# Temporal Tissue Assessment in non-Human Primate Cerebral Ischemia using Diffusion-weighted MR Imaging and ISODATA Cluster Analysis

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**Introduction:** Understanding the serial evolution of focal ischemic lesion pattern seen in the diffusion weighted MR images is important for better diagnosis and management of stroke patients [1,2,3]. However, human studies are bound by the limited temporal MRI scanning protocol and experimental rodent models have shown intrinsically different stroke evolution patterns when compared to human and non-human primates [4]. We therefore speculate that serial non-human primate models can potentially provide better insight into ischemic lesion evolution and provide more accurate parallels in the study of human stroke. An earlier study investigated changes in the DWI parameters in both permanent and transient models of stroke in cynomologous macaques using a region of interest (ROI) analysis and found that lesion evolution was more consistent with that observed in humans than in rodent models [5]. However, even carefull regional analyses may be subject to errors due to partial-volume averaging which may obscure variations in measured parameters. The use of cluster analysis may therefore be beneficial in staging, identifying and quantifying progressive ischemic change as a result of stroke [6]. This study aims to use a voxel-by-voxel cluster analysis for evaluating the evolutionary changes in the acute, sub-acute, and chronic stages after stroke. The first part of this study was to determine whether unique ADC/FA patterns could be identified during lesion evolution. The second part of the study involved evaluating temporal differences in DWI parameters between brains with transient vs permanent occlusion.

**Methods:** Serial diffusion magnetic resonance imaging data from five adult male macaques (Macacca fasicularis) were included in this study. All procedures were approved by our institution's animal care committee. Stroke was induced by either injection of a small volume of cyanoacrylate thrombus (permanent occlusion) or by mechanical obstruction of the M1 branch of the middle cerebral artery. The catheter was removed after 3 hours to produce a transient occlusion model. MRI were acquired on a 1.5-T MRI-scanner (GE Signa) within an average of 53 minutes after stroke. Dual echo T2-weighted imaging, DWI, and perfusion-weighted MRI were acquired at intervals for up to six hours after stroke-onset. Follow-up (fu) studies were performed at set timepoints (1,3,6,10,17,30 days). Images were acquired according to protocols previously described [5]. Trace apparent diffusion coefficient (ADC), and fractional anisotropy (FA) were calculated on a voxel-by-voxel basis and normalized with respect to the contra-lesional hemisphere. The volumetric serial diffusion studies at the acute and follow-up stages were co-registered using semiautomatic co-registration software (MNI, Autoreg) [8] after segmenting out the skull and muscle tissue from brain tissue using the brain extraction tool (BET) [7]. Edema induced voxel distortions were largely compensated for using non-linear co-registration to the first recorded timepoint [8]. Diffusion parameters over time were simultaneously analyzed using an iterative self-organizing data analysis (ISODATA) [8, 9] with spatial contiguity weighting [10]. The analysis was limited to artifact free parenchyma. The resulting temporal patterns were evaluated using a coefficient of variance analysis. Patterns with deviation larger than 0.005 were considered abnormal. The resulting evolution signatures were then compared using a Spearman's rho correlation test. Signatures were evaluated based on their relative evolution: increasing, decreasing, or pseudo-normalization. FA and ADC patterns that corresponded ($p<0.05$) were grouped as one distinct signature. Visual comparison of the ADC values at the first acquired timepoint and last chronic timepoint grouped signatures to either core or rim tissue. Core tissue was defined as abnormal ADC at the acute as well as chronic timepoint; rim tissue was at first abnormal, but returned to normal values. Co-registered T2-wt images acquired at the last recorded timepoint were additionally used to confirm sustained ischemic damage in the core regions.

**Results:** Figure 1 shows ADC/FA signatures of a brain with a transient occlusion before (a) and after variance pruning (b). The pruned signatures (b) correlated with the lesion core (purple) and rim region (yellow) visible in the 3 hours after stroke onset ADC (c) and FA (d) image. The 30 day follow-up T2 image (e) clearly designated increased T2 indicating sustained damage in the core region (blue arrow) as opposed to normal values (red arrow) for the rim region. Five datasets were analyzed up to 10 days after stroke onset, and the cluster analysis showed 12 – 19 distinct signatures per analyzed brain. After variance pruning this signature range was reduced to between 3 to 9 signatures. Good correspondence was found for almost all identified signatures. Two signatures were excluded because they corresponded to sustained contra-lateral ventricular distortions. Five corresponding signatures were identified from the resulting signatures. Figure 2 shows the FA signatures (a) and ADC signatures (b) ($n=5$, $p<0.05$) identified from this analysis. These signatures clearly show a correlation of increasing ADC with decreasing FA (signatures A and D) in the ischemic core region. Decreasing FA with more rapidly increasing ADC returning to pseudo-normalized values (signatures B and C) can be assigned to the perilesional rim (see table 1). Table 1 also shows a higher percent cluster distribution for the lesion core in the brains with permanent occlusions while the transiently occluded brains displayed more coherence within the peri-lesional rim.

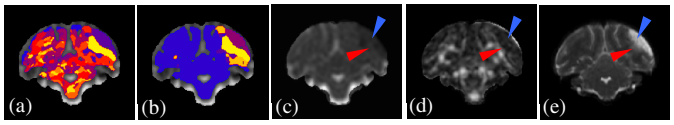


*Figure 1: Cluster result (a, b), ADC (c) and FA (d) 3hrs after onset, T2 fu at 30 days after stroke (e).*

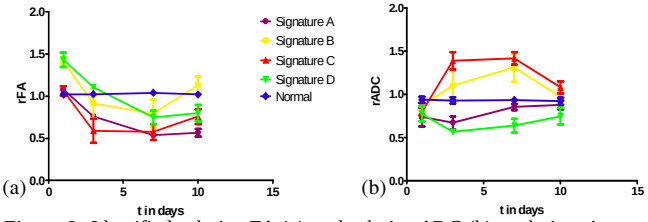


*Figure 2: Identified relative FA (a) and relative ADC (b) evolution signatures up to 10 days after stroke. Error bars resemble standard error of the mean*

| Signature | Core | Rim | Permanent | Transient |
|---|---|---|---|---|
| A | 77 | 29 | 70 | 33 |
| B | - | 29 | - | 33 |
| C | - | 29 | - | 33 |
| D | 23 | 13 | 30 | - |

*Table 1, Cluster distribution (%) in regions of increased temporal variance ($t > 0.005$)*

**Discussion:** Our results demonstrate that a voxel-by-voxel analysis allows us to elucidate evolutionary signatures corresponding to different ischemic stages after stroke. Not only were we able to differentiate patterns between brain tissue regions which reperfused and those that did not, we were also able to differentiate pathophysiological stages. The distinct ADC/FA signatures identified in this study were consistent with earlier pre-clinical studies investigating serial DWI evolution [3,5]. Although the initial number of clusters was predominantly defined by the heterogeneous values of FA, resulting in a high initial cluster load, variance analysis was successfully employed to identify those evolutionary patterns which corresponded with the ischemic tissue regions. Despite retrospective analysis, this study shows great potential for evaluating novel stroke therapies using a clinically relevant primate model, particularly since voxel-by-voxel analysis allows for identifying the ischemic core regions without making any assumptions about possible 'viability' thresholds. Although promising, the limitation of this study was the reliance on the focal DWI patterns. Future study will include T2 and perfusion parameters in order to better differentiate ischemic tissue fates.

**References:** 1. Kidwell CS, et al. Ann. Neurol. 2000; 47, 462-9; 2. Dijkhuizen RM, et al. Stroke. 1998; 29, 695-704; 3. Li F, et al. Ann. Neurol. 1999; 46, 333-42; 4. Tagaya M, et al. Stroke. 1997; 28, 1245-54; 5. Liu Y, et al. Stroke. 2007; 38, 138-45; 6. Jacobs MA, et al. Stroke. 2001; 32, 943-9; 7. Smith, S. HBM. 2002; 17, 143-155; 8. Collins DL, et al. J. Comput. Assist. Tomogr. 1994; 18, 192-205. 9. Wu, O, et al. Stroke. 2007; 38:492-3. 10. Cai W, et al. Pat. Recogn. 2007; 40(4):825-38.