Perfusion/Diffusion mismatch in stroke: what about the hematocrit?

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Purpose: In a stroke lesion, determining the tissue which can recover from the one which will become necrotic remains challenging. The diffusion/perfusion mismatch, assessed by MRI, is used to localize: 1) the ischemic core (low diffusion and perfusion) and 2) the penumbra, i.e. the severely ischemic but still viable brain tissue (normal diffusion and low perfusion) [1]. *In vivo* perfusion techniques however assess the plasmatic flow: the contrast agent is plasmatic and therefore do not reflect the behavior of red blood cells, and thereby the tissue oxygenation status. In this preclinical study, MRI and a molecular imaging technique (autoradiography) were associated to map the perfusion/diffusion mismatch and the local hematocrit level (Hct) of rats with stroke.

Material and methods: ***-Animal model-*** Rats (n=7) underwent a permanent focal cerebral ischemia by occlusion of the right middle cerebral artery (MCAo) [2]. ***-MRI-*** Then, in the 30 min post occlusion animals were imaged at 4.7T on a Bruker Avance 3 console using a volume/surface cross coil configuration (IRMaGe facility). All data were acquired with the same geometry (5 contiguous, 800µm-thick slices, FOV=30x30mm; matrix=128x128). The acquisition protocol was: T2w, Inversion recovery, pseudo-Continuous Arterial Spin Labeling (pCASL), and diffusion-weighted (EPI-DWI sequence; b=800s/mm²) sequences. The entire MRI protocol lasted 15 min per animal. Apparent diffusion coefficient of water (ADC) and cerebral blood flow (CBF) maps were then computed from EPI-DWI and pCASL sequences, respectively. ***-Molecular imaging-*** Thereafter anesthetized animals were transferred in nuclear medicine facility for autoradiography to map the Hct (within the 2h post occlusion). Briefly, brains were excised 15 minutes following i.v. injection of a mixture of ^{99m}Tc-labelled red blood cells (RBCs) (37MBq) and ^{125}I-labelled albumin (3.7MBq). Two autoradiographic images were then successively performed on several 100µm-thick slices using a phosphor-imager (Fuji BASS 5000) to first map ^{99m}Tc+^{125}I and, after ^{99M}Tc decay (i.e. one week later), ^{125}I only. Fractional RBCs, plasmatic volumes, and eventually Hct were then derived. ***-Image analysis-*** The *ex-vivo* Hct maps were then co-registered to the corresponding MR images (T2w, ADC and CBF). Data, averaged across rats, are presented for 3 regions of interest delineating the stroke lesion on the ADC (red), CBF (blue) and on the Hct (yellow) maps and for 1 ROI in the contralateral hemisphere (green). The sizes of the ADC and of the Hct ROIs were normalized to the size of the CBF ROI, animal per animal. Paired and unpaired student t-tests were used to assess differences between stroke lesion and healthy tissues and between ROI sizes, respectively.

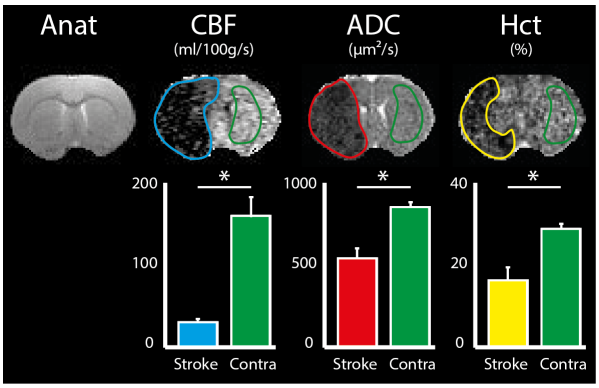

Fig. 1: Delineations of the stroke lesion on CBF, ADC and Hct maps (blue, red and yellow, respectively) and on a healthy ROI (green). Mean CBF, ADC and Hct values are presented on the bottom. Mean±SD. *: p<0.01.

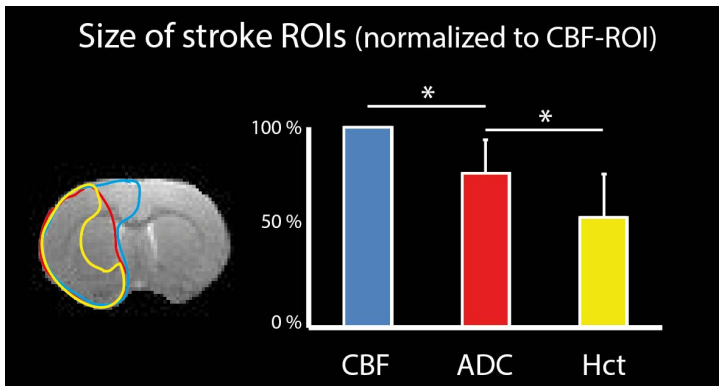

Fig. 2: Three different stroke delineations drawn on CBF, ADC and Hct maps (blue, red and yellow, respectively) overlayed on a T2w image. CBF, ADC, and Hct ROI sizes, normalized to the CBF ROI size (Mean±SD). *: p<0.01

Results: The mean CBF, ADC and Hct values measured in the stroke lesion were significantly lower than those estimated in the contralateral tissue (CBF: 30.1±3.8 *vs.* 164.8±21.6 mg/100g/s; ADC: 544±57 *vs.* 849±36 µm²/s; Hct: 16.3±3.3 *vs.* 28.8±1.4 % respectively, p<0.01; Fig. 1). For all animals, we also observed that: 1) the stroke lesion ROIs drawn on the CBF maps were significantly larger than the ones drawn on the ADC maps and 2) the stroke lesion ROIs drawn on the ADC maps were significantly larger than the ones drawn on the Hct maps (Fig. 2).

Discussion: Our results indicate that within the 2h post MCAo occlusion, major cellular (ADC) and vascular (CBF) modifications occur in the injured hemisphere. We could observe 3 different sizes of stroke lesion depending on the parameter studied. As expected, the diffusion lesion was smaller than the perfusion lesion. Interestingly, the stroke lesion defined on the Hct maps was always smaller and included in the lesion defined on the ADC map. Moreover, Hct values were not homogeneous within the CBF-ROI stroke lesion (and therefore in the ADC stroke lesion). Indeed, for example 35.4±15.6% of the CBF lesion had a normal Hct value (i.e. ≥ mean contralateral value minus 1SD). The mismatch between CBF and Hct also suggests that plasmatic and red blood cell flows are not equivalent. These experimental findings may contribute to refine advanced physiological models of stroke such as the one developed by Jespersen et al [3]. The ADC/Hct mismatch can be used to determine a new region of tissue, which could be able to recover. Therefore, in a future development, it would be interesting to obtain non invasive Hct maps by MRI, e.g. using red blood cells labeled with gadolinium, to monitor, *in vivo*, the tissue outcome of the Hct/CBF mismatches and of the Hct/ADC mismatches.

References: [1] Baron JC, et al. 1999 May;30(5):1150-3 [2] Longa EZ, et al. Stroke 1989;20: 84–91 [3] Jespersen SN, et al. JCBFM. 2012