of ¹⁹F with

32×32 pixels. Image overlay of ^{19}F on the corresponding ^1H MR image was performed to confirm HFB distribution.

The quantitative measurements of site-to-site pO_2 made from ^1H and ^{19}F MRI were compared by calculation of the intra-class correlation coefficient, linear regression analysis, and the method of Bland and Altman (37). All ^1H and ^{19}F MRI data and statistical analysis were processed using in-house Matlab (MathWorks Inc., Natick, MA) scripts. Statistical significance was defined as $p < 0.05$. In all linear regression analyses, power analysis at the 5% level of significance was performed to verify the acceptability of the statistical results.

Results

Multi-parametric ^1H and ^{19}F MRI data and associated fitted curves are shown for one representative rat (AT1; Fig. 1). The quantitative T_1 , T_2 , and fp together with derived Y and pO_2 results showed no significant differences ($P > 0.5$) between the tumor sublines and are grouped (Table 1). Mean and standard deviation for the eight rats are given for the whole tumor and muscle ROIs. Fig. 2 shows representative ^1H MRI-derived parameter maps obtained from one animal (MAT-Lu; No. 7). The tumors were found to have longer T_1 and T_2 relaxation times compared with muscle. The tumor showed spatially heterogeneous blood volume, Y and pO_2 distributions (Fig. 2).

Corresponding site-to-site pO_2 measurements obtained using ^1H and ^{19}F methods are shown in Table 2. A strong linear relationship (regression line slope = 1.002, intercept = -1.6, $r = 0.95$; $p < 0.0001$) and correlation (intra-class correlation coefficient, 0.98; $p < 0.0001$) was observed between quantitative pO_2 measurements obtained using ^1H and ^{19}F MRI (Fig. 3). Bland-Altman analysis (Fig. 3b) indicated a mean difference in pO_2 (^1H MRI minus ^{19}F MRI) between methods was 1.6 mm Hg, with a slight, but insignificant positive bias (bias line slope = -0.03, intercept = 2.1, $r=0.01$; $p=0.78$).

Repeat measurements over a period of 70 minutes indicated fluctuations in whole tumor and muscle parameters, as shown in Figure 4 for all tumors ($n = 8$). Each parameter showed fluctuations with greatest variation in fp and pO_2 . Tumor T_1 ranged from 1668 ms to 2415 ms and T_2 ranged from 36.0 ms to 66.8 ms, while tumor fp ranged from 0.10 to 0.56 and pO_2 ranged from 16 mm Hg to 72 mmHg. By comparison, the muscle showed less variation in T_1 (from 1338 ms to 1692 ms), T_2 (from 25.4 ms to 32.8 ms) and pO_2 (from 29 mm Hg to 59 mmHg), even though fp had relative greater changes (from 0.13 to 0.39).

Discussion

Tumor tissue pO_2 can be measured non-invasively using ^1H MRI derived multi-parametric maps and the correlation with ^{19}F MRI oximetry confirmed the accuracy of tumor pO_2 measurements. Quantitative noninvasive assessment of pO_2 appears particularly significant since multiple reports indicate that hypoxic tumors are more aggressive and respond less well to therapy; most notably, radiation (1). Local pO_2 has been assessed using electrodes and fiber optic probes each of which has been used extensively in pre-clinical studies of tumors including Dunning prostate tumors growing in rats(38,39), but both methods are invasive and only sample specific locations. ^{19}F MRI of HFB (so-called FREDOM) has

provided the reference here to validate the new multi-parametric ^1H MRI approach, but it has not been applied to humans due to lack of clinical ^{19}F MRI and the need to inject a reporter molecule. Hypoxia, as opposed to pO_2 may be assessed using various radionuclide reporters (1,2). The proposed method (MOXI) has multiple potential advantages including being entirely non-invasive, avoiding the need for an exogenous reporter molecule, avoiding radioactivity and providing pO_2 estimates throughout the tumor.

Due to the dependence of blood R_2 relaxation rate on the inter-echo spacing measured using CPMG sequence, the Luz-Meiboom chemical exchange and diffusion models have been proposed to estimate blood oxygenation status (16,21). Previous studies also showed that the diffusion model can better describe the dependency of blood R_2 on oxygen saturation than Luz-Meiboom chemical exchange model (40). In this study, we assumed that signal in a voxel is influenced by contributions from blood and non-blood components. To examine the oxygen-sensitive CPMG T_2 changes in tissue, the tissue microcirculation must be evaluated accurately and two-site proton exchange (41) and IVIM models (32) were used to estimate the fraction of the two components. High accumulation of iron in tumors could cause local susceptibility changes in the magnetic fields affecting the accuracy of the two-site proton exchange model for estimation of the fraction of two components. By comparison with IVIM models the diffusion signal is restricted to two major compartments: blood flow within the vascular space and water diffusion in the extra-vascular space. The vascular compartment exhibits higher mobility and therefore persists only at low b-values. The ADC measurement based on signal at low b-values has been implemented to map flow and volume changes in microvascular networks (42). It is expected that perfusion fraction, f_p , provides more purely intravascular information than the longer time scale perfusion scan that includes extravasations (43). However, the blood volume fraction measured by IVIM is also different from that measured using intravascular tracers (*e.g.*, contrast media) or estimated from relative CBF change using Grubb's CBF-total CBV relationship (44), because such perfusion estimates are based on tissue uptake or removal of the injected tracer (45). The results demonstrate that pO_2 values were heterogeneous, consistent with previous studies of solid tumors (1,2,39,46). It is particularly reassuring that the pO_2 values estimated using the new approach closely match the ^{19}F MRI estimates across the whole range of pO_2 values from 4 to 67 mm Hg (Table 2 and Figure 3). The overall pO_2 distributions also coincide with previous reports for these tumor types based on various technologies (39,47-49). The rat muscle tissue had a mean baseline pO_2 of 39 ± 5 mm Hg, which is comparable to earlier studies by others using EPR oximetry (40 ± 8 mm Hg) (50).

It is important to note limitations of the proposed ^1H method for pO_2 estimation. First, while most oxygen in the vasculature is carried by hemoglobin several factors affect the oxygen dissociation curve including plasma pCO_2 , pO_2 and pH, as well as temperature, lactate and hematocrit, which we assumed to be constant for all rats. Second, our measurement of blood volume was based on a modified IVIM model with the major contributions from arterioles. Tumor blood vessels differ from normal vasculature and the microvessel fractions for arterioles, capillaries, and venules are unknown. Although the fast diffusion component suppressed using low b-values is sensitive to micro-capillary perfusion, other bulk flow phenomena, such as tubular flow or glandular secretion can contribute to the signal

attenuation and may be difficult to distinguish from perfusion effects (32). While our approach taken as a whole produced successful estimation of pO₂ values, the derived perfusion fraction values in skeletal muscle are somewhat high, though within the range of reported values in the literature of human skeletal muscle (51). Future work may consider potential biases from low SNR or non-gaussian diffusion effects at high b-values in these measurements. Third, the proposed method may be limited by some assumptions introduced in the signal calculation given by Eq. 2-5 and Eq.8, namely blood relaxation times at specific field, Hct and blood cell size. Ultimately, translating current observations and findings into clinical practice or preclinical studies with different species should take such assumptions into consideration.

There is an increasing interest in exploiting multiple oxygen-sensitive ¹H MRI parameters, such as T₂* and T₁ contrast to examine tissue oxygenation (52,53). The integration of multiple parameters to estimate pO₂ itself represents the future direction. Noting the developing interest in tumor hypoxia and evidence for predictive value in terms of response to therapy and disease free survival, this new non-invasive approach should become a valuable asset in the armamentarium of the radiologist.

In conclusion, our findings confirm that non-invasive measurement of tumor pO₂ using ¹H MRI derived multi-parametric maps is feasible and it could become a valuable tool to evaluate tumor oxygenation.

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Figure 1.

Data quality and curve fitting for multi-parametric ^1H and ^{19}F MRI data. Examples are shown for ROI of representative AT1 tumor (No.1). In each case the experimental data ($\bullet$) are shown together with fitted curve (solid line). (a) Tissue $T_1 = 2204$ ms (adjusted $R^2 = 0.986$) obtained by mono-exponential fitting of the signal intensities for different TI values obtained using IR turbo-FLASH sequence (Eq. 6). (b) The tissue $T_2 = 63$ ms (adjusted $R^2 = 0.989$) obtained from mono-exponential fitting of the multi-TE CPMG sequence (Eq.7). (c) The blood volume fraction f_p of tissue ($f_p = 0.63$, adjusted $R^2 = 0.996$) was calculated using biexponential fitting model of the multi-b FSE-DWI sequence (Eq. 8) ($\text{SNR} > 2.6$). For direct comparison the mono-exponential curve fitting of $S = S_0 e^{-b\text{ADC}}$ is also shown in (d) (adjusted $R^2 = 0.976$). After f_p was determined, a biexponential fit on the CPMG sequence (c) was applied to Eq. 1 to determine $R_{2\text{ex}} = 13.4$, (adjusted $R^2 = 0.990$). (f) The ^{19}F R_1 was estimated using a three-parameter mono-exponential fit to the FREDOM data yielding $R_1 = 0.2085 \pm 0.0164 \text{ s}^{-1}$ (adjusted $R^2 = 1$ indicating $\text{pO}_2 = 67 \pm 9 \text{ mm Hg}$).

Figure 2.

Representative ^1H MRI parameter maps, derived Y and pO_2 maps from a MAT-Lu tumor (No. 7). (a) T_2 -weighted image; (b) T_1 map; (c) T_2 map; (d) Blood volume fraction (f_p) map; (e) derived oxygen saturation (Y) map and (f) pO_2 map. Compared to muscle (M), the tumor (T) showed longer T_1 and T_2 relaxation times, heterogeneous blood vessels, Y and pO_2 distributions.

Figure 3.

Comparison of pO₂ measurements obtained using with ^1H MRI and ^{19}F MRI. **(a)** linear correlation between ^1H and ^{19}F MRI oximetry: regression line slope = 1.002; intercept = -1.6 ($r = 0.953$; $p < 0.0001$). Intraclass correlation coefficient = 0.98 ($p < 0.0001$). **(b)** Bland-Altman plot shows limits of agreement. For difference (^1H minus ^{19}F pO₂), 95% CI is -7.6 to 10.7 mm Hg. At statistical power of 0.95, no significant bias was detected (bias line slope = -0.03; intercept = 2.1; $r = 0.01$; $p = 0.78$).

Figure 4.

Mean temporal fluctuations observed over a period of 70 minutes for the group of 8 rats across whole tumors (•) and muscles (□)(a) T_1 , b) T_2 , c) f_p , d) Y and e) pO_2 .

Table 1Quantitative Measurements of T_1 , T_2 , f_p , Estimated Y and pO_2 in rat muscle and whole tumor by 1H MRI

ROI	T_1 (ms)	T_2 (ms)	f_p	Y	pO_2 (mm Hg)
Muscle	1480±86	29±2	0.22±0.03	0.49±0.06	39±5
Tumor	1980±186	59±9	0.23±0.10	0.53±0.12	36±15

Note: Data are mean ± standard deviation for the group of eight tumors (4 AT1 + 4 MAT-Lu).

Table 2

The site-to-site pO₂ comparison of ¹H MRI and ¹⁹F MRI in each rat

Animal No.	Tumor Subline	¹ H MRI	¹⁹ F MRI	P value
1	AT1	66±5	67±4	0.94
2	AT1	39±3	39±3	0.77
3	AT1	10±2	11±4	0.52
4	AT1	13±3	18±4	0.12
5	MAT-Lu	5±3	4±2	0.24
6	MAT-Lu	9±4	8±3	0.41
7	MAT-Lu	17±5	12±4	0.33
8	MAT-Lu	24±14	14±7	0.23

Note. Values are mean pO₂ (mm Hg) for the corresponding ROIs with standard error reflecting variations over five successive determinations. P values are two-tailed probability values of a Student's t-test. The linear correlation, intra-class correlation coefficient, Bland-Altman plot between ¹H MRI and ¹⁹F MRI are shown in Fig.3.