

Identification of High Intensity Focused Ultrasound treated tumor tissue using a multiparametric MRI protocol and ISODATA analysis

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Introduction

To advance the clinical applicability of ablation of malignant tumors by High Intensity Focused Ultrasound (HIFU) it is of key importance that residual or recurrent tumor tissue can be distinguished from successfully ablated tumor tissue. The goal of this study was to identify a set of clinically translatable Magnetic Resonance Imaging (MRI)-based biomarkers that can be used to evaluate HIFU treatment. To that aim a time-efficient and quantitative multiparametric MRI protocol was developed, which was used to longitudinally assess tumor tissue status after HIFU treatment in a murine tumor model. Image clustering techniques were used to identify HIFU-treated and non-treated tumor tissue. Histology of whole tumors was performed to correlate non-viable tumor fractions with HIFU-treated tumor fractions derived from the multiparametric MRI data.

Materials and methods

Tumor model

10-12 weeks-old Balb/c mice were inoculated subcutaneously in the right hind limb with 2×10^6 CT26 colon carcinoma cells. MRI was performed 24h before ($n=11$), directly after ($n=11$) and 3 days after ($n=6$) ablation. MRI of a control group ($n=2$) that did not undergo HIFU treatment was done at the same three time points.

MRI protocol

MRI was performed with a 6.3T Bruker scanner. The multi-slice imaging protocol consisted of Look-Locker-based T_1 mapping, MLEV-prepared T_2 mapping, double-spin-echo prepared Apparent Diffusion Coefficient (ADC) mapping and magnetization transfer rate (MTR) mapping. For all sequences signal read-out was performed with echo planar imaging (EPI) to allow for fast acquisition. The acquisition time of the entire protocol was approximately 50 minutes.

Ablation protocol

Tumor ablation was performed with an 8-element preclinical therapeutic ultrasound transducer (TIPS, Philips). Ablation settings were: acoustic power 12 W, frequency 1.4 MHz, duty cycle 50%, treatment time 30s, wait time 120s. The treatment area was defined by a 16 mm² square grid of 25 treatment points.

MRI data analysis

T_1 , T_2 , ADC and MTR values were calculated using the acquired images. Based on the parameter values, the tumor pixels originating from all the performed MRI experiments were segmented into clusters using the Iterative Self Organizing Data Analysis (ISODATA) technique.¹ Clusters of which the fraction of pixels assigned to them was significantly (Student's t-test, $p < 0.1$) increased post ablation as compared to pre ablation were identified as HIFU-treated (ablation-associated) tumor tissue. The remaining clusters were assumed to represent non-treated tumor tissue.

Quantitative histological analysis

Excised tumors ($n=5$) were sectioned into 5 μ m sections with an inter-section distance of 300 μ m. Sections were stained for nicotinamide adenine dinucleotide (NADH) diaphorase activity, which is a marker for cell viability. ROIs of entire tumor tissue and non-viable tumor tissue were manually drawn on the brightfield microscopy images. From these ROIs the fractions of non-viable tumor tissue were calculated.

Results

Representative T_2 -weighted images pre and post ablation are shown in Figure 1 A and B. After ablation a heterogeneous, hypo-intense region emerged. Results of the clustering analysis are shown in Figure 1 C and D. After ablation a region appeared in which pixels were assigned to clusters that were identified as HIFU-treated tumor tissue. Mean values of the different MRI parameters are listed in Table 1. A significant increase in R_1 , R_2 , ADC and MTR was observed in HIFU-treated tumor tissue as compared to non-treated tumor tissue. A representative microscopy image of a section of ablated tumor tissue stained for NADH-diaphorase activity is shown in Figure 2. A clear white region is visible, which represents non-viable tumor tissue. A strong correlation ($r=0.96$, $p < 0.01$) was observed between histologically derived non-viable tumor fractions and HIFU-treated tumor fractions estimated from MRI (Figure 3).

MRI parameter	Non-treated tumor tissue	HIFU-treated tumor tissue
R_1 [s ⁻¹]	0.46±0.05	0.54±0.15 **
R_2 [s ⁻¹]	21.3±5.4	24.6± 10.0 **
ADC [mm ² s ⁻¹]	0.83±0.41	1.19±0.44 **
MTR [%]	23.5±5.5	24.9±7.7 **

Table 1 Mean±SD values of MRI parameters for non-treated and HIFU-treated tumor tissue following ISODATA segmentation.

** indicates significant difference between non-treated and HIFU-treated tumor tissue, Wilcoxon rank-sum test, $p < 0.001$.

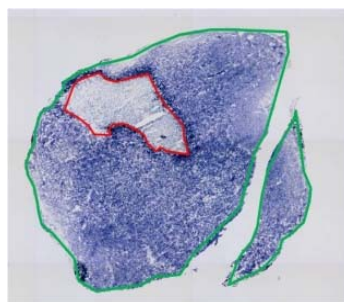


Figure 2: Representative microscopy image of a NADH-diaphorase stained tumor section. The green lines delineate the ROI of the tumor tissue. The red lines delineate the ROI of non-viable tumor tissue.

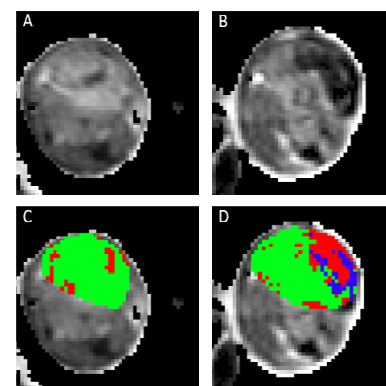


Figure 1 A,B: representative T_2 -weighted images pre and post ablation respectively. C,D: same images with results of ISODATA clustering analysis overlaid on tumor pixels. Green: tumor tissue pixels from clusters representing non-treated tumor tissue; red: tumor tissue from clusters identified as HIFU-treated tumor tissue; blue: pixels excluded from analysis because of low SNR (mainly associated with HIFU-induced hemorrhage).

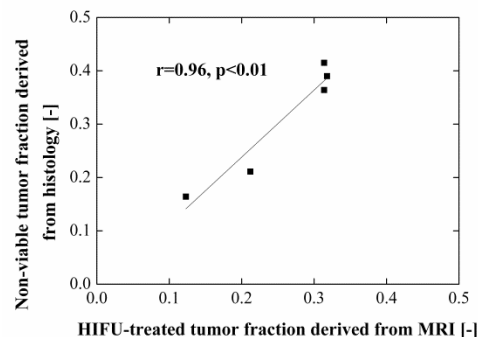


Figure 3: Correlation plot of histologically derived non-viable tumor fractions and HIFU-treated tumor tissue fractions derived from MRI.

Conclusion

These results show that HIFU-treated tumor tissue can be distinguished from non-treated tumor tissue using a time-efficient multiparametric MRI protocol combined with an ISODATA clustering algorithm. A strong correlation was observed between MRI-derived HIFU-treated tumor fractions and histologically derived non-viable tumor tissue fractions.

Acknowledgement

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¹ Jacobs et al. *Stroke* 2001;32:950-957