

## Serial pH-weighted imaging using amide proton transfer in acute ischemic stroke

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**Target audience:** Stroke physicians and research scientists with an interest in stroke or metabolic imaging

### Purpose

Amide proton transfer (APT) MRI quantifies the intracellular transfer of protons between amide groups and water using a chemical exchange saturation transfer (CEST) technique.<sup>1</sup> APT is base-catalysed and hence dependent on intracellular pH. Since acidosis occurs during ischemia prior to irreversible infarction it has been hypothesized that this imaging technique might be used to identify tissue at risk of infarction in the context of acute ischemic stroke.<sup>2</sup> APT MRI can be performed on standard clinical imaging hardware without need for exogenous contrast agents widening its appeal as an imaging biomarker in acute stroke. We report early data using APT MRI in acute stroke and demonstrate its feasibility, potential utility and limitations in this context.

### Methods

Patients with ischemic stroke (<6 hours from symptom onset) were recruited into an observational cohort study following informed consent or agreement from a representative, according to the protocol agreed by the UK National Research Ethics Service committee (ref:12/SC/0292). Serial MRI scans were performed (at presentation, 2 hours, 1 day, 1 week and 1 month). A 3.0T Siemens Verio scanner was used and scanning protocol included single slice CEST image to estimate the APT (first 3 scans only, 3.4x3.4x5mm, TR=5000ms, TE=23ms, 30 saturation offsets, 2 reference images from -4.5 to 4.5ppm), quantitative arterial spin labeling perfusion imaging, diffusion-weighted imaging with automated apparent diffusion coefficient (ADC) calculation, T1-weighted imaging and fluid attenuated inversion recovery (FLAIR) imaging. Analysis was restricted to patients with lesions greater than 5mm axial diameter on ADC image in whom serial imaging was acquired. Images were coregistered to structural space, before slice selection, masking and registration into APT image space. A pH-weighted signal was quantified using a Bayesian model-based analysis to calculate the APT ratio (APTR\*) relative to a contralateral region of interest (ROI).<sup>3</sup> Final infarct (FLAIR at 1 month or 1 week) was defined by a clinician using semi-automated software. Regional analyses were also performed, using the Harvard-Oxford cortical atlas to define objective ROIs, to explore correlations between cerebral blood flow, APTR\* and ADC at presentation. Matlab (Mathworks Inc.) or the FMRIB Software Library were used for all analyses.<sup>4-6</sup>

### Results

Of 11 patients, 2 died precluding follow up and for 3 patients APT imaging was severely affected by artefact (motion, ringing and partial volume effects). 17 APT MRI scans from 6 patients were included in the serial data analysis with a median symptom onset to initial MRI time of 2 hours 59 minutes. 3 patients reperfused within 24 hours and 3 did not. APTR\* demonstrated abnormal signal within the ischemic regions not limited to tissue destined to infarct by 1 month (Figure 1). Serial APTR\* analysis showed that for all patients there was a significantly lower APTR\* within the infarct ROI either at presentation or within 2 hours of the initial scan (Figure 2). The subsequent pattern of APTR\* change varied across patients. 2 of the patients in whom reperfusion occurred showed early normalization of APTR\*, with an overshoot in one patient with very early reperfusion. Complete normalization of APTR\* was not observed in non-reperfused patients. Regional analyses of predefined anatomical areas at presentation showed a statistically significant, although poorly predictive, correlation between regional CBF and APTR\* ( $r=0.32$ ,  $p=0.0003$ ), which was not seen between CBF and ADC ( $r=0.16$ ,  $p=0.089$ ).

### Discussion

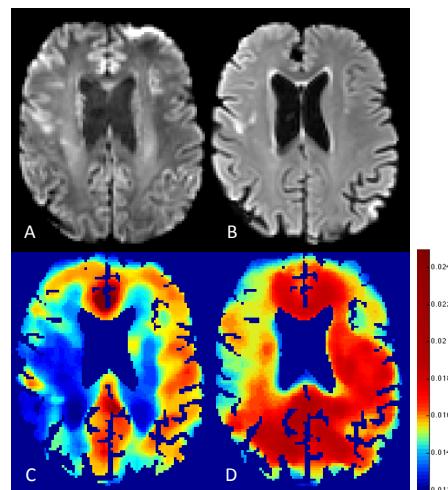
APTR\*, a pH-weighted signal, appears to consistently fall within ischemic regions compared to contralateral tissue. Abnormal APTR\* significantly correlates with the degree of hypoperfusion, although the correlation is weak suggesting that additional factors affect cellular metabolism and hence intracellular pH. Importantly, low APTR\* is reversible with early reperfusion and is not restricted to non-viable tissue, a necessary requirement for an imaging biomarker that might identify tissue at risk rather than predicting inevitable infarction. Interestingly, in 1 case early reperfusion was associated with an elevated APTR\*, which may represent an alkalosis, a phenomenon observed in animal models of early reperfusion.<sup>7</sup>

### Conclusion

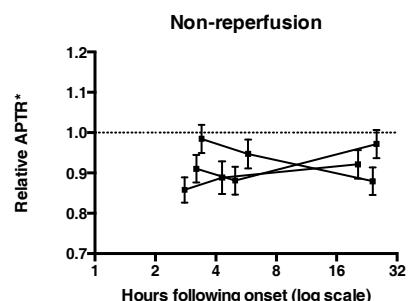
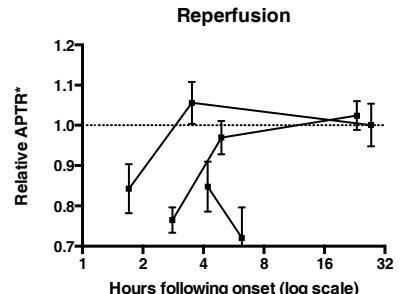
APT MRI provides a means to study the intracellular pH of cerebral parenchyma and hence to investigate the metabolic activity of cells during ischemia. These data suggest that APT MRI is feasible in acute stroke and can add information about metabolism that has not been previously possible. These early results are consistent with some observations from preclinical studies and may allow both improved translation of therapies from preclinical to clinical studies and an improved understanding of the pathophysiology of ischemic stroke in patients. However, there are limitations to APT MRI, including susceptibility to artefact, and more data are required to better understand the natural history of this imaging biomarker. Recruitment in this study is ongoing.

### References

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**Figure 1** - Coregistered images of presenting b1000 DWI (A), 1 month FLAIR image (B), ASL perfusion (C), and APTR\* (D) with colour bar. There is marked right hemisphere hypoperfusion with associated acidosis. The infarct on presentation (A) and follow up (B) is small following reperfusion.



**Figure 2** – Serial values of APTR\* relative to the contralateral hemisphere within the coregistered final infarct volume (error bars represent relative 95%CI)