The complementary value of arterial spin labeling next to contrast-enhanced MRI in the diagnosis of brain tumor invasion in mouse models

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Target audience – Neuroradiologists, Neurooncologists, Neurosurgeons

Purpose – Arterial spin labeling (ASL) provides valuable information about blood hemodynamics, e.g. cerebral blood flow (CBF), by using blood water molecules as an endogenous tracer. We aimed to first evaluate ASL to diagnose diffuse infiltrative glioma in well characterized xenografts mouse models and then combine it with the commonly used method, i.e. contrast-enhanced (CE) MRI.

Methods – We investigated female athymic Balb/c nu/nu mice carrying orthotopic glioma xenografts derived from:[1] glioblastoma (E468 (n=6), E473 (n=7) and E98 (n=8)) and anaplastic oligodendroglioma (E434, n=6). When tumor-related symptoms became apparent, MRI experiments were performed using an 11.7T BioSpec scanner (Bruker, Germany). To visualize the brain anatomy a gradient echo sequence was used. Pulsed-ASL data were acquired by a flow sensitive alternating inversion recovery (FAIR) EPI sequence with the following parameters: TR=18s, TE=12.9ms, FOV=25×25mm^2, slice thickness=1mm, matrix=128×96, and acquisition time (TA)=12min. All data were analyzed using ParaVision 5.2 and MATLAB (Mathworks, USA). Circular regions of interest (ROIs) of equal size were selected in the tumors and in the normal appearing brain tissue (NABT) by visual identification of tumor on the T_2-w images confirmed in retrospect on corresponding haematoxylin & eosin (H&E) stained sections. Pre- and post-contrast MRI was performed on E98 xenografts, which present with both compact and invasive tumor features, by injecting gadolinium-DOTA (0.3 mmol/Kg) using a RARE T_1-w sequence with the following parameters: TR=1500ms, TE=7.5ms, FOV=30×30mm^2, slice thickness=1mm, matrix=256×256, TA=1min. Directly after the MRI experiments, brains were formalin-fixed and paraffin-embedded. Sections (4µm) were stained with H&E. Immunohistochemistry was performed using Ki67 to detect tumor cell proliferation. For statistical analyses Prism software (GraphPad Software Inc, CA) was used. We used one-way analysis of variance (ANOVA) for group comparison and paired two-tailed t-test for comparing quantities in tumor versus NABT within a group and p-values less than 0.05 were considered significant.

Results – In all xenograft models CBF was markedly decreased (Figs. 1b, 2, and 3, p<0.001). The location and extent of areas with lower CBF were in good agreement with presence of tumor as evidenced by histopathology. Mean tumor CBF in the highly diffuse infiltrative xenograft model E468 was higher than in E473 (p<0.05) and E434 (p<0.001) and differed significantly between E473 and E434 (p<0.05, Fig 2). Interestingly similar to previous observations[2-4], in all tumor models we found a significant decrease in blood flow in the xenografts compared to normal brain. CE MRI experiments showed that only compact parts of E98 xenografts can be detected post-contrast while ASL detected both compact and diffuse parts of tumors (Fig. 3). The Ki67 proliferation index, that can be considered as an indirect measure of metabolic activity, was highest in the most compact xenograft line E434 (mean of 79.8%). Ki67 index in E468 and E473 xenografts were 64.2% and 76.3%, respectively (Fig. 4). Proliferation indices differed significantly between the most invasive model E468 and the most compact model E434 (p<0.05).

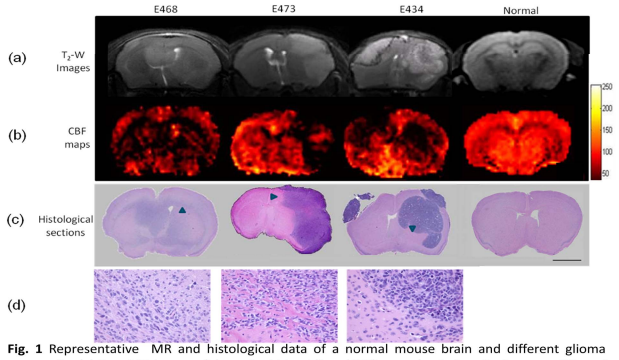

Fig. 1 Representative MR and histological data of a normal mouse brain and different glioma xenografts. (a) T_2-w MR images, (b) cerebral blood flow (CBF) maps (scale bar displays quantities of blood flow), (c) H&E histological sections (scale bar represents 2mm). (d) enlarged H&E images of regions pointed with arrowhead in Fig. 1c.

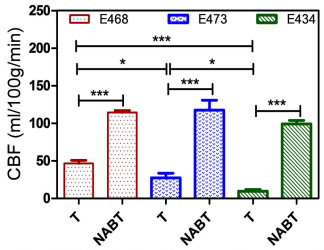

Fig. 2 CBF is significantly decreased in tumor (T) areas of all glioma models as compared to normal appearing brain tissue (NABT, p<0.001) and tumor CBF values in three different xenograft models are significantly different.

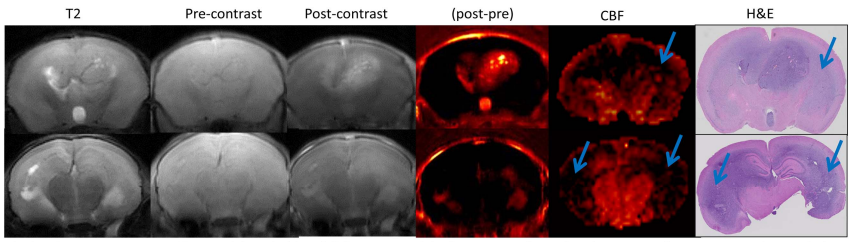

Fig. 3 Each row represents an E98 mouse injected with gadolinium. Top panel: both compact and more invasive tumor regions are present; CE MRI nicely detects the compact part of the tumor but fails in diagnosing invasive part. Bottom panel: this tumor has massively diffused with minimal solid parts; gadolinium again fails to detect tumor invasion. In both cases, CBF maps are nicely in agreement with histology showing tumor invasion (arrows).

Discussion – CBF maps were consistent with the histopathology results (Fig. 1 and 3). CBF decreased in all models compared to the NABT. This can be explained by tumor expansion without accompanying vessel formation and only limited vasodilatation, as evidenced by the combined vessel density and surface area measurements (data not shown). Due to the lack of vascular leakage, CE MRI failed to detect invasive parts of tumor while ASL was powerful to diagnose them by detecting alterations in the CBF. This has been shown in a human case-report with antiangiogenic therapy.[5]

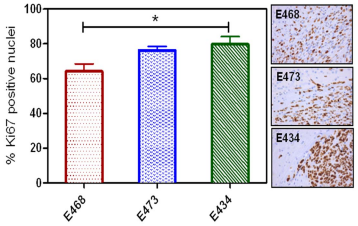

Fig. 4 Mean tumor cell proliferation in different xenograft models. Sections from each orthotopic xenograft were stained for Ki67 as a marker for proliferation. A representative Ki67 staining for each model is presented on the right side of the graph. Proliferation in the most invasive model E468 is significantly lower than that of the most compact line E434.

Conclusion – ASL has potentials to detect diffuse infiltrative parts of tumors and therefore could be considered as a complementary value to the CE MRI by which compact growing part is diagnosed. Our findings also suggest that ASL may be considered as a suitable alternative to the dynamic susceptibility contrast (DSC) MRI for investigation of brain perfusion. Notably, in contrast to DSC, ASL is a quantitative method that measures absolute blood flow and does not require contrast agent injection, making it a safe and repeatable diagnostic technique specially when it comes to patients with contraindication to contrast materials.

References – [1] A. Claes, J., et al., Brain Pathol, vol. 18, pp. 423-33, 2008. [2] Silva AC, et al., MRM, 2000;44:169-73. [3] Jerome NP, et al., JMRI, 2013. [4] Sun Y, et al., MRM, 2004;51:893-9. [5] Fellah S, Journal of clinical oncology, 2011;29:e308-11.