Signal Evolution and Infarction Risk for Apparent Diffusion Coefficient Lesions in Acute Ischemic Stroke Are Both Time- and Perfusion-Dependent

Hongyu An, DSc*; Andria L. Ford, MD*; Katie Vo, MD; William J. Powers, MD; Jin-Moo Lee, MD, PhD†; Weili Lin, PhD†

Background and Purpose—This study aimed to examine the temporal relationship between tissue perfusion and apparent diffusion coefficient (ADC) changes within 6 hours of ischemic stroke onset and how different reperfusion patterns may affect tissue outcome in ADC lesions.

Methods—Thirty-one participants were sequentially imaged at 3 hours, 6 hours, and 1 month post-stroke. Three regions of interest (ROIs) were defined within initial ADC lesions: ROI (1)reperf_3hour hyperacute reperfusion (within 3 hours), ROI (2)reperf_6hour acute reperfusion (3 to 6 hours), and ROI (3)nonreperf no reperfusion (by 6 hours). For each ROI, changes in ADC (ΔADC) from 3 to 6 hours and risks of infarction were examined.

Results—The magnitude of initial ADC reduction was similar in all 3 ROIs (P=0.51). ΔADC was strongly associated with reperfusion (P<0.0001) but not with initial ADC reduction (P=0.83). ΔADC in ROI (1)reperf_3hour and ROI (2)reperf_6hour was significantly larger than that of ROI (3)nonreperf (P<0.05). Positive ΔADC was obtained from 3 to 6 hours in ROI (1)reperf_3hour that had restored perfusion before 3 hours, demonstrating a temporal delay between reperfusion and ADC changes. Risks of infarction were significantly higher in ROI (3)nonreperf than those in ROI (1)reperf_3hour and ROI (2)reperf_6hour.

Conclusions—Improvement in ADC did not occur coincidently with reperfusion but showed a temporal delay. Regions with similar initial ADC reductions at 3 hours had different evolution of ADC and infarction risks depending on when or if tissue reperfused. These findings provide a physiological basis for the observation that a single ADC measurement at a fixed time after stroke onset may not accurately predict tissue outcome. (Stroke. 2011;42:00:00.)

Key Words: ADC recovery ■ ischemic stroke ■ reperfusion ■ risk of infarction

Magnetic resonance diffusion-weighted imaging (DWI) is widely used in clinical practice to depict acute ischemic stroke lesions. It has been demonstrated that compromised blood flow leads to a reduction in the apparent diffusion coefficient (ADC) during ischemia. ADC reduction may be observed as early as minutes after stroke onset. Conversely, DWI lesions have been found to reverse in various settings, including shortly after thrombolysis or a few days after stroke onset. However, the temporal behavior of ADC lesion improvement after reperfusion during the first hours after stroke onset has not been documented in humans. Moreover, it has not been thoroughly investigated whether the presence of reperfusion and its timing directly affect the final fate of an ADC lesion. In a rapid sequential DWI study in cats, Davis et al found that ADC reduction and recovery did not occur concurrently with stroke onset or reperfusion but rather evolved progressively over 5 to 10 minutes after these events. Based on this animal study, we hypothesized that a temporal delay may also exist between tissue reperfusion and ADC improvement in acute human stroke. This temporal delay may explain, in part, why ADC reduction is not a reliable predictor of ischemic tissue outcome, particularly because DWI images are usually acquired at a single time point after stroke onset in current clinical practice. An improved understanding of the relationship between perfusion and diffusion changes may aid in clinical decision-making using MRI.

In this study of sequential MRI in patients with acute ischemic stroke, we examined the temporal evolution of abnormal ADC in brain regions exhibiting 3 different reperfusion patterns during the hyperacute phase of ischemia. The infarction risk for each of these patterns was measured and compared.

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**Participants and Methods**

**Participants and Inclusion Criteria**

This is a retrospective analysis of data from a prospectively collected observational study of serial MRIs performed in patients with acute ischemic stroke at a large tertiary care referral center admitting >800 patients with ischemic stroke per year. After Institutional Review Board approval, the study enrolled consecutive patients within 3.5 hours of stroke onset based on the following prespecified inclusion criteria: clinically suspected acute cortical ischemic stroke; age ≥18 years; National Institutes of Health Stroke Scale score ≥5; and patient or patient’s next of kin capable of informed consent. Exclusion criteria included bilateral strokes or any acute endovascular or surgical intervention. Both tissue plasminogen activator (tPA)-treated and untreated patients were included. Patients were given intravenous tPA according to the National Institute of Neurological Disorders and Stroke tPA trial protocol. In tPA-treated patients, tPA administration was begun before all MR imaging studies without causing any delay in time to tPA treatment or any deviation from standard monitoring practices.

**Magnetic Resonance Imaging**

Thirty-one participants were serially scanned with MRI at 3 time points (tp): within 3.5 hours (tp1), at 6 hours (tp2), and at 1 month (tp3) after stroke onset. T1 scan was acquired as early as possible. Participants treated with tPA were imaged immediately after initiation of tPA infusion. One-month follow-up scans were obtained in 26 participants; the remaining 5 participants were not available due to long-term follow-up or patient’s next of kin’s refusal. Patients were given intravenous tPA according to the National Institute of Neurological Disorders and Stroke tPA trial protocol. In tPA-treated patients, tPA administration was begun before all MR imaging studies without causing any delay in time to tPA treatment or any deviation from standard monitoring practices.

**Data Analysis**

In perfusion-weighted images, the change of MR signal induced by the bolus passage of contrast agent was first converted to ΔR²/Δt concentration curve. Subsequently, voxels within the middle cerebral artery of the contralateral hemisphere were manually chosen to obtain an arterial input function. A singular value decomposition method was used to calculate cerebral blood flow, cerebral blood volume, and derive the mean transit time (MTT=cerebral blood volume/cerebral blood flow). Six parameter rigid image registration was performed to align MTT, ADC, and fluid-attenuated inversion recovery images from the same participant across all time points using FSL 3.2 (FMRIB, Oxford, UK). To minimize the inclusion of cerebrospinal fluid, voxels with an ADC value >100×10⁻⁶ mm²/s were removed from data analysis. Blood from early hemorrhagic transformation may affect ADC at tp2. To minimize its effect on ADC, we examined the T2*-weighted dynamic susceptibility contrast images at tp2 for each patient. Hypointense regions were then manually outlined as hemorrhagic transformation and excluded from data analysis. Mean MTT and ADC values were obtained from a region of interest (ROI) that encompassed the whole contralateral hemisphere for each participant.

Voxels with ADC values <mean−2SD of the contralateral hemisphere were defined as abnormal. Three ROIs were defined within tp1 abnormal ADC regions corresponding to 3 different reperfusion patterns to evaluate (1) the temporal evolution of each of these 3 ADC lesions; and (2) their respective tissue outcomes. MTT was chosen to define perfusion status because MTT is uniform across gray and white matter (unlike cerebral blood flow or cerebral blood volume), allowing for use of a single threshold across both gray and white matter. Hyperfusion was defined using MTT >4 seconds longer than the mean contralateral hemispheric. Figure 1 shows a schematic representation of the definition of 3 ROIs. ROI (1), defined as voxels with abnormal ADC at tp1 but normal MTT at tp1 and tp2 (labeled as “1,” Figure 1), representing an ADC lesion that reperfused before tp1 imaging (<3 hours). ROI (2), defined as voxels with abnormal ADC at tp1 and abnormal MTT at tp1 and tp2 (labeled as “2,” Figure 1), representing an ADC lesion that reperfused between tp1 (3 hours) and tp2 (6 hours). ROI (3)* defined as voxels with abnormal ADC at tp1 and normal MTT at tp1 and tp2 (labeled as “3,” Figure 1), representing an ADC lesion that did not reperfuse. Note our definition of ADC lesion was based on tp1 ADC maps only and 3 ROIs were defined using tp1 ADC and tp1 and tp2 MTT. Therefore, no ADC lesion is delineated at tp2 in Figure 1 to avoid confusion. In all ROIs, isolated regions <1 mL were removed to minimize artifacts due to potential misalignment and spurious findings caused by random noise.

Mean ADC values from these ROIs at tp1 and tp2 were obtained to examine the temporal evolution of ADC between the 2 tps on an individual participant basis. ADC changes from tp1 to tp2 were...
Table. Participant Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n=31</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, mean±SD</td>
<td>61±14</td>
</tr>
<tr>
<td>Women, no. (%)</td>
<td>13 (42%)</td>
</tr>
<tr>
<td>Stroke syndrome</td>
<td>R MCA: 17, L MCA: 13, L ICA: 1</td>
</tr>
<tr>
<td>tp1-treated, no. (%)</td>
<td>23 (72%)</td>
</tr>
<tr>
<td>NIHSS</td>
<td></td>
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<tr>
<td>Admission</td>
<td>15±6</td>
</tr>
<tr>
<td>tp1, hours</td>
<td>2.9±0.8</td>
</tr>
<tr>
<td>tp2, hour</td>
<td>6.3±0.3</td>
</tr>
<tr>
<td>tp3, day</td>
<td>29±10</td>
</tr>
<tr>
<td>Time from symptom onset</td>
<td></td>
</tr>
<tr>
<td>Admission</td>
<td>0.9±0.6</td>
</tr>
<tr>
<td>Treatment (OTT)</td>
<td>1.9±0.4</td>
</tr>
<tr>
<td>tp1, hours</td>
<td>2.9±0.8</td>
</tr>
<tr>
<td>tp2, hour</td>
<td>6.3±0.3</td>
</tr>
<tr>
<td>tp3, day</td>
<td>29±10</td>
</tr>
<tr>
<td>Time from tPA treatment to scan</td>
<td></td>
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<tr>
<td>tp1, hour</td>
<td>0.7±0.4</td>
</tr>
<tr>
<td>tp2, hour</td>
<td>4.0±1.2</td>
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<tr>
<td>ROI volumes, mL</td>
<td></td>
</tr>
<tr>
<td>ROI (reperf 3 hours)</td>
<td>11.3±14.0</td>
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<tr>
<td>ROI (reperf 6 hours)</td>
<td>6.8±4.3</td>
</tr>
<tr>
<td>ROI (nonreperf)</td>
<td>35.6±37.0</td>
</tr>
</tbody>
</table>

TTP indicates tissue plasminogen activator; NIHSS, National Institutes of Health Stroke Scale; tp, time point; OTT, onset-to-treatment time; ROI, region of interest; R, right; L, left; MCA, middle cerebral artery, ICA, internal cerebral artery.

computed as \( \Delta ADC = ADC_{tp2} - ADC_{tp1} \) in all 3 ROIs. A positive \( \Delta ADC \) indicates an improvement of ADC. A generalized linear model (SAS 9.2; SAS Institute Inc, Cary, NC) was used to perform an analysis of covariance to evaluate whether reperfusion status (reperfusion <3 hours, reperfusion 3 to 6 hours, and no reperfusion) and/or tp1 ADC values might affect \( \Delta ADC \). \( \Delta ADC \) was the dependent variable, whereas tp1 ADC values and reperfusion status were the independent variables in the model.

Final lesions were manually outlined as hyperintense regions on tp3 fluid-attenuated inversion recovery. The defined final lesions were mapped onto the tp1 and tp2 images. Based on the overlap between final lesion and each ROI, risk of infarction was calculated as the ratio of the number of infarcted voxels to the total number of voxels in a specific ROI on an individual patient basis. To evaluate whether the risk of infarction differed among all 3 ROIs, 1-way analysis of variance with Newman-Keuls multiple comparison post-test was performed. \( P<0.05 \) was considered statistically significant.

Results

Thirty-one patients with acute ischemic stroke were enrolled after obtaining written informed consent. Their characteristics are summarized in the Table. At tp1, the mean ADC of contralateral hemispheres was 81.1±2.0×10^{-5} mm²/s, which is similar to published reports.5,10 The threshold for abnormal ADC, defined as 2 SDs below the normal value at tp1, was 64.3±3.9×10^{-5} mm²/s (approximately 21% below normal values).

Representative tp1 and tp2 MTT, tp1 and tp2 ADC, 1 month fluid-attenuated inversion recovery, and overlaid ROIs from 3 participants are shown in Figure 2.

Temporal Change of ADC

In this study, 2 sequential MR scans were used to determine reperfusion status within the first 6 hours after stroke onset in both tPA-treated and untreated patients. Given that only 8 patients did not receive tPA treatment in our study cohort, we did not perform separate analyses for tPA-treated and untreated patients.

Tp1 ADC values were similarly reduced in all 3 ROIs \( (P=0.51) \) regardless of the initial or different subsequent reperfusion status (Figure 3A–C). The median and interquartile range (IQR) of tp1 ADC were 54.2 (IQR, 52.5 to 56.9) \( \times 10^{-5} \) mm²/s, 52.3 (IQR, 49.8 to 55) \( \times 10^{-5} \) mm²/s, and 52 (IQR, 48.9 to 55.8) \( \times 10^{-5} \) mm²/s for ROI (1)reperf_3hour, ROI (1)reperf_6hour, and ROI (3)nonreperf, respectively.

ROI (1)reperf_3hour, representing hyperacute reperfusion (<3 hours), was identified in 12 participants of which 2 (17%) were not treated with tPA (marked in red, Figure 3A). ROI (2)reperf_6hour representing reperfusion between tp1 (3 hours) and tp2 (6 hours), was detected in 9 participants of which 1 (11%) was not treated with tPA (marked in red, Figure 3B). ROI (3)nonreperf, representing initially abnormal ADC and abnormal perfusion at tp1 and without evidence of reperfusion at tp2, was detected in 19 participants of which 5 (26%) were untreated patients.

Scatterplots of \( \Delta ADC \) in the 3 predefined ROIs are given in Figure 3D. \( \Delta ADC \) was strongly associated with reperfusion status \( (P<0.0001, \text{analysis of covariance}) \) but was not associated with tp1 ADC \( (P=0.83) \). The median (IQR) \( \Delta ADC \) were 15.7 (IQR, 8.4 to 20.1) \( \times 10^{-5} \) mm²/s, 14.3 (IQR, 3.9 to 21.1) \( \times 10^{-5} \) mm²/s, and -2.3 (IQR, -4.4 to 0.6) \( \times 10^{-5} \) mm²/s in ROI (1)reperf_3hour, ROI (2)reperf_6hour, and ROI (3)nonreperf, respectively. \( \Delta ADC \) were not significantly different for ROI (1)reperf_3hour and ROI (2)reperf_6hour but \( \Delta ADC \) in both reperfused ROIs were significantly larger than that of ROI (3)nonreperf \( (P<0.05) \). That ROI (1)reperf_3hour showed a similar increase in ADC to ROI (2)reperf_6hour although already reperfused at 3 hours demonstrates that a temporal dissociation between reperfusion and ADC change occurred in this tissue. The small number of non-tPA-treated patients prevents us from discerning whether tPA-induced reperfusion or spontaneous reperfusion might have a different impact on subsequent ADC evolution.

Risk of Infarction

Risks of infarction were plotted for all ROIs in Figure 4. The median and IQR risks of infarction were 64.2% (IQR, 56.2% to 82.7%) for ROI (1)reperf_3hour, 84.3% (IQR, 80.2% to 88.5%) for ROI (2)reperf_6hour, and 94.4% (IQR, 83.5% to 97.9%) for ROI (3)nonreperf (Figure 4). One-way analysis of variance showed significantly different risks of infarction among the 3 ROIs \( (P<0.01) \). Newman-Keuls post-tests further revealed that risks of infarction in ROI (3)nonreperf were significantly higher than those in ROI (1)reperf_3hour and ROI (2)reperf_6hour \( (P<0.05) \). The fact that all 3 groups had similar initial ADC reductions but different risks of infarction highlights the failure of a single ADC measurement at 3 hours after stroke onset to accurately reflect tissue outcome and the importance of concurrent and subsequent perfusion status in this determination.
Discussion

DWI is widely used in the clinical setting of acute stroke to delineate ischemic injury. Given its common use, it is of critical importance to improve our understanding of ADC evolution during and after ischemia. Consistent with previous reports, we found that acute changes in ADC are dependent on tissue reperfusion but not on initial severity of ADC reduction. Moreover, a temporal delay between reperfusion and ADC improvement was observed in some tissue. Regions with similar ADC reductions at 3 hours had different tissue outcomes depending on whether or when reperfusion occurred.

Temporal Delay Between Reperfusion and ADC Improvement

Despite the clinical use of ADC to identify ischemic injury, challenges remain to explain why acute ADC lesions might or might not undergo infarction and why an absolute ADC threshold for infarction has not been identified. We have shown that similar ADC abnormalities at tp1 correspond to different tissue fates, depending on concurrent and subsequent perfusion status (Figure 4). Positive ΔADC was obtained from tp1 to tp2 in ROI (1)reperf_3hour that had restored perfusion before tp1 (Figure 3A). It demonstrates that depending on the elapsed time after reperfusion, different ADC values might be obtained (eg, tp1 and tp2 ADC in ROI (1)reperf_3hour) in tissue with already improved perfusion (Figure 3A). Our findings are consistent with a previous animal study showing ADC reversal subsequent to tissue reperfusion and previous MR spectroscopy and positron emission tomography studies demonstrating heterogeneous cellular metabolic injury in regions with similar ADC. Taken together, this evidence may help to explain why a single time point ADC threshold to predict tissue outcome has not been identified.

The biophysical mechanisms of ADC lesion reversal have not been fully determined. Previous animal studies demonstrated that extracellular [K+] began to revert to normal approximately 40 minutes after reperfusion. Meanwhile, electrical excitability was shown to recover after 8 to 15 minutes; synaptic excitability and low-frequency spontaneous electrocortical activity was restored after 30 to 60 minutes and 1 to 2 hours, respectively, after reperfusion. This progressive recovery of energy metabolism and ion homeostasis after reperfusion may be related to delayed ADC improvement after reperfusion.

ADC Improvement Versus Tissue Recovery

The median risks of infarction for the 2 ROIs that exhibited reperfusion, ROI (1)reperf_3hour and ROI (2)reperf_6hour, were 64.2% and 84.3%, respectively, indicating that regions with acute ADC improvement (positive ΔADC) within 6 hours after symptom onset still showed a high probability of infarction. This finding is consistent with previous animal and human studies.

Several mechanisms may be responsible for the discrepancy between acute ADC increase and 1-month tissue infarc-
tion. Ringer et al examined the histological condition of ischemic tissue exhibiting ADC reversal in rats after 30 minutes of middle cerebral artery occlusion followed by successful reperfusion. MRI scans were compared with histology using neuronal, astrocytic markers, and heat shock protein 72. Their results suggested that neurons already exhibited structural damage and stress despite ADC lesion reversal, whereas astrocytes were morphologically intact. Other studies reported that reversed ADC regions had varying degrees of neuronal injury. Moreover, several factors such as calcium overload, free radical formation, and lactic acidosis might trigger a delayed mitochondrial dysfunction leading to the death of these regions with normalized ADC.

Study Limitations and Other Issues
In this study, only 2 time points were acquired during the hyperacute phase of ischemia to characterize lesion evolution. Although additional time points will be needed to fully document the temporal relationship between perfusion and diffusion changes during hyperacute and acute stages of ischemia, this is impractical for human studies. In addition, the number of non-tPA-treated patients was too few to determine whether tPA treatment might uniquely impact our findings. Given these limitations, to the best of our knowledge, this study is the first to reveal that a temporal delay exists between reperfusion and ADC improvement in human acute stroke.

Of note, there is an important difference between the previously reported pseudonormalization and the ADC evolution observed in our study. Pseudonormalization (normal ADC, but subsequent infarct evolution on T2-weighted images) occurs between 1 and 7 days after stroke onset in humans. This phenomenon might be due to increased cerebral
water content associated with vasogenic edema. In contrast, ADC improvement in our study, which occurred during the first 6 hours after ischemia onset, is unlikely caused by vasogenic edema but rather in response to tissue reperfusion. Therefore, the underlying pathophysiological mechanism of the acute ADC improvement in this study likely reflects a different process from ADC pseudonormalization observed days after stroke.

**Clinical Implications**

Regions with a similar ADC reduction at 3 hours had different final outcomes depending on whether and when reperfusion occurred. In regions with reperfusion within 3 hours after stroke onset, <40% of tissue survived. In regions with reperfusion between 3 and 6 hours after stroke onset, risk of infarction varied in a large range with a median risk of infarction >80%. Because ADC may still increase over time in tissue that already reperfused, different ADC values could be obtained depending on the elapsed time after reperfusion. Therefore, a single time point ADC measurement does not fully reflect the disease process and outcome. Beyond diagnosing acute ischemia, interpretation of ADC for tissue outcome, particularly in the presence of reperfusion, must be performed with caution.

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**Disclosures**

None.

**References**

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