# DWI-FLAIR mismatch for the identification of patients with acute ischaemic stroke within 4·5 h of symptom onset (PRE-FLAIR): a multicentre observational study

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## Summary

**Background** Many patients with stroke are precluded from thrombolysis treatment because the time from onset of their symptoms is unknown. We aimed to test whether a mismatch in visibility of an acute ischaemic lesion between diffusion-weighted MRI (DWI) and fluid-attenuated inversion recovery (FLAIR) MRI (DWI-FLAIR mismatch) can be used to detect patients within the recommended time window for thrombolysis.

**Methods** In this multicentre observational study, we analysed clinical and MRI data from patients presenting between Jan 1, 2001, and May 31, 2009, with acute stroke for whom DWI and FLAIR were done within 12 h of observed symptom onset. Two neurologists masked to clinical data judged the visibility of acute ischaemic lesions on DWI and FLAIR imaging, and DWI-FLAIR mismatch was diagnosed by consensus. We calculated predictive values of DWI-FLAIR mismatch for the identification of patients with symptom onset within 4·5 h and within 6 h and did multivariate regression analysis to identify potential confounding covariates. This study is registered with ClinicalTrials.gov, number NCT01021319.

**Findings** The final analysis included 543 patients. Mean age was 66·0 years (95% CI 64·7–67·3) and median National Institutes of Health Stroke Scale score was 8 (IQR 4–15). Acute ischaemic lesions were identified on DWI in 516 patients (95%) and on FLAIR in 271 patients (50%). Interobserver agreement for acute ischaemic lesion visibility on FLAIR imaging was moderate (κ=0·569, 95% CI 0·504–0·634). DWI-FLAIR mismatch identified patients within 4·5 h of symptom onset with 62% (95% CI 57–67) sensitivity, 78% (72–84) specificity, 83% (79–88) positive predictive value, and 54% (48–60) negative predictive value. Multivariate regression analysis identified a longer time to MRI (p<0·0001), a lower age (p=0·0009), and a larger DWI lesion volume (p=0·0226) as independent predictors of lesion visibility on FLAIR imaging.

**Interpretation** Patients with an acute ischaemic lesion detected with DWI but not with FLAIR imaging are likely to be within a time window for which thrombolysis is safe and effective. These findings lend support to the use of DWI-FLAIR mismatch for selection of patients in a future randomised trial of thrombolysis in patients with unknown time of symptom onset.

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## Introduction

International guidelines and approval criteria for use of intravenous thrombolysis exclude patients with stroke whose time of symptom onset is unknown.[1,2] However, many patients who have had a stroke present with unknown time of symptom onset (eg, if symptoms occur during sleep and are recognised only when the patient awakes) and, as with myocardial infarction, patients are more likely to have a stroke in the morning.[3,4] An estimated 25% of ischaemic strokes occur during sleep,[4–6] which means that this large group of patients are precluded from thrombolysis—the only approved and effective specific treatment for acute ischaemic stroke. This dissatisfying situation has raised great interest in surrogate markers of lesion age. Moreover, clinical and imaging findings in patients waking with stroke symptoms were reported to be much the same as in patients with symptom onset known to be within 3–6 h,[5–7] indicating that some of these patients might benefit from thrombolysis.

Multiparametric MRI has been suggested as a means to identify patients who are likely to benefit from thrombolysis, by use of different sequences that are sensitive to different aspects of tissue pathophysiology in acute cerebral ischaemia.[6,8,9] Alterations of water diffusion can be detected with diffusion-weighted imaging (DWI) within 3 min from onset of ischaemia,[10] whereas a net increase of water can be detected as an increase of T2 signal within 1–4 h from onset of ischaemia.[11,12] Fluid-attenuated inversion recovery (FLAIR) imaging is a T2-weighted imaging sequence[13] and an integral part of common multiparametric stroke MRI protocols. FLAIR

imaging is highly sensitive to subacute ischaemic brain lesions,[14] but typically cannot be used to detect ischaemic lesions within the first few hours.[15,16]

The mismatch of visibility of an acute ischaemic lesion between DWI and FLAIR has been suggested to enable identification of patients with acute ischaemic stroke who are likely to be within the 3-h time window. In a single centre study,[9] a DWI-FLAIR mismatch identified patients within 3 h of symptom onset with high specificity (93%) and positive predictive value (PPV; 94%), although sensitivity and negative predictive value (NPV) were low. These findings have been reproduced in studies from other groups.[17–19] All these studies showed a clear time dependency, with acute ischaemic lesion visibility on FLAIR imaging increasing during the first 6 h after stroke onset and approaching nearly 100% thereafter, a finding that is well known from experimental stroke.[11] Taken together, the results from these studies are promising with regard to the use of DWI-FLAIR mismatch to estimate lesion age in patients with unknown time of stroke onset.

In some centres, multiparametric MRI incorporating the absence of a lesion on FLAIR imaging is already used to decide whether thrombolytic drugs should be given to patients who wake up with symptoms of stroke.[8,20,21] However, neither safety nor efficacy of MRI-based thrombolysis in patients who awake with stroke have been shown. Thus, more data on the benefits of DWI-FLAIR mismatch in clinical practice are needed. Four reliable but retrospective single-centre studies lend support to such use of DWI-FLAIR mismatch,[6,17–19] but several questions remain unanswered—eg, reproducibility in a multicentre setting and the effect of potential confounding factors such as leukoaraiosis, lesion volume, and infarct location. Before DWI-FLAIR mismatch can be used to guide use of thrombolysis in patients with unknown time of symptom onset, its safety and efficacy should be assessed in a randomised controlled trial, and before such a trial, the diagnostic accuracy of this approach needs to be confirmed in a large multicentre study. In the PREdictive value of FLAIR and DWI for the identification of patients with acute ischaemic stroke within 4·5 h of symptom onset (PRE-FLAIR) study, we aimed to test whether DWI-FLAIR mismatch can be used to identify patients within 4·5 h of symptom onset with high specificity and PPV, and thus to assess the possibility of using DWI-FLAIR mismatch in a future randomised controlled trial of MRI-based thrombolysis.

## Methods

### Study design and patients

The PRE-FLAIR study was a multicentre observational study of patients with acute ischaemic stroke with known time of symptom onset who had undergone MRI with DWI and FLAIR within 12 h of symptom onset. We tested the predefined hypothesis that DWI-FLAIR mismatch can be used to identify patients within 4·5 h of symptom onset with high specificity in a retrospective cohort of patients studied within 12 h of symptom onset, presenting between Jan 1, 2001, and May 31, 2009. The study was done by an international consortium of researchers within the Stroke Imaging Repository (STIR) and Virtual International Stroke Trials Archive (VISTA) Imaging research groups.[22] PRE-FLAIR included individual datasets from eight participating stroke centres and two studies: EPITHET (Echoplanar Imaging Thrombolysis Evaluation Trial), which was a phase 2 prospective, randomised, double-blinded, placebo-controlled, multinational trial,[23] and VIRAGE (Valeur predictive des parametres IRM à la phase aigue de l'accident vasculaire cerebral: application à la gestion des essais thérapeutiques), which was a national multicentre study.[24] Patients were enrolled if they had well defined symptom onset (ie, exact time of symptom onset was recorded and reported by either the patient or somebody who witnessed their symptom onset), MRI including DWI and FLAIR done within 12 h of symptom onset, and ischaemic stroke confirmed by follow-up imaging. During the study period in the participating centres, MRI was used as either a first diagnostic test for patients with acute stroke or after CT during the first 24 h. Study centres contributed consecutive eligible datasets; incomplete datasets (ie, those with incomplete MRI or missing information about stroke severity) were discarded. Details of the selection of patients for EPITHET and VIRAGE are reported elsewhere.[23,24] The study was approved by the local ethics committees at all centres. Either written or verbal informed consent was obtained for all patients, as required by local legislation. PRE-FLAIR is registered with ClinicalTrials.gov, number NCT01021319.

The primary outcome measure was the specificity and PPV of a DWI-FLAIR mismatch for the identification of patients with acute stroke within a sufficient period of time from symptom onset to allow intravenous thrombolysis with alteplase. At the time of initiation of PRE-FLAIR, the time window of interest was 3 h or less, in line with recommendations for intravenous thrombolysis at that time.[1,2] After publication of results from the third European Cooperative Acute Stroke Study (ECASS-3)[25] and extension of the recommended time window for intravenous alteplase treatment to 4·5 h or less in international guidelines,[26] we changed our time window of interest from 3 h or less to 4·5 h or less. In view of the potential benefit and safety of thrombolysis even up to 6 h from symptom onset, as shown in a meta-analysis of the large clinical trials of stroke thrombolysis,[27] we also tested the predictive values of DWI-FLAIR mismatch for the identification of ischaemic lesions within a time window of 6 h or less.

### Procedures

Multiparametric MRI was done in all contributing centres as part of institutional protocols for the diagnosis

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of acute stroke. Clinical MRI scanners with a field strength of 1·5 T or 3 T from three different manufacturers were used according to standardised acquisition protocols and in compliance with the parameters recommended by a consensus of international stroke MRI researchers (webappendix p 1).[22]

See Online for webappendix

Image analysis was done by two neurologists who were experienced in reading of brain images from patients with stroke (BC and MEb). When these judgments were conflicting, images were discussed together with a third investigator (GT) and consensus was reached between all three observers. Observers were masked to clinical information. The consensus judgment of DWI and FLAIR imaging was used for further analysis, and individual observers' judgments were used only for the calculation of interobserver agreement. Before image analysis we secured a high intra-observer reliability in a test dataset, which was assesed by both observers individually and again in a consensus rating together with a third observer.

DWI with strong diffusion weighting (b=1000) and spin-echo FLAIR images were anonymised, centrally stored, and analysed without further post-processing. We chose b=1000 images for DWI because of the high contrast of ischaemic lesions on these images and their signal similarities (hyperintensity) with FLAIR images early after stroke onset. Image analysis was done for DWI and FLAIR together—ie, the presence of acute ischaemic lesions on FLAIR images was judged with knowledge of the presence of lesions on DWI. In cases of doubt, observers were instructed to verify the acuity of the lesion detected on DWI on apparent diffusion coefficient maps. For each patient, observers judged image quality (separately for DWI and FLAIR), whether and in which region they were able to identify an acute ischaemic lesion on DWI, and whether and in which region they were able to identify a corresponding acute ischaemic lesion on FLAIR imaging. Regarding FLAIR images, observers were instructed to judge lesion visibility by only parenchymal hyperintensity, disregarding other possible signs on FLAIR images indicative of acute ischaemic stroke, such as hyperintense vessels. A lesion was judged as visible on FLAIR MRI (FLAIR positive) when traceable parenchymal hyperintensity was present in a region corresponding to the acute ischaemic lesion on DWI. DWI-FLAIR mismatch was diagnosed when a visible acute ischaemic lesion was present on DWI with no traceable parenchymal hyperintensity in the corresponding region on FLAIR imaging (FLAIR negative). Additionally, one observer (BC) assessed for presence of leukoaraiosis on FLAIR images with an adapted scale of Fazekas and Schmidt.[28] This scale can be used to classify white matter hyperintensities: periventricular changes are scored as 1 for caps or pencil-thin lining, 2 for smooth halo, and 3 for irregular lesions extending into deep white matter; the extent of deep white matter changes is scored as 0 for no lesion, 1 for punctuate foci, 2 for beginning confluent foci, and 3 for confluent lesions. For further analysis, we defined severe leukoaraiosis as a score of 2–3 in any of the two subscales. The counting of lesion numbers in the original scale of Fazekas and Schmidt was disregarded in our study. Furthermore, DWI lesion volume was calculated by use of a semi-automatic thresholding approach with in-house software (Analysis Tool for Neuro Image Data [AnToNIa]). In brief, DWI lesions were manually surrounded with a generous safety margin at each affected slice, and a second region of interest was drawn in the unaffected hemisphere. This second area was then used to calculate the corresponding mean and SD of the signal intensities within this area. Finally, intensity thresholding was applied to refine the defined lesion area. We retained all voxels that were part of the defined lesion area with a signal intensity exceeding the mean signal intensity of the unaffected hemisphere by more than two SDs and rejected all others.

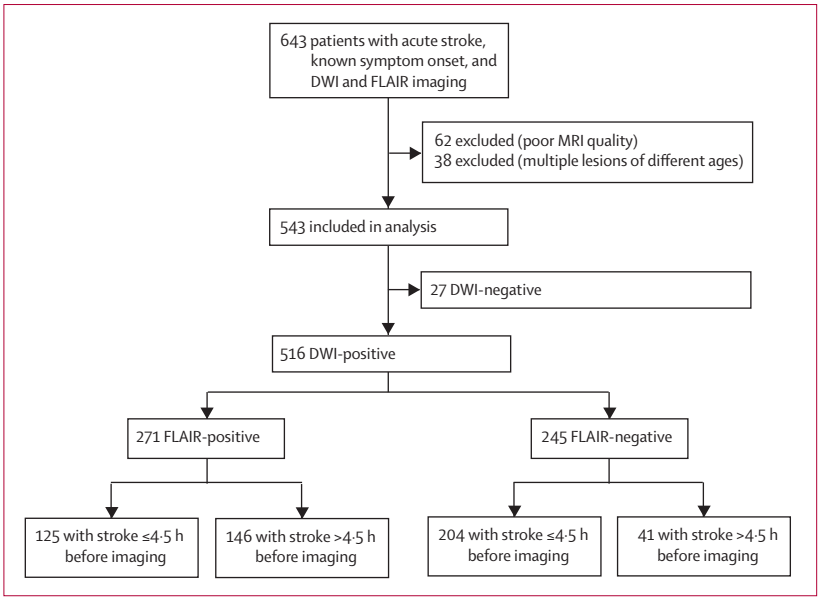


***Figure 1:*** **Study profile**
DWI=diffusion-weighted imaging. FLAIR=fluid-attenuated inversion recovery.

Image analysis was done on two workstations with identical high-resolution screens (thin-film transistor liquid-crystal display, 20·1 inch, 1600×1200 resolution; Samsung Electronics, Seoul, South Korea) with commercial software (eFilm Workstation [version 3.1], Merge Healthcare, Milwaukee, WI, USA). Observers were allowed to modify the window and level to display images with optimum contrast for lesion identification. Before image analysis, all observers were trained on independent pilot datasets until they were accustomed to the rating procedure. Only datasets with both DWI and FLAIR images judged to be of sufficient quality and assessable by both observers were included in the final analysis. Images indicative of multiple acute and subacute ischaemic lesions of different ages, precluding the

attribution of symptom onset to one specific lesion, were also excluded from the final analysis.

We recorded demographic data, time from symptom onset to MRI, severity of neurological deficit on admission as assessed with the National Institutes of Health Stroke Scale (NIHSS),[29] stroke cause according to Trial of Org 10172 in Acute Stroke Treatment (TOAST) definitions,[30] and blood glucose and systolic blood pressure on admission.

### Statistical analysis

We calculated the extent of interobserver agreement for the identification of acute ischaemic lesions on DWI and on FLAIR imaging. Group comparison between FLAIR-negative and FLAIR-positive cases was done with multivariable models with the centre as a random effect. We graphically checked values for each continuous factor and, in cases with asymmetric distribution, we log transformed parameters before group comparison. For parameters with asymmetric distribution, median (IQR) values are reported with the geometric mean for descriptive purposes. We entered parameters with $p<0·1$ in a univariate analysis into a multivariable logistic regression analysis including the centre as a random effect and with positive FLAIR imaging being the dependent variable. We calculated sensitivity, specificity, PPV, and NPV for the identification of patients within 4·5 h and 6 h of symptom onset who had negative FLAIR scans, with exact 95% CI, for all patients with a lesion on DWI and an assessable FLAIR image. We also calculated predictive values for subgroups of patients, with the aim of identifying a population who might be eligible for thrombolysis (on the basis of clinical criteria) and a population in which the DWI-FLAIR mismatch seemed to be reliable (on the basis of previous experience). We arbitrarily defined a relevant neurological deficit on the basis of an NIHSS score of >3—a cutoff that has been used in previous stroke trials.[23,31] In line with the improved performance of DWI-FLAIR mismatch recorded in previous studies,[18,20] for the secondary analysis we also excluded very small (<5 mL) DWI lesions and DWI lesions outside the middle cerebral artery [MCA] territory.

We also did a multivariable logistic regression analysis including FLAIR-DWI mismatch together with potential confounding covariates (age, severe leukoaraiosis, and DWI lesion size) with time window (≤4·5 h and ≤6 h) as a dependent variable in a backward selection model and calculated the area under the curve as a measure of model performance. Statistical analysis was done with SAS (version 9.2), the statistical package R (version 2.11.1), and SPSS (version 13.0). The report of this study follows the Standards for the Reporting of Diagnostic accuracy studies statement.[32]

### Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

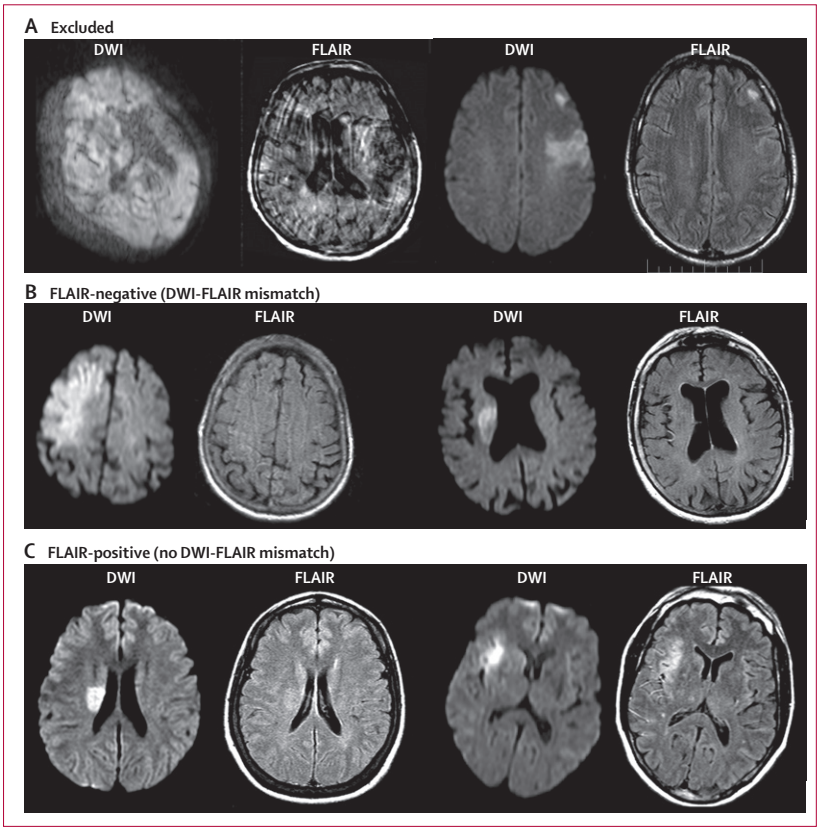


***Figure 2:*** **Examples of DWI and FLAIR images**
(A) Diffusion-weighted imaging (DWI) and fluid-attenuated inversion recovery (FLAIR) images excluded from the final analysis because of poor quality (left) or the presence of multiple acute and subacute ischaemic lesions of different ages, precluding the attribution of symptom onset to one specific lesion (right). (B) Pairs of images showing acute ischaemic lesions on DWI but not on FLAIR imaging (FLAIR-negative, DWI-FLAIR mismatch). (C) Pairs of images showing acute ischaemic lesions on DWI together with a corresponding subtle (left) or obvious (right) parenchymal hyperintensity on FLAIR imaging (FLAIR-positive, no DWI-FLAIR mismatch).

## Results

Figure 1 shows the study profile. Figure 2 shows examples of DWI and FLAIR images, including poor quality MRI scans for which some patients were excluded. At least one observer judged imaging to be of poor quality on DWI in 26 cases (4·0%), on FLAIR in 29 cases (5%), and on both DWI and FLAIR in 7 cases (1%). Table 1 shows baseline characteristics of the study sample.

Interobserver agreement for the detection of acute DWI was 93·9% with a κ of 0·506 (95% CI 0·361–0·651). Interobserver agreement for the detection of corresponding FLAIR lesions was 77·9% with a κ of 0·569 (0·504–0·634). We recorded an increasing proportion of FLAIR-positive findings with increasing time between symptom onset and MRI (figure 3). All further analysis was restricted to patients with a visible DWI lesion (DWI positive).

| | Included in final analysis (n=543) | Excluded from final analysis (n=100) |
|---|---|---|
| Age (years, mean [95% CI]) | 66·0 (64·7–67·3) | 69·0 (66·4–71·5) |
| Female | 251 (46%) | 47 (47%) |
| NIHSS score on admission | 8 (4–15)* | 11 (5–17) |
| Time to MRI (min) | 201 (110–321)* | 152 (96–271) |
| Field strength 3 T | 86 (16%) | 12 (12%) |

Data are number (%) or median (IQR) unless otherwise stated. NIHSS=National Institutes of Health Stroke Scale. *Data missing for five patients.

***Table 1:*** **Baseline characteristics**

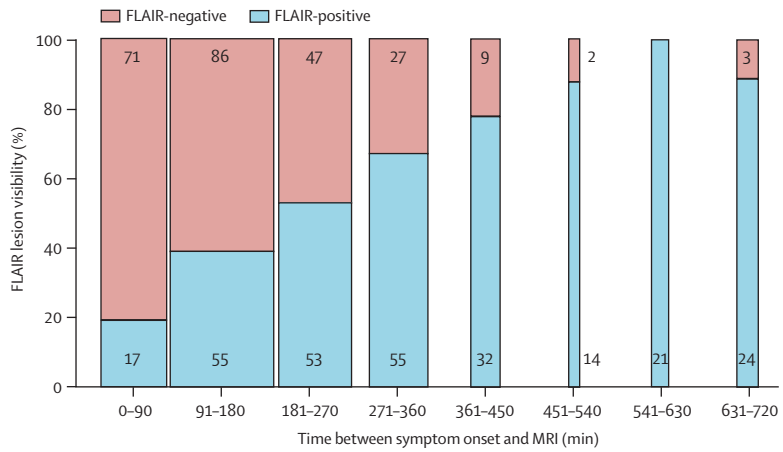


***Figure 3:*** **FLAIR lesion visibility in relation to time from symptom onset**
Visibility of acute ischaemic lesions on FLAIR images in relation to time from symptom onset. Numbers are patients within each time interval, which also relate to the widths of the columns. FLAIR=fluid-attenuated inversion recovery.

FLAIR-positive patients were younger than FLAIR-negative patients, had larger DWI lesion volumes, and had a lower frequency of severe leukoaraiosis (table 2). The event-to-MRI time in FLAIR-positive patients was longer than that in FLAIR-negative patients (table 2). Groups were much the same regarding sex, NIHSS score on admission, side of infarction, systolic blood pressure, blood glucose, and cause of stroke (table 2).

In the multivariate regression analysis, longer time to MRI, lower age, and larger DWI lesion volume were identified as independent predictors of visibility of acute ischaemic lesions on FLAIR images, but leukoaraiosis was not (table 3). In view of the correlation between age and leukoaraiosis ($r$=0·503, p<0·0001), we also tested a model excluding age. This model identified longer time to MRI, larger DWI lesion volume, and less severe leukoaraiosis as independent predictors of visibility of acute ischaemic lesions on FLAIR imaging. The odds for a positive FLAIR scan increased by 22% for every 30 min from symptom onset to MRI, and by 7% for every 10 mL increase in DWI lesion volume, although it decreased by 39% in the presence of severe leukoaraiosis (table 4).

Table 4 shows the predictive values of DWI-FLAIR mismatch for the identification of patients within either 4·5 h or 6 h of symptom onset. Restriction of the analysis to subgroups of patients with ischaemic lesions in the MCA territory, MCA stroke and NIHSS scores of greater than 3, and MCA stroke and DWI lesion of greater than 5 mL resulted in slight increases in specificity and PPV (table 4).

We also did an exploratory subgroup analysis on the basis of TOAST classifications. Data on cause of stroke were available in 428 (79%) of 543 patients. The percentage of acute ischaemic lesions identified on FLAIR imaging was much the same between patients with large artery atherosclerosis (67 of 145; 46%) and those with cardioembolism (69 of 155; 45%). Predictive values were also much the same between both groups (data not shown). Other subgroups (small-vessel occlusion [n=22], other determined cause of stroke [n=40], and undetermined cause of stroke [n=66]) were too small and heterogeneous to allow subgroup analysis.

In the multivariable regression analysis, the area under the curve was 0·8080 for use of DWI-FLAIR mismatch to identify ischaemic lesions within 4·5 h of symptom onset, and none of the additional covariates tested (age, severe leukoaraiosis, and DWI lesion volume) improved the model. For the identification of ischaemic lesions within 6 h of symptom onset, the area under the curve for DWI-FLAIR mismatch was 0·8305 and, similarly, none of the additional variables improved the model.

## Discussion

Our assessment of a large multicentre dataset yielded three main findings. First, we showed that the DWI-FLAIR mismatch can be used to identify patients within 4·5 h of symptom onset with high specificity and high PPV. This finding substantiates those from some of the previous smaller single-centre studies,[9,17,19] lending support to the use of DWI-FLAIR mismatch as a surrogate marker to identify patients with acute stroke who are eligible for intravenous thrombolysis (panel). Second, the sensitivity of DWI-FLAIR mismatch to identify patients within 4·5 h of symptom onset was low, as previously reported,[9,18] showing the need for future studies of other imaging parameters. Third, our study provides further insight into potential confounding variables that interfere with the diagnostic accuracy of DWI-FLAIR mismatch, such as lesion volume, leukoaraiosis, image quality, and interobserver agreement.

Since DWI-FLAIR mismatch was suggested as a surrogate marker to identify patients eligible for intravenous thrombolysis,[9] three studies[17–19] have reported a time dependency of the visibility of acute ischaemic lesions on FLAIR imaging. In these studies, for the identification of patients within 3 h of symptom onset, specificity was between 71% and 97% and PPV was between 64% and 97%, and for the identification of lesions within 4·5 h of symptom onset, specificity was between 73% and 89% and PPV was between 86% and

97%.[9,17–19] Some differences in these predictive values can be explained by different sample characteristics. Restriction of analysis to clinical subgroups—eg, by the exclusion of infratentorial and lacunar infarcts[17] or very small DWI lesions[18]—reduced the variance and increased the predictive values. For the identification of ischaemic lesions at less than 4·5 h of symptom onset, the predictive values recorded in the PRE-FLAIR study (specificity=78% and PPV=83%) are within these previously reported ranges, and both increased slightly in analysis of a subgroup of patients with MCA stroke and a relevant neurological deficit (specificity=81% and PPV=87%). The PPV of 0·87 is likely to be the relevant predictive value for clinical practice because this subgroup of patients with acute ischaemic stroke is a typical cohort in whom thrombolysis is considered according to previous stroke trials and guidelines.[1,2,25] Specificity and PPV were even higher when analysis was extended to the identification of patients within 6 h of symptom onset—such an increase in specificity and PPV has also been recorded elsewhere.[17]

The clinical use of DWI-FLAIR mismatch as a surrogate marker of lesion age could enable the extension of thrombolysis use to a new population of patients who are likely to benefit from recanalisation treatment. However, patients should not be offered such treatment beyond a time from onset of symptoms during which thrombolysis is safe and effective. In view of these considerations, high specificity and PPV are essential. Intravenous thrombolysis is effective and is recommended up to 4·5 h after symptom onset.[1,25,33] Furthermore, combined analyses of acute stroke thrombolysis trials suggest a beneficial effect or at least no net harm from thrombolysis up to 6 h after symptom onset.[27,33] In our study, patients with MCA stroke and an NIHSS score of greater than 3 presenting with a DWI-FLAIR mismatch had an 87% probability of being within a time window of unequivocally proven efficacy of intravenous thrombolysis, and a 95% probability of being within a time window for which there is evidence for a potential benefit of thrombolysis together with proven safety. These values seem sufficiently high to begin the testing of DWI and FLAIR MRI in a prospective clinical trial of thrombolysis in patients with unknown time of symptom onset.

Although specificity and PPV were high, sensitivity and NPV were low. A low sensitivity for the absence of a lesion on FLAIR imaging to identify patients with hyperacute stroke has been reported elsewhere,[9,18] and results from the high proportion of patients within 4·5 h of symptom onset with an acute ischaemic lesion already visible on FLAIR imaging (46% of patients ≤4·5 h in PRE-FLAIR). This finding is explained by the pathophysiological basis of T2 signal changes that are indicated by FLAIR MRI. Depending on the extent and severity of ischaemia, a net increase of tissue water occurs and can be detected with T2-weighted MRI as early as within the first 2 h of stroke.[11,12] Higher sensitivity of DWI-FLAIR mismatch to identify patients within 3 h of symptom onset was reported in a study in which observers were advised to disregard subtle FLAIR lesions restricted to the cortex in patients with large DWI lesions.[19] The low sensitivity of the DWI-FLAIR mismatch approach calls for further studies to assess other MRI indices such as quantitative T2,[34] T1-rho,[35] or indices resulting from diffusion tensor imaging.[36]

Our study provides further insight into potential confounding factors of acute ischaemic lesion visibility on FLAIR MRI beyond lesion age alone. As noted elsewhere,[9] lesion size was a strong and independent predictor of lesion visibility in PRE-FLAIR, increasing the odds that an acute ischaemic lesion was identified on FLAIR imaging by about 7% per 10 mL lesion volume. This finding would have been expected because FLAIR

| | FLAIR negative (n=245) | FLAIR positive (n=271) | p value |
|---|---|---|---|
| Age (years) | 69·2 (66·7–71·8) | 62·6 (60·0–65·1) | <0·0001 |
| Female | 117/245 (48%) | 123/271 (45%) | 0·8003 |
| NIHSS score on admission*† | | | |
| Median [IQR] | 9 (4–16) | 8 (4–14) | .. |
| Geometric mean (95% CI) | 8·7 (6·5–11·8) | 8·5 (6·3–11·4) | 0·6323 |
| Time to MRI (min)* | | | |
| Median (IQR) | 129 (88–230) | 281 (176–420) | .. |
| Geometric mean (95% CI) | 149 (123–181) | 244 (202–295) | <0·0001 |
| Field strength 3 T | 36/245 (15%) | 40/271 (15%) | 0·3994 |
| Side of infarction on DWI | | | |
| Left | 112/245 (46%) | 121/271 (45%) | 0·6169 |
| Right | 125/245 (51%) | 137/271 (51%) | .. |
| Bilateral | 8/245 (3%) | 13/271 (5%) | .. |
| Anterior circulation infarct | 230/245 (94%) | 246/271 (91%) | 0·4020 |
| Systolic blood pressure (mm Hg)‡ | 155·8 (149·3–162·4) | 155·4 (148·4–162·0) | 0·9093 |
| Blood glucose (mmol/L)§ | 7·1 (6·6–7·5) | 7·3 (6·9–7·7) | 0·3328 |
| Cause¶ | | | |
| Large-artery atherosclerosis | 78/222 (35%) | 67/206 (33%) | 0·6269 |
| Cardioembolism | 86/222 (39%) | 69/206 (34%) | .. |
| Small-vessel occlusion | 11/222 (5%) | 11/206 (5%) | .. |
| Other determined | 17/222 (8%) | 23/206 (11%) | .. |
| Undetermined | 30/222 (14%) | 36/206 (17%) | .. |
| DWI lesion volume* | | | |
| Median (IQR) | 5·5 (2·0–18·0) | 11·6 (3·1–35·0) | .. |
| Geometric mean (95% CI) | 5·7 (3·3–9·9) | 10·4 (6·0–18·2) | <0·0001 |
| Leukoaraiosis | | | |
| Fazekas and Schmidt scale sum | 2·0 (1·7–2·5) | 1·4 (1·1–1·7) | <0·0001 |
| Severe leukoaraiosis‖ | 91/245 (37%) | 66/271 (24%) | 0·0026 |

Data are mean (95% CI) or n (%), unless otherwise stated. Group comparison was done with multivariable models with the centre as a random effect. DWI=diffusion-weighted imaging. FLAIR=fluid-attenuated inversion recovery. NIHSS=National Institutes of Health Stroke Scale. *Because of asymmetric distribution, logarithmic transformation was applied before group comparison and the geometric mean (95% CI) is given, together with median (IQR) for descriptive purposes. †Data for 244 patients with a FLAIR-negative scan and 267 with a FLAIR-positive scan. ‡Data for 122 patients with a FLAIR-negative scan and 128 with a FLAIR-positive scan. §Data for 164 patients with a FLAIR-negative scan and 200 with a FLAIR-positive scan. ¶Classification according to TOAST (Trial of Org 10172 in Acute Stroke Treatment) definitions. ‖Leukoariosis defined as a score of >1 on either of the subscales (deep white matter changes or periventricular white matter changes) of the adapted scale by Fazekas and Schmidt.[28]

***Table 2:*** **Comparison by study group**

| | Univariate | | Multivariate | |
|---|---|---|---|---|
| | OR (95% CI) | p value | OR (95% CI) | p value |
| **Model A (including age, time to MRI, DWI lesion volume, and leukoaraoisis*)** | | | | |
| Time to MRI (per 30 min) | 1·230 (1·170–1·293) | <0·0001 | 1·215 (1·154–1·279) | <0·0001 |
| Age (per 5 years) | 0·857 (0·803–0·915) | <0·0001 | 0·880 (0·817–0·949) | 0·0009 |
| DWI lesion volume (per 10 mL) | 1·103 (1·043–1·167) | 0·0006 | 1·068 (1·009–1·129) | 0·0226 |
| Leukoaraoisis (severe *vs* not severe) | 0·540 (0·362–0·805) | 0·0026 | 0·842 (0·525–1·350) | 0·4747 |
| **Model B (including time to MRI, DWI lesion volume, and leukoaraoisis*)** | | | | |
| Time to MRI (per 30 min) | 1·230 (1·170–1·293) | <0·0001 | 1·216 (1·155–1·279) | <0·0001 |
| DWI lesion volume (per 10 mL) | 1·103 (1·043–1·167) | 0·0006 | 1·071 (1·013–1·133) | 0·0162 |
| Leukoaraiosis (severe *vs* not severe) | 0·540 (0·362–0·805) | 0·0026 | 0·608 (0·396–0·933) | 0·0229 |

Odds ratios refer to the intervals given in parentheses. In model B, age was excluded because of a strong correlation between age and leukoaraiosis ($r$=0·503, p<0·0001). DWI=diffusion-weighted imaging. FLAIR=fluid attenuated inversion recovery. OR=odds ratio. *Leukoaraiosis rated by the sum of the subscales (deep white matter changes, periventricular white matter changes) of the adapted scale by Fazekas and Schmidt.[28]

***Table 3:*** **Predictors of visibility of acute ischaemic lesions on FLAIR imaging**

| | Sensitivity (95% CI) | Specificity (95% CI) | PPV (95% CI) | NPV (95% CI) |
|---|---|---|---|---|
| **Identification of patients within 4·5 h of symptom onset** | | | | |
| DWI-positive (n=516) | 62% (57–67) | 78% (72–84) | 83% (79–88) | 54% (48–60) |
| MCA (n=469) | 63% (57–68) | 79% (37–86) | 85% (80–90) | 53% (47–60) |
| MCA+NIHSS >3 (n=408) | 64% (58–70) | 81% (74–87) | 87% (81–91) | 53% (46–60) |
| MCA+DWI lesion >5 mL (n=280) | 58% (51–66) | 84% (75–90) | 86% (78–91) | 55% (47–63) |
| **Identification of patients within 6 h of symptom onset** | | | | |
| DWI-positive (n=516) | 56% (51–61) | 87% (80–93) | 93% (91–97) | 34% (28–39) |
| MCA (n=469) | 56% (51–61) | 87% (80–94) | 95% (92–98) | 33% (27–39) |
| MCA+NIHSS >3 (n=408) | 57% (52–62) | 88% (78–94) | 95% (92–98) | 32% (25–39) |
| MCA+DWI lesion >5 mL (n=280) | 52% (45–59) | 92% (82–97) | 96% (90–99) | 34% (27–42) |

DWI=diffusion-weighted imaging. PPV=positive predictive value. NPV=negative predictive value. MCA=middle cerebral artery. NIHSS=National Institutes of Health Stroke Scale.

***Table 4:*** **Predictive values of DWI-FLAIR mismatch for the identification of patients within either 4·5 h or 6 h of symptom onset**

signal change is only subtle during the first 2–3 h of acute ischaemia and this low contrast limits the visibility of FLAIR signal changes, especially in small ischaemic lesions. Moreover, greater age of patients was associated with lower odds of lesion visibility on FLAIR scans—a confounding factor that was hitherto unrecognised. This effect probably results from the occurrence of more frequent and more severe white matter changes with increasing age, which is shown by the relation between age and leukoaraiosis in our sample. If age was excluded from our model, severe leukoaraiosis was a relevant confounding factor, decreasing the odds of acute ischaemic lesions being detected on FLAIR imaging by 28%. This finding also could have been expected, because a subtle parenchymal hyperintensity can be more easily overlooked against the background of pronounced white matter changes. A previous study[17] acknowledged this fact by excluding 2% of images because of severe leukoaraiosis, which was judged to preclude interpretation of FLAIR scans. Other parameters known to predict both clinical outcome and final infarct volume in acute stroke, such as sex, side of infarction, symptom severity assessed by the NIHSS, serum glucose, and systolic blood pressure, were not predictive of acute ischaemic lesion visibility on FLAIR.

In our study, nearly 10% of datasets had to be excluded from final analysis because of poor image quality. The proportion of images deemed to be of poor quality was much the same between DWI and FLAIR. This percentage is much the same as the rate of poor quality of FLAIR images previously reported in single-centre studies,[9,17] and is likely to be the real-life situation of acute MRI in a population of patients who have been severely affected by acute stroke.

Patients whose imaging was done more than 6 h after symptom onset and who were misclassified as having hyperacute stroke lesions warrant further discussion. In our final analysis, FLAIR scans were rated as negative in 14 patients with time from symptom onset greater than 6 h. In 12 of these patients the false-positive finding of DWI-FLAIR mismatch might be explained by insufficient contrast or image resolution in cases of very small punctual and subtle DWI lesions (n=9) or by severe leukoaraiosis (n=3) hampering the identification of parenchymal hyperintensity on FLAIR MRI resulting from an acute ischaemic lesion.

Our study has limitations. Although a consensus was reached in all cases, observers had some disagreement in their initial judgment of acute ischaemic lesions on FLAIR imaging. Interobserver agreement for the detection of acute ischaemic lesions on FLAIR scans is within the range of interobserver agreement reported in previous studies, with κ between 0·46[9] and 0·65.[17] Poor image quality of scans of some patients and the moderate agreement of observers in the detection of acute ischaemic lesions on FLAIR imaging in the first 12 h after stroke will have to be taken into account as potential limitations if FLAIR MRI is to be used as a surrogate marker of lesion age. The higher interobserver agreement reported in another single-centre study,[19] with κ of 0·97 for two experienced and trained observers, might be explained by inclusion of lesion size in the instructions for image judgment in that study. This descrepancy suggests the need for enhancement of interobserver agreement with dedicated instructions and training. Standardised training in image reading has been shown to improve the detection of early ischaemic signs on CT,[37] and might also improve reading of acute stroke FLAIR images. Quantitative measurements of FLAIR signal intensity ratios in regions of visible FLAIR hyperintensity have also been suggested, but results from pilot studies are contradictory.[18,19] Although FLAIR signal intensity ratios showed a high positive association with time from symptom onset and could be used to reliably identify patients within the first 3 h of symptom onset in one study,[19] no significant correlation with time from onset was seen in another study.[18] The time-consuming post-processing needed for quantitative signal analysis based on regions of interest and the absence of a

***Panel:* Research in context**

**Systematic review**

We searched Medline from Jan 1, 1950, to June 30, 2011, for studies reporting diffusion-weighted MRI (DWI) and fluid-attenuated inversion recovery (FLAIR) MRI findings in acute stroke using the search terms "acute stroke AND (FLAIR OR fluid attenuated inversion recovery) AND (DWI OR diffusion weighted imaging)". We included all papers of human studies reporting both DWI and FLAIR data from at least five patients. Case reports were disregarded. There were no language restrictions.

**Interpretation**

This study overcomes the limitations of previous smaller, single-centre studies reporting in part contradictory results. In four retrospective single-centre studies, predictive values were reported for symptom onset within either 3 h,[9,17–19] 4·5 h,[9,17,18] or 6 h,[17] and specificity values of a negative FLAIR scan for time from symptom onset within 3 h varied between 71% and 97%.[9,17–19] Sensitivity of DWI-FLAIR mismatch was low (<50%) in two studies,[9,18] but high (83–95%) in two other studies.[17,19] Our study provides conclusive evidence for the use of DWI-FLAIR mismatch as a surrogate marker to identify patients with unknown time of symptom onset in a large multicentre set of real-life data. Thus, the findings from this study provide the basis for the use of DWI and FLAIR MRI to be tested in a future randomised trial of intravenous thrombolysis in patients with unknown time of symptom onset.

reliable pathophysiological correspondent of FLAIR signal intensity might limit the use of this approach in acute stroke, but further studies are needed for a final judgment.

Field strength can also affect the visibility of acute ischaemic lesions on both DWI and FLAIR MRI. In our study, most patients were scanned at 1·5 T, but a subgroup of patients were studied at 3 T. In the group of DWI-positive patients, predictive values for the identification of patients within 4·5 h of symptom onset were much the same between the 76 patients studied at 3 T and the 440 patients studied at 1·5 T (data not shown). Vessel occlusion and the resulting perfusion deficit, as well as the presence of collateral vessels, are other factors with a crucial effect on the evolution of the acute ischaemic lesion and might also affect the dynamics of FLAIR hyperintensity in acute stroke. However, we did not collect data on vessel occlusion or perfusion in the PRE-FLAIR study. Future research should try to elucidate a potential relation between these factors and acute ischaemic lesion visibility on FLAIR imaging.

PRE-FLAIR was designed to test the diagnostic accuracy of DWI-FLAIR mismatch as a surrogate marker of lesion age. Our study does not provide evidence for efficacy and safety of MRI-based thrombolysis in patients with unknown time of symptom onset. Such use of DWI-FLAIR mismatch should be tested in a randomised controlled trial of thrombolysis in patients with unknown time of symptom onset, with DWI and FLAIR MRI used to enrol patients.

**Contributors**

GT and CG co-chaired PRE-FLAIR and had the idea for and designed the study. BC, MEb, QH, VD, OW, JSK, LB, OCS, SC, IG, TE, MK, D-WK, and JBF collected data. BC, MEb, and GT did image analysis, assisted by NDF. AT was the independent statistician, designed the statistical analysis, and did the statistical analysis of the database. GT, BC, MEb, SW, MR, MEn, TT, AGS, DSL, and JF analysed and interpreted the data. GT and BC drafted and edited the paper, assisted by AT, CG, MEb, NDF, SW, MK, D-WK, JBF, MR, MEn, TT, AGS, DSL, and JF. All authors have seen and approved the final version of the paper and take full responsibility for the content of the paper.

**STIR and VISTA Imaging steering committee**

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*Databases*—EPITHET (SC); VIRAGE (VD).

**Conflicts of interest**

VD has received a national grant from the French Government. MEn has received grant support from Astra Zeneca and Sanofi-Aventis, has participated in advisory board meetings of Boehringer Ingelheim, Pfizer, Bristol-Myers Squibb, and Sanofi-Aventis, and has received honoraria from Novartis, Pfizer, Bayer, Astra Zeneca, Boehringer Ingelheim, Sanofi-Aventis, Trommsdorff, Berlin-Chemie, GlaxoSmithKline, and Bristol-Myers Squibb. JBF has received fees as a board member, consultant, or lecturer from Boehringer Ingelheim, Lundbeck, Siemens, Syngis, and Synard. CG has received fees as a consultant or lecture fees from Bayer Vital, Boehringer Ingelheim, EBS Technologies, GlaxoSmithKline, Lundbeck, Pfizer, Sanofi-Aventis, Silk Road Medical, and Union Chimique Belge. DSL has received fees as a consultant for CoAxia and Concentric Medical. AGS was supported by grants from the National Institutes of Health, has received fees as a board member, consultant, or lecturer from the American College of Radiology Imaging Network, Biogen, Genzyme, Mitsubishi, and has received royalties from General Electrics and Olea. GT has received a research grant from the Else Kröner-Fresenius-Stiftung. TT has received a national grant from the French Government (PHRC). OW was supported in part by grants from the National Institutes of Health. LB, BC, SC, MEb, TE, JF, NDF, IG, QH, D-WK, JSK, MK, MR, OCS, AT, and SW declare that they have no conflicts of interest.

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