Association Between Time From Stroke Onset and Fluid-Attenuated Inversion Recovery Lesion Intensity Is Modified by Status of Collateral Circulation

Anke Wouters, MD; Patrick Dupont, PhD; Soren Christensen, PhD; Bo Norrving, MD; Rico Laage, PhD; Götz Thomalla, PhD; Greg Albers, MD; Vincent Thijs, MD; Robin Lemmens, MD

Background and Purpose—In patients with acute stroke, the intensity of a fluid-attenuated inversion recovery (FLAIR) lesion in the region of diffusion restriction is associated with time from symptom onset. We hypothesized that collateral status as assessed by the hypoperfusion intensity ratio could modify the association between time from stroke onset and FLAIR lesion intensity.

Methods—From the AX200 for ischemic stroke trial, 141 patients had appropriate FLAIR, diffusion-weighted imaging, and perfusion-weighted imaging. In the region of nonreperfused core, we calculated voxel-based relative FLAIR (rFLAIR) signal intensity. The hypoperfusion intensity ratio was defined as the ratio of the $T_{\text{max}} >10$ s lesion over the $T_{\text{max}} >6$ s lesion volume. A hypoperfusion intensity ratio threshold of ≤0.4 was used to dichotomize good versus poor collaterals. We studied the interaction between collateral status on the association between time from symptom onset and FLAIR intensity.

Results—Time from symptom onset was associated with the rFLAIR intensity in the region of nonreperfused core ($B=1.05$; 95% confidence interval, 1.0–1.1). We identified an interaction between this association and collateral status; an association was present between time and rFLAIR intensity in patients with poor collaterals ($r=0.53$), but absent in patients with good collaterals ($r=0.17; P=0.04$).

Conclusions—Our findings show that the relationship between time from symptom onset and rFLAIR lesion intensity depends on collateral status. In patients with good collaterals, the development of an rFLAIR-positive lesion is less dependent on time from symptom onset compared with patients with poor collaterals. (Stroke. 2016;47:1018-1022. DOI: 10.1161/STROKEAHA.115.012010.)

Key Words: collateral circulation ■ magnetic resonance imaging ■ pathophysiology ■ perfusion imaging ■ stroke

Therefore, in the first hours after stroke onset the difference in signal intensities between DWI and FLAIR, the DWI/FLAIR mismatch is proposed as a predictor for time from symptom onset before 4.5 hours, the time window for thrombolysis.11-14 The sensitivity and specificity of the DWI/FLAIR mismatch vary between studies with sensitivity ranging between 40% and 80% and specificity between 78% and 89%, respectively.7 These differences can be explained by various factors, for example, variation in imaging techniques used to assess the mismatch or size of the diffusion lesions,11 but other pathophysiological variables influencing the intensity of the FLAIR lesion have not been extensively studied. Collateral circulation plays an important role in timely progression of

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In 10% to 25% of patients with acute stroke, the exact time from symptom onset cannot be obtained.1-5 Most commonly, these patients wake up with stroke symptoms or they are aphasic, hampering the ability to inform others on the exact onset time.

When duration of stroke symptoms is uncertain, multiparametric magnetic resonance imaging can be used to estimate the age of the lesion.6,7 Minutes after stroke onset cytotoxic edema can already develop causing a net decrease in water diffusion as visualized by an increased signal on diffusion-weighted imaging (DWI). Hours after stroke onset vasogenic edema gradually develops causing a visible hyperintensity on fluid-attenuated inversion recovery (FLAIR) imaging.8-10 Therefore, in the first hours after stroke onset the difference in signal intensities between DWI and FLAIR, the DWI/FLAIR mismatch is proposed as a predictor for time from symptom onset before 4.5 hours, the time window for thrombolysis.11-14 The sensitivity and specificity of the DWI/FLAIR mismatch vary between studies with sensitivity ranging between 40% and 80% and specificity between 78% and 89%, respectively.7 These differences can be explained by various factors, for example, variation in imaging techniques used to assess the mismatch or size of the diffusion lesions,11 but other pathophysiological variables influencing the intensity of the FLAIR lesion have not been extensively studied. Collateral circulation plays an important role in timely progression of
Collateral Status Modifies FLAIR Lesion Intensity

Wouters et al

Materials and Methods

Clinical and neuroimaging data from the AX200 for ischemic stroke trial (AXIS 2) were used. This was a large phase multicenter, placebo-controlled, randomized, and double-blinded trial. The clinical efficacy of recombinant granulocyte colony-stimulating factor (AX200) in patients with acute ischemic stroke was tested.

Granulocyte colony-stimulating factor failed to meet the primary and secondary endpoints of the trial. The full methodology of the trial has been described previously. Patients were included in a time window of 69 hours after the patient was last seen normal. Magnetic resonance imaging (FLAIR, T2, T2*, DWI, time-of-flight magnetic resonance angiography, and perfusion-weighted imaging) was mandatory before inclusion. A minimum DWI lesion size of 15 mL was required. Previous treatment with intravenous tissue-type plasminogen activator was allowed if patients fulfilled all criteria to be eligible for thrombolysis. The main exclusion criteria were signs of severe stroke on imaging (carotid T occlusion, ischemic lesions larger than two thirds of the middle cerebral artery territory, and signs of midline shift), hemorrhagic, and lacunar strokes.

For this study, we only included patients in whom perfusion-weighted imaging, DWI, and FLAIR lesion volumes could be obtained. In addition, a perfusion deficit within a DWI lesion needed to be present for this analysis of the nonperfused core. Quantitative relative FLAIR maps (rFLAIR) were calculated in a voxel-based manner using in-house developed software. For each voxel, the relative signal intensity was determined by the ratio of the mean signal intensity in that voxel and the median of the signal intensity in a sphere with radius 15-mm positioned around the homolog voxel in the other hemisphere. The midplane used for mirroring every voxel to the contralateral hemisphere was semiautomatically defined. To achieve accurate mirroring, points were manually chosen and were used to define the 3-dimensional midplane. A secondary analysis was done with a radius of 10 and 20 mm to investigate if the chosen radius would influence our results (Figure 1 in the online-only Data Supplement).

RAPID software was used to calculate perfusion-weighted imaging and DWI lesion volumes. $T_{\text{max}}$ (time to the maximum of the residue function obtained by deconvolution) was used to define regions of hypoperfusion. The irreversibly injured tissue (core) was defined by the ratio of the mean signal intensity of the ROI over the mean signal intensity of the mirrored ROI. The nonperfused core (DWI lesion volume within a region of $T_{\text{max}}$≤6 s perfusion deficit) was selected as the region of interest (ROI) to study the pathophysiology of FLAIR signal intensity and calculate the mean FLAIR relative signal intensity (Figure 1). We compared our results with a more commonly used nonvoxel-based technique to determine the mean FLAIR relative signal intensity in the nonperfused core. First, this region was mirrored to the contralateral hemisphere. Second, the relative signal intensity of the ROI was defined by the ratio of the mean signal intensity of the ROI over the mean signal intensity of the mirrored ROI. Patients with white matter lesions in $T_{\text{max}}$≤6 s without diffusion restriction were excluded from the analysis. Collateral status was assessed by using the HIR, which is defined as the proportion of the $T_{\text{max}}$>6 s lesion volume with $T_{\text{max}}$>10 s delay. Good and poor collateral circulation were dichotomized based on the predefined threshold of 0.4, with poor collateral status defined by an HIR>0.4.

We assessed the interaction between collateral status and the association between time from symptom onset and FLAIR intensity in a linear regression model with rFLAIR as dependent variable. As additional variables, age, sex, National Institute Stroke Scale (NIHSS) score, DWI lesion volume, and arterial occlusion site were tested in univariate linear regression. We postulated variables with an $\alpha$<0.1 to be included in the multivariate model. Collateral status (dichotomized in poor versus good collaterals defined by the HIR), time from symptom onset, and an interaction term were included as independent variables. In a second approach, the collateral status in the regression model defined by the HIR was replaced by the mean $T_{\text{max}}$ of the whole region of hypoperfusion, assuming higher values to be associated with poorer collateral status.

The qualitative DWI/rFLAIR mismatch was visually rated by 2 readers (A.W. and V.T.) blinded to clinical information as previously described. The Youden Index defined the most optimal threshold of rFLAIR to predict symptom onset before 4.5 hours. We compared the sensitivity and specificity for symptom onset before 4.5 hours between rFLAIR and the qualitative DWI/rFLAIR mismatch.

Clinical characteristics between different groups were compared using the Mann–Whitney U test for continuous variables and a $\chi^2$ test for categorical variables. A value of $P$<0.05 was considered significant. Statistical testing was performed with IBM SPSS Statistics software version 22.

Results

Patient Population and Clinical Characteristics

From the original 323 patients of the AXIS 2 trial, in 206 (63.8%) all imaging sequences were available and of sufficient quality for analysis. Furthermore, 12 patients (5.2%) were excluded because of confluent or large FLAIR lesions in the contralateral hemisphere or leukoaraiosis overlapping the acute lesion; in 53 (25.7%) patients, the core was reperfused and could, therefore, not be analyzed. Here, we report the results of the total of 141 included patients. The interobserver reliability for visual DWI/rFLAIR mismatch was good ($kappa$=0.70). Collateral status, assessed by the HIR, was poor in 87 patients (61.7%) and good in 54 patients (38.3%). Patients with poor collaterals had more severe stroke symptoms at baseline as determined by the NIHSS (NIHSS 14 versus NIHSS 11, $P$=0.01), larger DWI lesion volumes (47.2 versus 14.6 mL, $P$<0.01) and larger perfusion ($T_{\text{max}}$>6 s) volumes (91.5 versus 45.8 mL, $P$=0.01). The rate of excellent functional outcome at 90 days, modified Rankin Scale 0 to 1, was

![Figure 1. Regions of interest.](http://stroke.ahajournals.org/)

A. Presentation of the different regions of interest. I, region of apparent diffusion coefficient <620 mm²/s and II, region of $T_{\text{max}}$>6 s. Green: region of $T_{\text{max}}$>6 s without diffusion restriction=Penumbra. Red: region of diffusion restriction with $T_{\text{max}}$>6 s=Nonreperfused core, and Pink: the area of diffusion restriction without hypoperfusion=Reperfused core. B. Eighty-two-year-old patient with right-sided stroke. The image shows the different regions of interest.
higher in patients with good collaterals (31%) versus patients with poor collaterals (17%;  P=0.05; Table 1).

**Comparison of Quantitative Versus Visual DWI/FLAIR Mismatch**
The correlation between rFLAIR and time from symptom onset was moderate (r=0.4,  P<0.01). The optimal threshold of rFLAIR to predict symptom onset before 4.5 hours was 1.16. Using the threshold of 1.16, rFLAIR had a sensitivity of 88% (95% confidence interval, 75%–95%) and a specificity of 57% (95% confidence interval, 46%–67%) to predict symptom onset before 4.5 hours. The visual DWI/FLAIR mismatch had a sensitivity of 66% (95% confidence interval, 51%–78%) and a specificity of 70% (95% confidence interval, 60%–79%) to predict symptom onset before 4.5 hours. The Pearson Correlation coefficient between the quantitative and visual mismatch was 0.5.

**Effect of Collateral Status on the Association Between Time From Symptom Onset and FLAIR Intensity**
The predictive value of time for rFLAIR intensity was moderate in patients with poor collateral circulation (R² = 0.28), but poor in patients with a good collateral circulation (R²=0.03; Figure 2). From all other variables, only the DWI lesion volume was associated with rFLAIR in univariate linear regression analysis (P=0.04), but was excluded from the model because of its poor explanatory value (R² = 0.03).

The relationship between time from symptom onset and rFLAIR was stronger in patients with poor collaterals compared with patients with good collateral status as measured by HIR (P for interaction=0.04; Table 2). When modifying the sphere diameter from 15 mm to 10 or 20 mm to calculate the rFLAIR, the results remained unaltered (Table 2). Using the nonvoxel-based approach for calculating FLAIR relative signal intensity, no interaction was identified (P=0.12).

We performed an additional analysis with the mean Tₘₐₓ in the region of the perfusion deficit as a measurement of hypoperfusion severity. A strong interaction between this severity on the association between time and rFLAIR intensity was identified (P=0.001), confirming the initial results that collateral status modifies the relationship between time from symptom onset and rFLAIR intensity.

**Discussion**
This study shows that the relationship between time from symptom onset and rFLAIR lesion intensity is dependent on the severity of hypoperfusion (which is a reflection of collateral status). In this study population, no association was identified in individuals with good collaterals between time from symptom onset and rFLAIR lesion intensity. In contrast, in patients with poor collaterals, FLAIR lesion intensity increased over time.

Animal and human studies have shown that rFLAIR lesion intensity gradually increases with time after stroke onset.¹¹,²⁷,²⁸ The temporal difference between the presentation of a DWI lesion and FLAIR intensity after stroke onset, the DWI/FLAIR mismatch, has been proposed to determine eligibility for thrombolysis in patients presenting with unknown time from stroke onset.¹¹,²⁷,²⁸ One of the limitations of this imaging pattern is the intersubject variability when determining an FLAIR-positive lesion.²⁹ In acute ischemic stroke because of large vessel occlusion, collateral status is a known modifier of infarct growth and is associated with NIHSS at presentation and outcome after reperfusion therapy.¹⁶,³⁰–³³ Here, we show that collateral status is a predictor of rFLAIR intensity over time. In severely hypoperfused lesions, the development of vasogenic edema seems to have a strict relationship with time as opposed to in patients with good collaterals. These findings could be clinically relevant when FLAIR intensity is used to select patients for thrombolysis (eg, in clinical trials) because patients with good collaterals might be selected after the 4.5-hour time window from stroke.

**Table 1. Characteristics Comparison by Collateral Quality**

<table>
<thead>
<tr>
<th></th>
<th>Good Collaterals (n=54)</th>
<th>Poor Collaterals (n=87)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>68 (61.8–77)</td>
<td>72 (62–76)</td>
<td>0.40</td>
</tr>
<tr>
<td>Sex (F)</td>
<td>27 (50.0%)</td>
<td>39 (44.8%)</td>
<td>0.60</td>
</tr>
<tr>
<td>Time from symptom onset, min</td>
<td>326.5 (258.3–413)</td>
<td>317 (229–381)</td>
<td>0.10</td>
</tr>
<tr>
<td>IV tPA</td>
<td>34 (63.0%)</td>
<td>56 (64.4%)</td>
<td>0.90</td>
</tr>
<tr>
<td>NIHSS</td>
<td>11 (8–14.3)</td>
<td>14 (10–18)</td>
<td>0.01</td>
</tr>
<tr>
<td>DWI volume, mL</td>
<td>14.6 (8.6–26.3)</td>
<td>47.2 (24.6–87.8)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Tₘₐₓ &gt;6 s volume, mL</td>
<td>45.8 (24.2–99.9)</td>
<td>91.5 (42.2–146.4)</td>
<td>0.01</td>
</tr>
<tr>
<td>DWI/FLAIR mismatch</td>
<td>19 (35.2%)</td>
<td>41 (47.1%)</td>
<td>0.20</td>
</tr>
<tr>
<td>rFLAIR</td>
<td>1.15 (1.11–1.23)</td>
<td>1.14 (1.09–1.22)</td>
<td>0.20</td>
</tr>
<tr>
<td>mRS D90 (0–1)</td>
<td>17 (31.0%)</td>
<td>15 (17.0%)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Data are median (interquartile range) or n (%). Collateral grading is based on hypoperfusion intensity ratio (HIR). HIR >0.40 represents poor collateral circulation. Group comparison was done by χ² test for categorical variables and Mann–Whitney U test for continuous variables. DWI indicates diffusion-weighted imaging; FLAIR, fluid-attenuated inversion imaging; IV tPA, intravenous tissue-type plasminogen activator; mRS, modified Rankin Scale; NIHSS, National Institute Stroke Scale; and rFLAIR, relative FLAIR calculated with the voxel-based method (sphere diameter 15 mm).
onset. It is unclear whether these patients, that is, patients with good collaterals in an extended time window, will have the same benefit from thrombolysis, as patients with poor collaterals but within 4.5 hours.

The optimal threshold of rFLAIR to predict symptom onset before 4.5 hours was 1.16. This value was higher than previously reported, which is most likely related to the different method used to determine the rFLAIR intensity.\(^3^5\) The prediction of stroke onset time using the quantitative method versus the visual assessment of the DWI/FLAIR mismatch did not improve which is a confirmation of previous findings.\(^3^5\)

Our study has certain limitations. First, in this data set digital subtraction angiography (DSA) was lacking. Therefore, collateral status was indirectly obtained by collateral grading based on HIR. This technique has a good correlation with DSA-assessed collateral circulation.\(^2^1\) Second, the HIR is used as a dichotomized scale, defining good versus bad collaterals, which could have resulted in loss of power to detect an interaction. To strengthen our findings, we performed an additional analysis with the mean \(T_{max}\) in the perfusion deficit as a marker of hypoperfusion severity, which confirmed the interaction between severity of hypoperfusion on the association between time from symptom onset and rFLAIR lesion intensity. Third, a new method to calculate rFLAIR was used. Previous studies used the contralateral lesion to calculate the relative ratio.\(^3^5,^3^6\) We think our voxel-based approach to be more accurate. To strengthen our results, we showed our findings not to be critically dependent on the sphere radius chosen. Finally, the exact time from onset of symptoms was not known for all patients and in these cases, time since last seen normal was used instead.

We conclude that collateral status rated by severity of hypoperfusion modifies the association between time from symptom onset and rFLAIR signal intensity. This could influence the accuracy of FLAIR signal intensity to predict stroke onset in patients with unknown time from onset. Clinical trials using the DWI/FLAIR mismatch to determine stroke onset ≤4.5 hours might, therefore, be enrolling a larger proportion of patients with good collateral status.

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### Table 2. Results of the Multivariate Linear Regression Model

<table>
<thead>
<tr>
<th>Radius</th>
<th>B (Time); 95% CI</th>
<th>B (HIR); 95% CI</th>
<th>(P) Value of Interaction Term</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mm</td>
<td>0.03 (0.02–0.04)*</td>
<td>0.01 (−0.02 to 0.05)</td>
<td>0.04</td>
</tr>
<tr>
<td>15 mm</td>
<td>0.03 (0.02–0.04)*</td>
<td>0.02 (−0.02 to 0.04)</td>
<td>0.04</td>
</tr>
<tr>
<td>20 mm</td>
<td>0.03 (0.02–0.04)*</td>
<td>0.02 (−0.01 to 0.05)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

This table shows the results of the multivariate linear regression model with mean rFLAIR in the nonreperfused core as dependent variable and time (per hour) and the quality of collaterals (defined by HIR) as explanatory variables. An interaction term of the 2 independent variables is included. The first 2 columns represent the B, the variable estimate, with the corresponding 95% CI. The last column shows the \(P\) value of this interaction. Radius represents the sphere radius used to calculate the rFLAIR value. CI indicates confidence interval; HIR, hypoperfusion intensity ratio; and rFLAIR, relative fluid-attenuated inversion imaging.

*Corresponding \(P\) value <0.01.
References


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A voxel (red) is mirrored to its homologue voxel in the contralateral hemisphere. The rFLAIR signal intensity is determined by the ratio of the signal intensity in that voxel and the median of the signal intensity in a sphere with radius 15 mm positioned around the homologue voxel in the other hemisphere.