White Matter Ischemic Changes in Hyperacute Ischemic Stroke
Voxel-Based Analysis Using Diffusion Tensor Imaging and MR Perfusion

Kambiz Nael, MD; Theodore P. Trouard, PhD; Scott R. Lafleur, BS; Elizabeth A. Krupinski, PhD; Noriko Salamon, MD, PhD; Chelsea S. Kidwell, MD

Background and Purpose—The purpose of this study was to evaluate changes in fractional anisotropy (FA), as measured by diffusion tensor imaging, of white matter (WM) infarction and hypoperfusion in patients with acute ischemic stroke using a quantitative voxel-based analysis.

Methods—In this prospective study, diffusion tensor imaging and dynamic susceptibility contrast perfusion sequences were acquired in 21 patients with acute ischemic stroke who presented within 6 hours of symptom onset. The coregistered FA, apparent diffusion coefficient, and dynamic susceptibility contrast time to maximum (Tmax) maps were used for voxel-based quantification using a region of interest approach in the ipsilateral affected side and in the homologous contralateral WM. The regions of WM infarction versus hypoperfusion were segmented using a threshold method. Data were analyzed by regression and ANOVA.

Results—There was an overall significant mean difference ($P<0.001$) for the apparent diffusion coefficient, Tmax, and FA values between the normal, hypoperfused, and infarcted WM. The mean±SD of FA was significantly higher ($P<0.001$) in hypoperfused WM (0.397±0.019) and lower ($P<0.001$) in infarcted WM (0.313±0.037) when compared with normal WM (0.360±0.020). Regression tree analysis of hypoperfused WM showed the largest mean FA difference at Tmax above versus below 5.4 s with a mean difference of 0.033 ($P=0.0096$).

Conclusions—Diffusion tensor imaging-FA was decreased in regions of WM infarction and increased in hypoperfused WM in patients with hyperacute acute ischemic stroke. The significantly increased FA values in the hypoperfused WM with Tmax≥5.4 s are suggestive of early ischemic microstructural changes. (Stroke. 2015;46:00-00. DOI: 10.1161/STROKEAHA.114.007000.)

Key Words: diffusion imaging ■ ischemia ■ magnetic resonance imaging ■ perfusion imaging ■ stroke
disorganization of tracts. However, FA changes are heterogeneous and variable between the infarction core and ischemic regions depending on the severity of ischemia and time of onset. Thus, additional study is required to delineate the use of these techniques in AIS.

The purpose of this study, therefore, was to perform a voxel-based quantitative analysis of combined use of DTI-FA and DSC-Tmax in WM infarction and hypoperfusion in patients with AIS to test the following hypotheses: (1) FA values are spatially different between the infarction core versus hypoperfusion versus normal WM; and (2) FA can identify microstructural changes associated with hypoperfused ischemic, but not yet infarcted, WM. To demonstrate this, we aimed to find a Tmax threshold at which the highest differences in FA could be identified.

**Methods**

**Patients**

This prospective study was conducted between December 2012 and July 2013. Patients with suspected AIS were enrolled. Inclusion criteria were (1) interval between the onset of neurological deficits to MRI of <6 hours; (2) image acquisition at 3.0T magnetic field with both DSC and DTI studies obtained; and (3) presence of infarction and perfusion–diffusion mismatch as identified by MRI. Patient demographic data, median time from last known well to first MRI, and perfusion–diffusion mismatch as identified by MRI. Patient demographic data, median time from last known well to first MRI, and baseline National Institutes of Health Stroke Scale scores were documented.

**Imaging Protocol**

All patients underwent MRI on a 3.0T Siemens Skyra MRI system (Siemens, Erlangen, Germany). The imaging protocol included DTI, fluid attenuation inversion recovery imaging, gradient recalled echo, MR angiography, and DSC perfusion imaging.

DTI was acquired using single-shot spin-echo echo-planar imaging (repetition time/echo time, 5500/82 ms; field of view, 22×22 cm; matrix, 128 mm; slices, 40×3 mm; voxel size, 1.5×1.5×3 mm). Diffusion gradients were applied along 20 noncollinear directions with a b value of 1000 s/mm² resulting in a 5-minute acquisition time. A generalized partial parallel acquisition technique with acceleration factor of 3 was used.

DSC perfusion was performed using a single-shot gradient-echo echo-planar imaging sequence with the following parameters: repetition time/echo time, 1450/22 ms; field of view, 22×22 cm; matrix, 128×128 mm, 30×4 mm slices; generalized partial parallel acquisition, 3). A total of 60 repetitions were acquired after intravenous injection of 0.1 mmol/kg of gadolinium contrast agent at a rate of 5 mL/s.

**Data Analysis**

DSC and DTI studies were processed using commercially available Food and Drug Administration–approved software (Olea Sphere; Olea Medical SAS, La Ciotat, France). DSC analysis was performed using a block-circulant singular value decomposition technique. The Tmax maps were then automatically generated and exported from the software for subsequent analysis. DTI analysis was also performed by the Olea DTI package, where FA and ADC maps were calculated using standard methods.

FA, ADC, and Tmax maps for each patient were coregistered with the Olea software using a 12 degree of freedom transformation and a mutual information cost function. This was followed by visual inspection to ensure adequate alignment. Coregistered images were exported into Matlab program for voxel-based quantitative analysis. An example of image analysis segmentation of WM infarction versus hypoperfusion is shown in Figure 1. A mask of the gray matter (FA threshold >0.15) was generated for each patient to ensure extraction of voxel values was limited only to WM. A map of the infarction core was also generated by a threshold method defined as an ADC value <600×10⁻⁶ mm²/s. For WM infarction, the quantitative values were calculated after applying the gray matter mask (FA>0.15) and within the regions with ADC<600×10⁻⁶ mm²/s. For the WM hypoperfusion, the quantitative values were calculated after applying the gray matter mask (FA>0.15) and within the regions with ADC>600×10⁻⁶ mm²/s. Regions of interest were placed over the area of perfusion abnormality using the coregistered Tmax maps and also in contralateral regions depending on the severity of ischemia and time of onset. Thus, additional study is required to delineate the use of these techniques in AIS.

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![Figure 1](image-url)
normal WM in the centrum semiovale. Perfusion deficit was defined as an area with visually perceptible increased Tmax when compared to the surrounding brain tissue and to the homologous contralateral hemisphere. For each regions of interest, the FA, ADC, and Tmax values in the regions of infarction, hypoperfusion, and normal WM were calculated and exported into an excel spreadsheet for statistical analysis.

Statistical Analysis
Mean values of ADC, FA and Tmax were first computed across voxels for each person and region. Then these person–region means were used in a mixed (repeated measure) ANOVA model. Examination of residual errors under this model confirmed that the residual errors had a normal distribution, justifying the use of a parametric model. Multiple comparison Tukey-adjusted P values were reported. The significance level was defined as P<0.05. Spearman correlation coefficients were computed, and scatter plots were examined to assess the association between ADC, FA, and Tmax in normal, hypoperfused, and infarcted WM. A regression tree analysis was used to find the value of Tmax that best splits FA into high and low values.

Results
Twenty-one patients (14M, 7F) with a mean age of 62.4 (range 47–83) years met our inclusion criteria. The baseline National Institutes of Health Stroke Scale scores ranged from 4 to 17 with a median of 7. The median time from last well known to first MRI was 4.7 hours (range 1–6 hours). The median volume of infarction based on the threshold ADC<600×10−6 mm2/s was 19.2 mL (range, 11–58 mL).

The mean±SD of the ADC, FA, and Tmax values for the normal, hypoperfused, and infarcted WM are shown in Table 1. Repeated measure ANOVA model revealed statistically significant differences between mean values of ADC, FA, and Tmax across all regions except for the ADC difference between normal versus hypoperfused WM (P=0.65; Table 2).

Scatter plots for association between ADC, FA, and Tmax in normal, hypoperfused, and infarcted WM are shown in Figure 2. Spearman correlations (r) were calculated. Figure 3 shows the mean FA value comparisons in hypoperfused and infarcted WM for Tmax<5.4 s versus Tmax≥5.4 s.

Table 1. Mean±SD of ADC, FA, and Tmax in Normal, Hypoperfused, and Infarcted WM

<table>
<thead>
<tr>
<th></th>
<th>Normal Contralateral WM</th>
<th>Hypoperfused WM</th>
<th>Infarcted WM</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADC (10−6 mm2/s)</td>
<td>811.60±66.0</td>
<td>835±84.0</td>
<td>487.0±49.0</td>
</tr>
<tr>
<td>FA</td>
<td>0.360±0.020</td>
<td>0.397±0.019</td>
<td>0.313±0.037</td>
</tr>
<tr>
<td>Tmax, s</td>
<td>1.4±0.85</td>
<td>4.93±1.50</td>
<td>8.48±5.46</td>
</tr>
</tbody>
</table>

Data are mean±SD. The overall F statistic is significant at P<0.001 for all 3 outcomes. ADC indicates apparent diffusion coefficient; FA, fractional anisotropy; and WM, white matter.

Table 2. Mean Differences for Paired-Wise Comparison Between ADC, FA, and Tmax Across Different WM Regions

<table>
<thead>
<tr>
<th></th>
<th>Normal vs Hypoperfused WM</th>
<th>Normal vs Infarcted WM</th>
<th>Hypoperfused vs Infarcted WM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Diff</td>
<td>P Value</td>
<td>Mean Diff</td>
<td>P Value</td>
</tr>
<tr>
<td>ADC (10−6 mm2/s)</td>
<td>18.5</td>
<td>0.65</td>
<td>328.9</td>
</tr>
<tr>
<td>FA</td>
<td>0.036</td>
<td>0.0002</td>
<td>0.047</td>
</tr>
<tr>
<td>Tmax, s</td>
<td>3.53</td>
<td>0.0012</td>
<td>7.08</td>
</tr>
</tbody>
</table>

Mean values are significantly different among all regions with the exception of ADC between hypoperfused and normal WM. ADC indicates apparent diffusion coefficient; FA, fractional anisotropy; and WM, white matter.

Discussion
Advanced imaging techniques, such as DSC perfusion and DTI, can be used to interrogate the spatial heterogeneity of infarction and ischemia in the setting of hyperacute ischemic stroke further. In this study, using a combination of DTI and DSC perfusion, we conducted a voxel-based analysis of DTI-measured FA and DSC-measured Tmax changes in the regions of WM infarction and hypoperfusion using defined thresholds. In addition, we compared FA and Tmax to identify a time-based threshold for the detection of microstructural changes. We note 2 primary findings.

The first is that FA values in the hypoperfused and infarcted WM are significantly different from normal WM, but in opposite directions. Reduced FA values in infarcted WM likely signify the loss of cellular integrity with irreversible cellular injury. On the contrary, we showed that FA values are significantly elevated in the hypoperfused (but not infarcted) WM in comparison with normal WM. This is in agreement with previous studies of animal models of brain ischemia. The elevation in FA occurs in the context of a reduction in the anisotropic tensor and therefore is a consequence of ratio-metric measurement. The acute increase in FA has been linked to cytotoxic edema without a significant change in structural coherence. Subsequent to cytotoxic edema, there could be an increase in tortuosity of the extracellular space and shift of water from the extracellular space to the more restricted intracellular space. Both of these models result in increased tortuosity of diffusion along the axon and hence increased apparent anisotropy.

Our second finding is that the FA values are significantly higher in the hypoperfused WM with Tmax≥5.4 s in comparison with regions with Tmax<5.4 s with a mean difference of 0.033. Parametric MR perfusion maps, such as Tmax, have commonly been used to identify the penumbral tissues in some clinical trials. One major drawback of using time-domain perfusion parameters, such as Tmax, is the fact that a perfusion deficit may represent any part of hemodynamic milieu from delayed perfusion to benign oligemia to hypoperfusion and likely a combination of all of the above. Thus, not all regions with Tmax delay are necessarily destined for infarction. Tmax solely provides an estimate of the delay in bolus arrival time between the arterial input function and a given voxel, without describing the hemodynamic status of
the tissue or degree of ischemia itself. Many investigators have attempted to identify a predefined threshold for Tmax that represents true ischemic penumbra, and as the result, Tmax values with threshold ranging from 2 to 8 s have been used in the literature. Most of these investigations were focused on the correlation with final infarction core size at day 7 or 30 after the ischemic event.

There has been relatively little attention on the role of combined DTI and MR perfusion to characterize ischemic tissue. In this study, we prospectively performed a voxel-based analysis of the FA values in regions of hypoperfusion to identify a Tmax threshold at which ischemic changes can be identified. We found statistically significant higher FA values in the hypoperfused WM with Tmax ≥ 5.4 s, suggesting that perhaps microstructural ischemic changes are largest above this threshold. Significant increase in FA values in the hypoperfused WM with Tmax ≥ 5.4 s supports the result of Diffusion and Perfusion Imaging Evaluation for Understanding Stroke Evolution (DEFUSE) trial, suggesting that Tmax > 6 s may represent a threshold for differentiating ischemic penumbra from benign oligemia. Recently, the hypoperfusion intensity ratio, defined as the proportion of

Figure 2. Scatter plots for fractional anisotropy (FA), Tmax, and apparent diffusion coefficient (ADC) mean values by region. The correlation pattern is different depending on the region. In the normal white matter (WM), FA, and ADC have a moderate negative correlation ($r_s = -0.590; P = 0.005$). In the hypoperfused WM, Tmax and FA have moderate positive correlation ($r_s = 0.561; P = 0.008$). In the infarcted WM, there are moderate negative correlations between Tmax and FA ($r_s = -0.539; P = 0.012$) and Tmax and ADC ($r_s = -0.681; P < 0.001$).

Figure 3. Mean fractional anisotropy (FA) value comparisons in hypoperfused and infarcted white matter (WM) for Tmax < 5.4 s vs Tmax ≥ 5.4 s. The mean±SD for FA values in hypoperfused WM is 0.390±0.014 for Tmax < 5.4 s when compared with 0.423±0.014 in Tmax ≥ 5.4 s ($P = 0.0096$). In the infarcted WM, the mean±SD for FA values are 0.328±0.023 for Tmax < 5.4 s compared with 0.295±0.042 in Tmax ≥ 5.4 s ($P = 0.023$).

```ruby
puts FA
```
Tmax>6 s lesion volume with a Tmax>10-s delay has been proposed as a good predictive measure of infarction growth and clinical outcome. It will be interesting to compare the FA values against the hypoperfusion intensity ratio in the future and in a more broad clinical setting.

This study has several limitations, including (1) a relatively small sample size drawn from a single institution possibly introducing a sample bias; (2) the FA values likely resulted from a combination of ischemic injury and edema-induced compression and distortion of WM tracts, making it difficult to differentiate the 2 underlying pathophysiology although within 6 hours of symptoms onset the effect of edema should be modest; (3) using FA>0.15 to generate the gray matter mask is suboptimal. Ideally, high-resolution T2- or T1-weighted compression and distortion of WM tracts, making it difficult from a combination of ischemic injury and edema-induced introducing a sample bias; (2) the FA values likely resulted against the hypoperfusion intensity ratio in the

Conclusions

DTI-measured FA is decreased in regions of WM infarction and increased in hypoperfused, but not infarcted, WM in patients with hyperacute AIS. The FA values are significantly higher in the hypoperfused WM with Tmax25.4 s suggestive of early and perhaps real microstructural changes related to ischemia.

Acknowledgments

We thank Jeff Gornbein/University of California, Los Angeles SBCC for statistical analysis support.

Disclosures

Dr Nael is a consultant to Olea Medical; honorarium, unrelated to the subject of this project. The other authors report no conflicts.

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Abstract

超急性期虚血性脳卒中における白質の虚血性変化
拡散テンソル画像と MR 灌流画像を用いたボクセル解析

White Matter Ischemic Changes in Hyper Ischemic Stroke
Voxel-Based Analysis Using Diffusion Tensor Imaging and MR Perfusion

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背景および目的: 本研究では、定量的なボクセル解析によって急性虚血性脳卒中患者の大脳白質 (WM) の梗塞部と低灌流部の拡散テンソル画像で測定した異方性比率 (FA) の変化を評価した。

方法: 本研究では、急性虚血性脳卒中発症後6時間以内の患者21例の拡散テンソル画像とdynamic susceptibility contrast (動的濃度差造影) 法による灌流画像を取得し、位置合わせをしたFA、みかけの拡散係数、および最高動的濃度差造影剤到達時間 (Tmax) のマップを用いて、WMの病巣の周囲と対側の関心領域でボクセル解析に基づく定量を行った。WMの梗塞部と低灌流部は関値法で分け、回帰分析とANOVAでデータの解析を行った。

結果: WMの正常部・低灌流部・梗塞部のみかけの拡散係数、Tmax、およびFA値には全体的な有意差が認められた (p < 0.001)。FAの平均±SDはWMの正常部 (0.360 ± 0.020) と比較して低灌流部 (0.397 ± 0.019) で有意に高く (p < 0.001)、梗塞部 (0.313 ± 0.037) では有意に低かった (p < 0.001)。WMの低灌流部を回帰フィッティング解析したところ、平均FAの最大差はTmaxが5.4秒の時で、Tmaxが5秒以上の時のFA値は5秒未満の時よりも有意に高く、平均差は0.035であった (p = 0.0096)。

結論: 超急性期虚血性脳卒中患者では、拡散テンソル画像のFAはWMの梗塞部で減少し、低灌流部で増加した。WMの低灌流部ではTmaxが5秒以上の時にFA値が有意に増加したことから、早期に虚血による微細構造の変化が生じたと考えられる。

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左側に突然片麻痺を生じた71歳女性。脳梗塞開始時の米国国立衛生研究所脳卒中スケールは17。その後の画像をMRAでは右中大脳動脈M1部の閉塞が示された（表示せず）。連続的に位置合わせをした拡散テンソル画像の異方性比率 (FA)、みかけの拡散係数 (ADC)、および動的濃度差造影法のdynamic contrast susceptibility: DSCのTmaxを示す。DSCのTmaxの画像では、右放線冠と島葉皮質下(subinsular region)に右中大脳動脈領域に沿って大きな低灌流を伴う急性梗塞がある。調査して位置合わせしたマップをMatlabプログラムに移した。DSCのTmaxを用いて低灌流領域の関心領域 (ROI) を描いた (A)。灰白質の遮蔽を除去した後に、白質ボクセルを含めたROIを作成した (B)。次に、< 600 x 10^4 mm^2/s 以上の閾値のADCマップを含めた後に低灌流部 (C) と梗塞部 (D) でそれぞれFA値とTmax値を算出した。