Relaxometry vs artefact volume measurements for estimating the number of iron-labelled macrophages: in vivo testing in the mouse brain

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Rationales and objectives:

In recent years, numerous studies have been published on the use of iron oxide nanoparticles for MRI assessment of macrophage infiltration in neuroinflammatory diseases [1]. Quantitative estimate of iron-labelled cells is highly desirable in such studies, as it could allow to monitor the effects of anti-inflammatory drugs. In-vitro studies performed at 4.7T [2] have shown that there was a linear relationship between iron concentration and 1/T2 and 1/T2* for concentrations < 0.36 mM of AMNP, a new class of anionic nanoparticles (CNRS UMR 7612, France) [3]. The aim of this study was to compare four quantitative methods for estimating the number of AMNP-labelled cells stereotaxically implanted in the mouse brain: T2 relaxometry [2], T2* relaxometry [4], and measurement of the volume of the artefact generated by AMNP-labelled cells, using negative and positive contrasts [5].

Materials and Methods:

Bone marrow-derived macrophages were incubated in the presence of AMNP ([Fe]=1-mM) for 12h [2]. Eight healthy mice were injected in the striatum with 3-µl saline solution containing a given number of iron-labelled cells ranging from 500 to 7,500 cells (2 injections per mouse, one in each hemisphere). MRI was performed on a Bruker Biospec 4.7T/10-cm magnet, using coronal T2-weighted spin-echo imaging (TE/TR 80/4000-ms), coronal T2*-weighted gradient-echo (T2*GE) imaging (TE/TR 10/30-ms, flip angle 30°, one echo), axial multi-slice multi-echo (MSME) sequence for T2 quantification (TE(interecho delay)/TR 15/4000-ms, 35 echoes) and axial multi-gradient echo (MGE) sequence for T2* quantification (TE/TR 3/450-ms, flip angle 20°, 12 echoes and ΔTE = 5 ms). Two positive contrast images were acquired by shifting the slice rephasing gradient values by ± 20 with regard to the T2*GE sequence [6]: one enhancing the pole of the artefact and one enhancing the equator. These images were then added, and T2*GE was subtracted to improve detection (Combined Positive Contrast CPC). A published methodology was used to estimate cell number based on T2 and T2* quantification, using *in vitro* calibration curves [2]. The volume of hypointense voxels was measured on all slices of T2*GE images, and the volume of hyperintense voxels was measured on all slices of CPC images. Bland-Altman plots and scatter diagrams were used to compare the T2 and T2*-based estimated number of cells, the artefact volumes, and the actual number of AMNP-labelled cells.
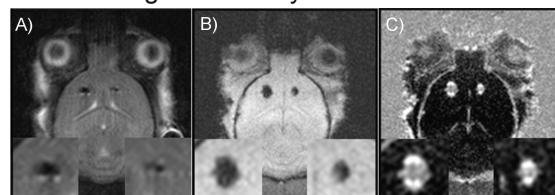

Fig. 1: Typical Coronal T2-weighted spin-echo (A), gradient-echo (B) and combined positive contrast (C) images for injections with 5,000 (left) and 1,000 (right) AMNP-labelled cells.
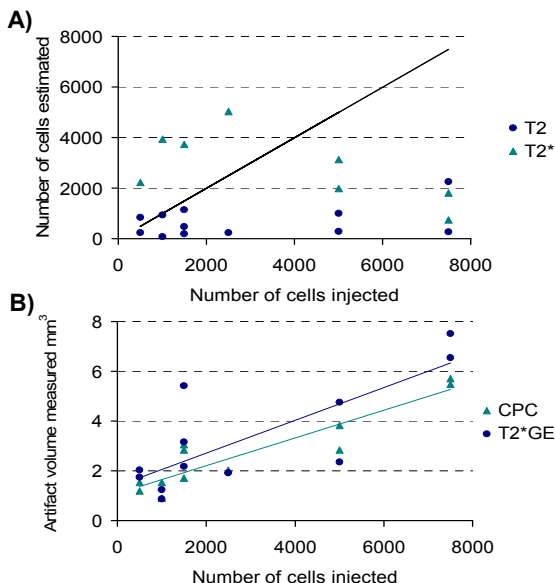

Fig. 2: Scatterplot of the number of cells estimated from T2 and T2* quantification (A) and of the volume of the artefact as measured on T2*GE and combined positive contrast (CPC) images (B), as a function of actual number of AMNP-labelled cells. Solid lines represent the line of equality in A and regression lines in B.

Results:

Iron-labelled cells were clearly detected on coronal images, with a typical arrowhead artefact on spin-echo images (Fig 1A) and cloverleaf artefact on gradient-echo images (Fig 1B). The pole and equator lobes of the cloverleaf were highlighted in the CPC image (Fig 1C). T2 quantification systematically overestimated the number of AMNP-labelled cells, while T2* quantification underestimated the number of cells in the range [500-2500] and overestimated it in the range [5000-7500] (Fig 2A). There was a fair correlation between the number of AMNP-labelled cells and the volume of the artefact for T2*-weighted images (R^2=0.62) and a good correlation for CPC images (R^2=0.85) (Fig 2B).

Discussion and Conclusion:

T2 and T2* quantification failed to estimate the number of AMNP-labelled cell *in vivo*, while measurement of the artefact volume gave promising results. The impact of different times of echo (TE) on this result remains to be investigated. To our knowledge, these results represent the first obtained in the brain using positive contrast techniques, with a range of cells that is far inferior compared to the literature outside the brain (a few thousands vs hundreds of thousands [5]). Such quantitative approaches should prove useful to enhance the quality of pre-clinical cellular imaging studies.

References :

[1] Corot et al, Invest Radiol 39, 619 (2004) [2] Brisset et al, Eur Radiol, (2009) [3] Wilhelm et al, Biomaterials 29, 3161 (2008) [4] Politi et al, Stem Cells 25, 2583 (2007) [5] Stuber et al, Magn Reson Med 58, 1072 (2007) [6] Mani et al, Magn Reson Med 55, 126 (2006).