Monitoring quantitative tumor blood volume in mouse brain under Bevacizumab by the RSST1-MRI method.

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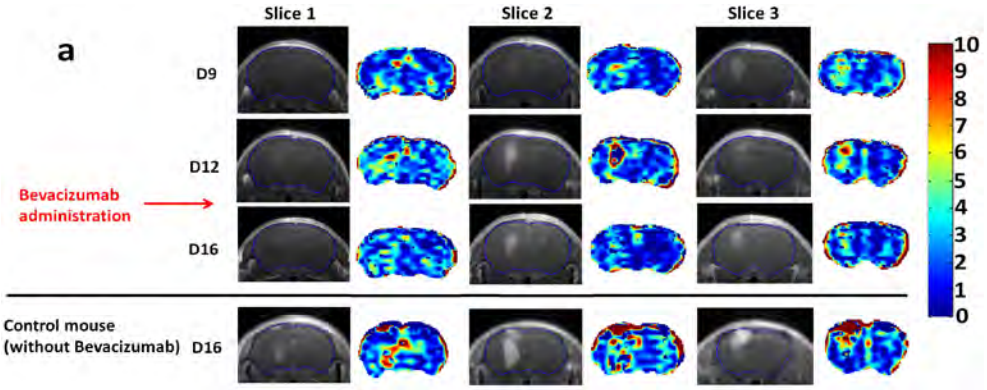
Introduction: The objective of this study is to demonstrate the sensitivity of the $RSST_1$-MRI method for quantifying the tumor blood volume fraction (BVf) and detection of vasculature changes during tumor growth and at an early stage after anti-angiogenic treatment with *Bevacizumab®*.

Materials and methods: U87 human glioblastoma cells (5.10^5) were injected in nude mice brain (n=7). MRI was performed at days 9, 12 and 16 (D9, D12 and D16) after tumor cell implementation. At D12, and after MRI, *Bevacizumab®* an antiangiogenic treatment was administrated at a dose of 10mg/kg intravenously (i.v) (n=3).

Mice were imaged at 4.7 T (47/40 Bruker Biospec). 8 contiguous slices of 1mm thickness, field of view of 16×16 mm^2, and a 32×32 matrix were used. The $RSST_1$ sequence for BVf quantification was a 3D inversion recovery sequence (TR=750ms, T_{inv}=305ms.) followed by a gradient echo (TE=2ms, TR=10ms). After 5 min of acquisition (S_{pre} signal), Gd-DOTA was i.v injected (0.7 mmol/kg) and S_{post} signal was acquired during 10 min. In absence of Gd-DOTA extravasation, BVf is derived from the $RSST_1$ signal (S_{norm}) according to ($BVf=S_{norm} = (S_{pre}–S_{post})/S_0$) [1], where S_0 is the fully relaxed magnetization, acquired for normalization. In presence of Gd-DOTA extravasation, BVf corresponds to S_{iv}, derived from the following mathematical model of the $RSST_1$ signal ($S_{norm}(t) = S_{iv} + S_L\ [1 – \exp(-\kappa_{model}*(t-t_0))]$ [2], where t_0 is the time of CA leakage starts, S_L is the volume fraction of the leakage compartment and κ_{model} is the parameter related to the endothelial permeability. Contrast-enhanced T_1-weighted MRI (CE-MRI) was acquired 20 min after Gd-DOTA injection.

Results: BVf changes were detected during tumor growth and after *Bevacizumab®* administration (Fig.1a). Between D9 and D12, tumor BVf increased significantly (3.6 ± 0.5 % and 5 ± 0.5 % respectively P_{value} = 0.016) and remained constant at D16 (Fig.2). As expected, a significant tumor BVf decrease was clearly detected at D16 (P_{value} = 0.019), i.e. 4 days after *Bevacizumab®* administration (Fig.1 and 2c). These results are in accordance with previous study [3]. During the tumor growth and after treatment, the tumor BVf increase appeared early, while CE-MRI didn't show any changes (Fig.1a, slice 1). Fig.1b shows the blood vasculature in the whole brain slice using two-photon microscopy and a new lipophilic tracer. A comparable blood volume distribution is observed with MRI images. Future work will be focused to correlate quantitatively BVf between the two methods [4]. Between D9 and D12, the permeability of tumor microvasculature also increased (0.038 ± 0.012 s^{-1} and 0.05 ± 0.01 s^{-1}), but at D16, it decreased significantly both in the case of treatment and controls, reaching the same values (0.027 ± 0.001 s^{-1} and 0.028 ± 0.008 s^{-1} respectively). In Fig.2a, the $RSST_1$ signal reached a constant value, a signature of regions without Gd-DOTA extravasation i.e. with intact BBB. In Fig.2b, an increase of $RSST_1$ signal is observed; it was due to Gd-DOTA extravasation in regions for which the BBB is damaged. During tumor growth, we observed an increase of the $RSST_1$ curve parameters (magnitude and slope) and their decrease after treatment. These parameters are obviously directly related to BVf and vessel permeability changes respectively; so as first analysis and before quantification, they can be directly used to assess the tendency of angiogenesis evolution.

Discussions and Conclusion: The sensitivity of the $RSST_1$ method to detect tumor BVf changes is demonstrated. In fact BVf changes were: i) increased at early stage of tumor growth, while CE-MRI didn't show yet any changes and (ii) decreased after *Bevacizumab®* administration. $RSST_1$ is suitable to assess the effect of new anti-angiogenic therapies and will help in simple way, physicians to take early decisions to stop, continue or change therapy strategies during treatment.



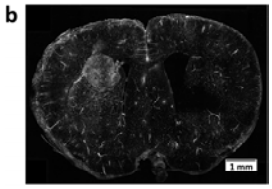


Fig.1: (a) U87 tumor growth follow-up from one typical mouse by CE- MRI and quantitative BVf (reconstructed matrix 128×128). (b) Typical two-photon image (mosaic z-projection) corresponds to slice 2, D16 with treatment.

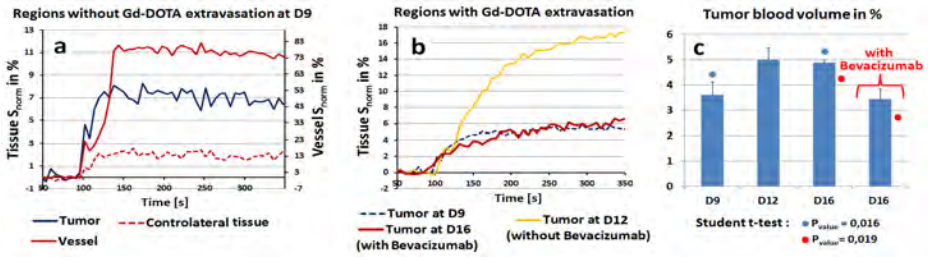


Fig.2 $RSST_1$ signals: (a) at D9, in regions without CA extravasation (vessels, contralateral and tumor region) and (b) in tumor regions with CA extravasation (D9, D12 and D16). (c) BVf histogram: evidence of BVf decrease after *Bevacizumab®* injection.

Reference: [1] Perles-Barbacaru et al JCBFM 2007. [2] Sarraf M et al, MRM 2014. [3] Kimberly R. Pechman et al, JMRI 2012. [4] Vérant P et al, JCBFM 2007.