Target Audience: The present work may be addressed to people interested in the use of advanced models of DWI data fitting to assess the development of brain tumor in animal models.

Purpose: Alterations in inflammation and microvasculature underlie some of the most complicated brain diseases, like cancer. A deeper characterization of the inflammatory microenvironment in tumoral processes would improve its diagnosis, prognosis and help in the validation of new-targeted therapies. Magnetic resonance imaging (MRI) is a very well established and powerful tool for obtaining structural, functional and molecular information\(^1\)-\(^3\). It is a widely used and versatile technique in neurooncology\(^4\) that provides information about blood flow/volume, edema and cellularity\(^5\). In this line, this work was focused on the identification of DW parameters that allow characterizing the evolution of the blood perfusion and inflammatory microenvironment in a tumor as it grows. Our last goal, is to improve the knowledge of pathophysiological and biochemical basis of the coupling between inflammation and microvasculature alterations. We used for that a glioblastoma multiforme -the most frequent, aggressive and lethal intracranial tumor- animal model, DWI measurements at low and high b values, and three different models of data fitting in order to analyze the diffusion behavior and the perfusion contribution to the signal.

Methods: Adult male Wistar rats (n=5, 220-250g) were intracranial injected with C6 glioma cells and imaged in two stages of tumoral growth: small tumors (smaller than 50 mm\(^3\)) and big tumors (bigger than 75 mm\(^3\)) (Fig. 1). DWI: Animals were anesthetized with 1.5% isoflurane/oxygen during MRI protocols. We used a 7T Bruker Biospec scanner equipped with a 90mm gradient coil insert (360 mT/m) and a rat head resonator. DWI was acquired with 4 shot EPI-read gradient and in the L-R, A-P and H-F directions. Acquisition parameters were: δ/Δ=4/20ms, TR/TE=3000/31ms and a 237x273 µm\(^2\) in plane resolution. We obtained 9 high b value (300<b<2000 s/mm\(^2\)) and 9 low b value (10<b<600) acquisitions across 5 imaging planes centred in the tumoral area. Data analysis: The high b diffusion data set was fitted (MATLAB v7a) pixel by pixel either to a monoexponential S(b)/S(0)=exp(-b·ADC) or to a biexponential model S(b)/S(0)=SDP·exp(-b·D\(_{\text{slow}}\))+FDP·exp(-b·D\(_{\text{fast}}\)), with slow (SDP) and fast (FDP) diffusion coefficients characterized by slow (D\(_{\text{slow}}\)) and fast (D\(_{\text{fast}}\)) diffusion. The calculated high b biexponential coefficients were used to obtain the perfusion components affecting the low b measurements, in which the signal decay was thus considered to be triexponential S(b)/S(0)=f\(_{\text{perf}}\)·exp(-b·D\(_*\))+SDP·exp(-b·D\(_{\text{slow}}\))+FDP·exp(-b·D\(_{\text{fast}}\)), with a fraction of water molecules contributing to perfusion (f\(_{\text{perf}}\)) with a pseudodiffusion coefficient (D\(_*\)). On each slice, two ROIs were studied, the tumor and an area of the contralateral (CL) region (Fig.1). Statistical tests were performed between conditions and area using ANOVA tests.

Results: Our results show significant differences between the mean values of ADC, SDP and f\(_{\text{perf}}\) in the glioma and CL areas (Fig. 2). ADC values are higher in the tumor regions than in the CL, especially in big tumors. On the contrary, the lowest SDP values are also found in big tumors. D\(_{\text{slow}}\) and D\(_{\text{fast}}\) coefficients showed some directionally dependent differences between areas and tumor conditions, with generalized higher D\(_{\text{fast}}\) values in tumor than in CL regions. In the DWI-perfusion analyses, small tumors showed the highest f\(_{\text{perf}}\) values. D\(_*\) values showed high SD between animals and did not report robust changes between areas.

Discussion/Conclusion: We report that DWI can be used with high and low b weightings to emphasize different tumoral properties. At high b values, both mono&biexponential models revealed increased diffusion coefficients in big tumors. SDP fractions indicate diminished fractions of slow diffusion in big tumoral areas, suggesting that ADC increase might come from a shift of the number of molecule with slow to fast diffusion. Low b analyses indicate that small tumor have the highest perfusion components. This may reflect a major angiogenic state in the initial stage of the glioma development and/or a higher necrosis in the last phase of the tumors.