
Anke Wouters, MD; Patrick Dupont, PhD; Bo Norrving, MD; Rico Laage, PhD; Götz Thomalla, MD; Gregory W. Albers, MD; Vincent Thijs, MD; Robin Lemmens, MD

Background and Purpose—Acute stroke patients with unknown time of symptom onset are ineligible for thrombolysis. The diffusion-weighted imaging and fluid-attenuated inversion recovery (FLAIR) mismatch is a reasonable predictor of stroke within 4.5 hours of symptom onset, and its clinical usefulness in selecting patients for thrombolysis is currently being investigated. The accuracy of the visual mismatch rating is moderate, and we hypothesized that the predictive value of stroke onset within 4.5 hours could be improved by including various clinical and imaging parameters.

Methods—In this study, 141 patients in whom magnetic resonance imaging was obtained within 9 hours after symptom onset were included. Relative FLAIR signal intensity was calculated in the region of nonreperfused core. Mean $T_{\text{max}}$ was calculated in the total region with $T_{\text{max}}>6$ s. Mean relative FLAIR, mean $T_{\text{max}}$, lesion volume with $T_{\text{max}}>6$ s, age, site of arterial stenosis, core volume, and location of infarct were analyzed by logistic regression to predict stroke onset time before or after 4.5 hours.

Results—Receiver-operating characteristic curve analysis revealed an area under the curve of 0.68 (95% confidence interval 0.59–0.78) for the visual diffusion-weighted imaging/FLAIR mismatch, thereby correctly classifying 69% of patients with an onset time before or after 4.5 hours. Age, relative FLAIR, and $T_{\text{max}}$ increased the accuracy significantly ($P<0.01$) to an area under the curve of 0.82 (95% confidence interval 0.74–0.89). This new predictive model correctly categorized 77% of patients according to stroke onset before versus after 4.5 hours.

Conclusions—In patients with unknown stroke onset, the accuracy of predicting time from symptom onset within 4.5 hours is improved by obtaining relative FLAIR and perfusion imaging. (Stroke. 2016;47:2559-2564. DOI: 10.1161/STROKEAHA.116.013903.)

Key Words: fluid-attenuated inversion recovery imaging ■ perfusion imaging ■ prediction ■ thrombolysis ■ time-window

Intravenous thrombolysis can be administered ≤4.5 hours after symptom onset.1 Unfortunately, the exact time of stroke onset cannot be determined in a large subset (20%) of patients.2,4 In this subgroup, the time when patients were last seen normal is used to safely estimate the stroke onset time. As a result, many of these patients are ineligible for any acute stroke treatment. Neuroimaging characteristics have been explored as surrogate markers to predict time after symptom onset as an alternative for witnessed stroke onset times. In the PRE-FLAIR study, a mismatch between diffusion-weighted imaging (DWI) and fluid-attenuated inversion recovery (FLAIR) hyperintensities was shown to be associated with time from symptom onset.5 The presence of a DWI/FLAIR mismatch predicted patients to be within a time interval of 4.5 hours after stroke onset, with 62% sensitivity and 78% specificity. Clinical implementation of the DWI/FLAIR mismatch is currently being explored in the WAKE-UP trial (Efficacy and Safety of MRI-Based Thrombolysis in Wake-Up Stroke). This study enrolls patients with unknown stroke onset time and randomizes the subgroup of patients with a visual DWI/FLAIR mismatch to placebo versus thrombolysis.6

Received April 26, 2016; final revision received July 29, 2016; accepted August 12, 2016.

From the Department of Neurosciences, Experimental Neurology and Leuven Research Institute for Neuroscience and Disease (LIND), KU Leuven–University of Leuven, B-3000 Leuven, Belgium (A.W., R. Lemmens); VIB, Vesalius Research Center, Laboratory of Neurobiology, B-3000 Leuven, Belgium (A.W., R. Lemmens); Department of Neurology, University Hospitals Leuven, B-3000 Leuven, Belgium (A.W., R. Lemmens); Laboratory for Cognitive Neurology, KU Leuven, Herestraat 49, 3000 Leuven, Belgium (P.D.); Department of Clinical Sciences, Section of Neurology, Lund University, Sweden (B.N.); Guided Development GmbH, Heidelberg, Germany (R. Laage); Universitätssklinikum Hamburg-Eppendorf, Klinik und Poliklinik für Neurologie, Kopf-und Neurozentrum, Hamburg, Germany (G.T.); Stroke Center, Stanford University, Palo Alto, CA (G.W.A.); and Department of Neurology Austin Health, and Melbourne Brain Center, Florey Institute of Neuroscience and Mental Health, Heidelberg, Australia (V.T.).

Guest Editor for this article was Liping Liu, MD, PhD.

The online-only Data Supplement is available with this article at http://stroke.ahajournals.org/lookup/suppl/doi:10.1161/STROKEAHA.116.013903/-/DC1.

Correspondence to Anke Wouters, MD, Herestraat 49, 300 Leuven, Belgium. E-mail anke.wouters@med.kuleuven.be

© 2016 American Heart Association, Inc.

Stroke is available at http://stroke.ahajournals.org

DOI: 10.1161/STROKEAHA.116.013903

2559
The predictive power of the DWI/FLAIR mismatch pattern to accurately classify patients in the appropriate time epoch is variable. Performing a quantitative analysis of FLAIR imaging by determining intensities in defined region of interests (ROI) did not improve the accuracy compared with the visual DWI/FLAIR mismatch. In addition, clinical and other neuroimaging parameters, for example, age and DWI volume, have been identified as modifiers of the DWI/FLAIR mismatch. Recently, we identified an interaction between collateral status and the association between FLAIR lesion intensity and stroke onset time. Based on these findings, we aimed to construct an improved model to determine stroke onset time within 4.5 hours by adding other predictive, clinical, and neuroimaging variables.

In addition, we studied the power of the predictive models for a time window within 3 and 6 hours after onset. The 3-hour time interval is of interest because it is used for thrombolysis selection of some patients, and the 6-hour time window is relevant in the decision to initiate endovascular treatment of acute stroke patients with large vessel occlusions. To our knowledge, the predictive value of the DWI/FLAIR mismatch has only been studied limited to correctly classify patients within 3 and 6 hours after stroke onset.

Methods
Clinical and neuroimaging data from a large Phase Ib, multicenter, placebo-controlled, randomized and double-blind trial the AXIS 2 (AX200 for ischemic stroke trial) were used. In AXIS 2, the clinical efficacy of recombinant granulocyte colony-stimulating factor (AX200) in acute ischemic stroke patients was evaluated. The primary and secondary end points of the trial were not met. The full details of the methodology of AXIS 2 have been described previously. In short, patients were included in a time window of 9 hours after symptom onset or after last seen normal. The main exclusion criteria used were signs of severe stroke on neuroimaging, hemorrhagic strokes, and lacunar infarcts.

The presence of a DWI/FLAIR mismatch was rated visually by 2 independent readers (Drs Wouters and Thijs) according to the criteria implemented in the ongoing WAKE-UP trial. The relative FLAIR (rFLAIR) signal intensity was calculated in voxel-based manner as described previously. For every voxel, the rFLAIR was calculated as the ratio of the FLAIR signal intensity in that voxel and the median FLAIR signal intensity of the sphere, with radius 15 mm around the homologue voxel in the contralateral hemisphere. Currently, we have developed prototype software, and rFLAIR is calculated by a Matlab script.

To validate our predictive model, we used the leave-one-out cross validation technique. We repeatedly calculated the coefficients of determination between time from symptom onset and rFLAIR were added as explanatory variables. This model was simplified by removing variables that are not sufficiently contributing according to the Akaike information criterion method. The smaller the Akaike information criterion, the better the model. Predictive tables with a threshold of 0.5 and receiver-operating characteristic curves were calculated for the different models. Areas under the curve (AUCs) were compared with the DeLong’s test. The same multivariate analysis was performed with symptom onset before or after 4.5 hours as dependent variable.

To validate our predictive model, we used the leave-one-out cross validation technique. We repeatedly calculated the coefficients of

Figure 1. Imaging parameters used in the multivariate predictive model. DWI (A) and FLAIR (B) imaging reveal a DWI/FLAIR mismatch. The perfusion imaging map (C) shows the \( T_{\text{max}} \) in the region of hypoperfusion with a \( T_{\text{max}} > 6 \) s, whereas in (D), the rFLAIR in the nonreperfused core is represented. DWI indicates diffusion-weighted imaging; FLAIR, fluid-attenuated inversion recovery imaging; and rFLAIR, voxelwise relative FLAIR.
the logistic regression model each time leaving one patient out and determined the cross-validated AUC.

Statistical analysis was done with R: A language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/) and Matlab_R2012B.

Results
In 206 patient from the original 324 patients of the AXIS 2 trial (63.8%), the necessary imaging sequences were available and of sufficient quality to be evaluated. As prespecified, 12 patients (5.2%) were excluded because confluent or large FLAIR lesions were present in the contralateral hemisphere or leukoaraiosis overlapped the acute lesion. No DWI lesion detected or complete reperfusion of the DWI lesion led to the exclusion of an additional 53 (25.7%) patients because a nonreperfused core was absent. Reasoning for including only patients with a nonreperfused core is mentioned in the methods, and in addition, in patients with complete reperfusion, the estimation of stroke onset seems less relevant because reperfusion therapy is no longer required.

Here we report the results of a total of 141 included patients. Baseline characteristics are shown in Table 1. Median time of symptom onset was 320 minutes. The time distribution of the whole study population can be found in Figure II in the online-only Data Supplement. When comparing the patients included in this analysis versus the excluded cohort, baseline National Institutes of Health Stroke Scale was more severe, and the percentage of good functional outcome at 90 days was slightly lower in the excluded cohort (Table I in the online-only Data Supplement).

Table 2 presents the models predicting delay since time of onset. In the final model, rFLAIR signal intensity, mean $T_{\text{max}}$ in the hypoperfused lesion, age, and the interaction terms were retained. These variables provided the highest predictive power for stroke onset within the time window of 4.5 hours (Table 2). When evaluating patients with the visual DWI/FLAIR mismatch rating, with good interobserver reliability (kappa=0.70), 69% patients were correctly classified with stroke onset before versus after 4.5 hours. This could be improved to 77% of the patients by the newly constructed predictive model (Table 3). The positive predictive value for this model was 0.75 (95% confidence interval [CI] 0.57–0.87), and the negative predictive value was 0.78 (95% CI 0.69–0.85) compared with 0.55 (95% CI 0.42–0.68) and 0.79 (95% CI 0.68–0.87) for the visual DWI/FLAIR mismatch. Interestingly, by only including perfusion imaging in the model with rFLAIR, the accuracy was already significantly improved compared with the visual mismatch rating (Figure III in the online-only Data Supplement). The visual DWI/FLAIR mismatch had an AUC of 0.68, which was improved to 0.82 ($P<0.01$) in the multivariate predictive model (Figure 2 and Table 3). The model was internally validated, resulting in a similar improvement of the accuracy (AUC of 0.79; $P=0.01$, Table II in the online-only Data Supplement). Figure IV in the online-only Data Supplement shows an example of a patient without a visual DWI/FLAIR mismatch being correctly classified by the multivariate predictive model.

In a sensitivity analysis to determine if the method used to obtain the rFLAIR would be critically important for our results, the rFLAIR was evaluated by the ROI technique. The same explanatory variables were retained in the final model with hypoperfused lesion volume as additional variable. This model resulted in correctly categorizing 77% of patients, and the accuracy showed a similar trend toward improvement (AUC 0.78; $P=0.06$).

In addition, we studied the power of a predictive multivariate model for stroke symptom onset before or after 3 and 6 hours. In our study, 15 patients (11%) presented within 3 hours after stroke onset and 92 (65%) within 6 hours. In the model to predict stroke onset before versus after 3 hours, the following explanatory variables were retained: rFLAIR, mean $T_{\text{max}}$, rFLAIR×mean $T_{\text{max}}$, and DWI lesion volume (Table 2). This multivariate model correctly classified 93% of the patients in the time epoch of 3 hours with a positive predictive value of 0.78 (95% CI 0.40–0.96) and a negative predictive value of 0.94 (95% CI 0.88–0.97). In this model, the AUC improved to 0.90 compared with 0.67 for the visual DWI/FLAIR mismatch ($P<0.01$; Figure 2).

To predict stroke onset before versus after 6 hours, the following explanatory variables were retained in the final model: rFLAIR, mean $T_{\text{max}}$, rFLAIR×mean $T_{\text{max}}$, and location of infarct (Table 2). This multivariate regression model correctly classified 67% of patients with a positive predictive value of 0.71 (95% CI 0.61–0.79) and a negative predictive value of 0.55 (95% CI 0.36–0.72). The AUC improved to 0.72 compared with 0.59 when using the visual DWI/FLAIR mismatch ($P=0.01$; Figure 2).

Discussion
In this analysis of acute neuroimaging in stroke patients, the accuracy of visual judgment of DWI/FLAIR mismatch to predict stroke onset within 4.5 hours was improved by combining quantitative FLAIR analysis (rFLAIR), hypoperfusion status, and age. This multivariate model correctly classified 77% of stroke patients in the time window before versus after 4.5 hours. Similarly, multivariate models that included clinical and neuroimaging parameters were more accurate in predicting stroke onset before or after 3 and 6 hours. Our results show
exists for qualitative assessments of the DWI/FLAIR mismatch.5 Measurements could eliminate the interrater variability that whether a qualitative visual rating or a potentially more objective time window, probably reflecting the increase in heterogeneity of the FLAIR intensity in the DWI lesion over time. Several factors may influence the predictive value of the DWI/FLAIR mismatch.10 These data suggested that the severity of hypoperfusion interacted with the association between time and FLAIR intensity.10 By including the severity of hypoperfusion, the accuracy to detect time of onset was further improved in our study.

Currently, the WAKE-UP trial16 is studying the effect of thrombolysis in patients with a DWI/FLAIR mismatch on clinical outcomes and 90 days. The results of this study could have potential implications for the treatment of patients with unknown time of stroke onset. Clinical implementation of the DWI/FLAIR mismatch is dependent on both the accuracy of the currently used visual rating versus the complexity of alternative methods used to improve the predictive value. Potentially, the automated quantitative measurements of FLAIR and perfusion imaging presented here could be of benefit to estimate duration of stroke symptoms. In the PRE-FLAIR study, the DWI-FLAIR mismatch was introduced to predict onset of stroke symptoms within 4.5 hours because this was the time window for the only acute stroke therapy: IV thrombolysis. In recent years, robust evidence has been provided for a beneficial effect of mechanical thrombectomy in patients with acute ischemic stroke and a large vessel occlusion within a time window of 6 hours after stroke onset.18 Here we show improvement of the accuracy of the multivariate model to estimate stroke onset in this particular time window compared with the visual DWI/FLAIR mismatch.

Our study has some limitations. We could not analyze all patients in AXIS 2 because the imaging parameters were not of adequate quality in all. Therefore, we assessed potential differences in baseline and outcome parameters in the included

### Table 2. Final Predictive Models

<table>
<thead>
<tr>
<th>Variable</th>
<th>Exp(B)</th>
<th>95% CI Exp(B)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>1.04</td>
<td>1.00–1.09</td>
<td>0.03</td>
</tr>
<tr>
<td>rFLAIR, %</td>
<td>2.18</td>
<td>1.19–4.00</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean T&lt;sub&gt;max&lt;/sub&gt;, s</td>
<td>2.44</td>
<td>1.33–4.48</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>rFLAIR×mean T&lt;sub&gt;max&lt;/sub&gt;</td>
<td>0.99</td>
<td>0.99–1.00</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>3 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DWI volume, mL</td>
<td>0.95</td>
<td>0.91–1.00</td>
<td>0.04</td>
</tr>
<tr>
<td>rFLAIR, %</td>
<td>4.15</td>
<td>1.74–9.88</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean T&lt;sub&gt;max&lt;/sub&gt;, s</td>
<td>4.63</td>
<td>1.95–10.99</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>rFLAIR×mean T&lt;sub&gt;max&lt;/sub&gt;</td>
<td>0.99</td>
<td>0.98–0.99</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>6 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location, GM</td>
<td>0.42</td>
<td>0.18–1.00</td>
<td>0.05</td>
</tr>
<tr>
<td>rFLAIR, %</td>
<td>1.56</td>
<td>1.02–2.37</td>
<td>0.04</td>
</tr>
<tr>
<td>Mean T&lt;sub&gt;max&lt;/sub&gt;, s</td>
<td>1.67</td>
<td>1.09–2.54</td>
<td>0.02</td>
</tr>
<tr>
<td>rFLAIR×mean T&lt;sub&gt;max&lt;/sub&gt;</td>
<td>1.00</td>
<td>0.99–1.00</td>
<td>0.02</td>
</tr>
</tbody>
</table>

This table represents the coefficients of the final logistic regression models with 4.5 h, 3 h, and 6 h time interval as dependent variables. CI indicates confidence interval; DWI, diffusion-weighted imaging; GM, gray matter; rFLAIR, relative fluid-attenuated inversion recovery imaging; and T<sub>max</sub>, time to the maximum of the residue function obtained by deconvolution.

Table 3. Prediction of Stroke Onset Before or After 4.5 h

<table>
<thead>
<tr>
<th>Time After Stroke Onset</th>
<th>Predicted Time Window</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Visual (n)</td>
</tr>
<tr>
<td></td>
<td>&gt;4.5 h</td>
</tr>
<tr>
<td>&gt;4.5 h (n=91)</td>
<td>64</td>
</tr>
<tr>
<td>≤4.5 h (n=50)</td>
<td>17</td>
</tr>
<tr>
<td>Correctly classified (total n=141)</td>
<td>97 (69%)</td>
</tr>
</tbody>
</table>

AUC

0.68 0.76 0.80 0.82

Actual and predicted patients within or after the 4.5 time window after stroke onset. The explanatory variables for every model are as follows: Visual, visual DWI/FLAIR mismatch; Model 1, rFLAIR; Model 2, rFLAIR+mean T<sub>max</sub>; Model 3, rFLAIR+mean T<sub>max</sub>+rFLAIR×mean T<sub>max</sub>; Model 3, rFLAIR+mean T<sub>max</sub>+rFLAIR×mean T<sub>max</sub>+age. AUC indicates area under the curve of the complementary ROC curve; DWI, diffusion-weighted imaging; mean T<sub>max</sub>, time to the maximum of the residue function obtained by deconvolution; rFLAIR, relative fluid-attenuated inversion recovery imaging; and ROC, receiver-operating characteristic.
versus excluded cohort and did not identify major differences except for stroke severity. Second, in the AXIS 2 study, exact stroke onset times were recorded when known, and when this was unclear, the time when patients were last seen normal was assumed as the onset time. Potentially, our results would have been even more striking if we could have limited our analysis to patients with exact stroke onset times, but unfortunately the method (exact versus last seen normal) of determining stroke onset was not documented. Third, the created predictive models could not be externally validated because we did not have access to an additional sample set of patients in whom all required imaging parameters have been obtained and stroke onset times are known. To partially overcome this limitation, we performed an internal validation that confirmed our results. Last, we would like to emphasize that, currently, we have developed prototype software to determine rFLAIR values. If clinical implementation of this model is considered, optimization of the postprocessing will be required to quickly predict the time window in patients with unknown stroke onset time.

Conclusions
We conclude that a multivariate predictive model integrating quantitative rFLAIR measurement, information on the severity of hypoperfusion, and age has greater accuracy than the visual judgment of DWI/FLAIR mismatch for determining stroke onset time within 4.5 hours. After further validation, this model may be useful for selecting patients for stroke therapies in whom the time of symptom onset is unknown.

Acknowledgments
Dr Lemmens is a Senior Clinical Investigator of FWO Flanders.

Sources of Funding
Dr Albers received grant funding from the National Institutes of Health.

Disclosures
Dr Wouters received grants from European Union. Soren Christensen reports consultant of iSchemaView. Dr Norving reports fees paid to the institution from SYGNIS for steering committee work in the AXIS2 trial. Dr Thomalla reports receiving grants from European Union. Dr Albers reports consultant of iSchemaView, Covidien, and Lundbeck. He has an equity interest in iSchemaView. Dr Thijs reports receiving fees for serving on the steering committee of the AXIS 2 trial. The other authors report no conflicts.

References


Anke Wouters, Patrick Dupont, Bo Norrving, Rico Laage, Götz Thomalla, Gregory W. Albers, Vincent Thijs and Robin Lemmens

Stroke. 2016;47:2559-2564; originally published online September 6, 2016;
doi: 10.1161/STROKEAHA.116.013903

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/47/10/2559

Data Supplement (unedited) at:
http://stroke.ahajournals.org/content/suppl/2016/09/15/STROKEAHA.116.013903.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/
**SUPPLEMENTAL MATERIAL**

**Supplementary methods I**

**Overview FLAIR analyzing techniques**

<table>
<thead>
<tr>
<th>Visual DWI/FLAIR mismatch</th>
<th>Voxel-based rFLAIR calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>The assessment of the visual DWI/FLAIR mismatch is in accordance with the protocol of the ongoing Wake-Up trial. A visual mismatch is defined as an acute DWI lesion without a marked parenchymal hyperintensity on FLAIR. Patients with leukoariosis overlapping with the DWI lesion are excluded (i.e. DWI/FLAIR mismatch negative).</td>
<td>For every voxel the relative FLAIR signal intensity was determined by the ratio of the signal intensity in that voxel and the median of the signal intensity in a sphere with a radius of 15 mm around the homologue voxel in the contralateral hemisphere. This proces was repeated for every voxel in the way that for all voxels the rFLAIR signal intensity was calculated.</td>
</tr>
</tbody>
</table>
### Supplementary table I

<table>
<thead>
<tr>
<th></th>
<th>Excluded (n=183)</th>
<th>Included (n=141)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>71 (65-78)</td>
<td>70 (62-76)</td>
<td>0.2</td>
</tr>
<tr>
<td>Gender (f)</td>
<td>89 (49%)</td>
<td>66 (47%)</td>
<td>0.8</td>
</tr>
<tr>
<td>Time from symptom onset (min)</td>
<td>310 (229-401)</td>
<td>320 (233-396)</td>
<td>0.8</td>
</tr>
<tr>
<td>IV tPA</td>
<td>121 (66%)</td>
<td>90 (64%)</td>
<td>0.7</td>
</tr>
<tr>
<td>NIHSS</td>
<td>11 (8-15)</td>
<td>13 (9-17)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>mRS D90 (0-2)</td>
<td>85 (46%)</td>
<td>49 (35%)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Data are median (IQR) or n (%). Group comparison was done by Chi Square test for categorical variables and Mann-Withney-U for continuous variables. IV tPA = intravenous tissue plasminogen activator, NIHSS= National Institute Stroke Scale, mRS = Modified Rankin Scale at day 90.
### Supplementary table II

**Prediction of stroke onset within 4.5 h after cross validation**

<table>
<thead>
<tr>
<th>Time after stroke onset</th>
<th>&gt;4.5h</th>
<th>&lt;4.5h</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;4.5h (n=91)</td>
<td>79</td>
<td>12</td>
</tr>
<tr>
<td>&lt;4.5h (n=50)</td>
<td>23</td>
<td>27</td>
</tr>
<tr>
<td>Correctly classified (total n=141)</td>
<td></td>
<td>106 (75%)</td>
</tr>
<tr>
<td>AUC</td>
<td></td>
<td>0.79</td>
</tr>
</tbody>
</table>

The predictive values in this table were obtained after internal validation with the Leave-One-Out approach. The AUC improved (0.79) compared to the visual DWI/FLAIR mismatch (0.68) as predictive model (p=0.01). AUC = area under the curve.
Supplementary figure I

Example of an excluded patient due to an old ischemic contralateral lesion.
A. FLAIR imaging
B. Diffusion weighted imaging
Supplementary figure II

Time distribution of stroke onset time as documented in the total study population.
Receiver operating characteristic curves with stroke onset before 4.5h as dependent variable. X-axis represent 1-specificity, Y-axis the sensitivity.

**Model 1**: rFLAIR (AUC= 0.76); p=0.08

**Model 2**: rFLAIR + meanTmax + rFLAIR*meanTmax (AUC = 0.80); p < 0.01

**Model 3**: rFLAIR + meanTmax + rFLAIR*meanTmax + age (AUC = 0.82); p < 0.01

**Model Mismatch**: DWI/FLAIR mismatch (AUC = 0.68)

p-values represent differences in AUC compared to the model with visual DWI/FLAIR mismatch as dependent variable. (DeLong’s test).
rFLAIR = relative Fluid Attenuated Inversion Recovery Imaging, DWI= diffusion weighted imaging, Mean T_max = mean T_max (= time to the maximum of the residue function obtained by deconvolution), AUC= area under the curve
Supplementary figure IV

Imaging example of a patient who presented 252 minutes after stroke onset. The patient had no visual mismatch since a FLAIR hyperintensity was present in the DWI lesion; therefore this patient was classified beyond the 4.5h time window. The multivariate model classified the patient correctly within 4.5h.

Panel A: Diffusion weighted imaging
Panel B: FLAIR imaging
Panel C: Tmax perfusion imaging
Panel D: rFLAIR map