

Assessing Response to Stroke Thrombolysis

Validation of 24-Hour Multimodal Magnetic Resonance Imaging

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Background: Imaging is used as a surrogate for clinical outcome in early-phase stroke trials. Assessment of infarct growth earlier than the standard 90 days used for clinical end points may be equally accurate and more practical.

Objective: To compare assessment of the effect of reperfusion therapies using 24-hour vs day 90 magnetic resonance imaging.

Design: Infarct volume was assessed on diffusion-weighted imaging (DWI) at baseline and 24 hours after stroke onset and on fluid-attenuated inversion recovery images at day 90. The DWI and fluid-attenuated inversion recovery lesions were manually outlined by 2 independent raters, and the volumes were averaged. Interrater consistency was assessed using the median difference in lesion volume between raters.

Setting: Referral center.

Patients: Imaging data were available for 83 patients; 77 of these patients received thrombolysis.

Main Outcome Measures: Infarct volume at 24 hours and 90 days.

Results: The 24-hour DWI infarct volume had a strong linear correlation with day 90 fluid-attenuated inversion recovery infarct volume ($r=0.98$, 95% confidence interval, 0.97-0.99). Recanalization had a significant effect on infarct evolution between baseline and 24 hours but not between 24 hours and day 90. Infarct growth from baseline was significantly reduced by recanalization, whether assessed at 24 hours or day 90. Infarct volume at either time point predicted functional outcome independent of age and baseline stroke severity. Interrater agreement was better for DWI than fluid-attenuated inversion recovery (1.4 mL [8%] vs 1.8 mL [17%]; $P=.002$).

Conclusions: Assessment of final infarct volume using DWI at 24 hours captures the effect of reperfusion therapies on infarct growth and predicts functional outcome similarly to imaging at day 90. This has the potential to reduce loss to follow-up in trials and may add early prognostic information in clinical practice.

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INFARCT GROWTH CAN BE USED AS a surrogate marker for clinical outcome in ischemic stroke trials.^{1,2} This provides a meaningful intermediary between preclinical studies, where drug effects are often assessed on the basis of infarct volume attenuation, and phase 3 clinical outcomes. In the Diffusion and Perfusion Imaging Evaluation for Understanding Stroke Evolution (DEFUSE) study and the Echoplanar Imaging Thrombolytic Evaluation Trial (EPITHET), recanalization and reperfusion have been demonstrated to significantly reduce infarct growth.^{2,3} Although thrombolysis did not significantly attenuate infarct growth in the primary EPITHET analysis, subsequent

analysis using more stringent optimized perfusion thresholds developed in positron emission tomography⁴ and magnetic resonance imaging^{5,6} has demonstrated reduced growth in patients who received thrombolysis.⁷ Traditionally, stroke trial

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clinical end points are assessed at 90 days following stroke. There has been less consensus around the timing of imaging outcome assessments. However, these have generally been delayed, eg, 30 days in the DEFUSE study¹ and 90 days in EPITHET.² However, these were arbitrary choices and have some distinct disadvantages. By days

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30 to 90, significant atrophy of the infarcted region has taken place.⁸⁻¹⁰ This leads to an underestimate of the infarct volume relative to the baseline brain topography.

There is also a real risk of loss to imaging follow-up given the requirement for in-person attendance at 1 to 3 months when the patient may be at home or in a nursing institution with limited access to transportation. Some loss to follow-up also occurs due to early mortality. In the DEFUSE study¹ and EPITHET,² 25% of patients were unable to participate in day 30 or day 90 imaging, respectively (16% died and 9% were unavailable). Assessment of final infarct volume at earlier time points may therefore be more accurate and yield major practical advantages.¹¹ Magnetic resonance diffusion-weighted imaging (DWI) has recently been confirmed as a reliable indicator of irreversible infarction.¹⁰ A 24-hour scan is commonly obtained in routine clinical practice to assess for hemorrhagic transformation after thrombolysis. We therefore examined the potential for 24-hour DWI to predict day 90 fluid-attenuated inversion recovery (FLAIR) lesion volumes and clinical outcome data. We also assessed the interrater variability in manually outlined lesion volumes using the 2 modalities.

METHODS

Multimodal magnetic resonance images (1.5 T) were obtained in consecutive patients with ischemic stroke at 2 institutions either as part of standard institutional thrombolysis imaging protocols or as part of imaging-based clinical trials.¹² Studies were approved by institutional review boards at both centers, and informed consent was obtained from all participants. All patients with imaging at 24 hours and 90 days after stroke onset were selected for this analysis, and baseline scans were also analyzed where available. Two stroke neurologists (B.C.V.C. and H.T.H.T., with >5 years of experience) independently outlined infarct regions of interest (ROIs) for each time point. For DWI, the B1000 trace-weighted image was used and the maximal visual extent of the acute infarct was manually outlined. The FLAIR ROIs were drawn with reference to DWI (to maximize detection of small FLAIR lesions) and baseline T2 hyperintensities (to distinguish preexisting leukoaraiosis and infarcts). When present, hemorrhagic transformation was included in the infarct ROI on both 24-hour and day 90 images. A visual inspection of the 24-hour and day 90 scans was performed to check for infarct growth beyond 24 hours that might not be detected by volumetric analysis due to confounding atrophy effects.¹⁰

Arterial patency was assessed using time-of-flight magnetic resonance angiography at baseline (where available) and at 24 hours and was graded independently by 2 stroke neurologists (B.C.V.C. and M.W.P., with >10 years of experience), who then reached consensus using an adapted Thrombolysis in Myocardial Infarction classification¹³: 0 indicates complete occlusion; 1, severe stenosis; 2, mild to moderate stenosis; and 3, normal arterial caliber. Recanalization was defined as an increase of 2 or more Thrombolysis in Myocardial Infarction grades. Arterial patency at 24 hours was defined as Thrombolysis in Myocardial Infarction 2 or 3 flow grade. Infarct growth from baseline to 24 hours and day 90 was assessed using both absolute and relative measurements. Clinical outcome was assessed using the modified Rankin Scale at 90 days.

Statistical analysis was performed using PASW version 18 statistical software (SPSS Inc, Chicago, Illinois). Interrater reliability for DWI vs FLAIR modalities was assessed using the median difference in lesion volume between raters along with Pearson correlation coefficient (*r*) and Bland-Altman plot. The lesion volumes created by the 2 raters were then averaged, and

Table. Clinical Characteristics of 83 Patients

Characteristic	Value
Age, mean (SD), y	69 (10)
Men, No. (%)	44 (53)
Hypertension, No. (%)	42 (51)
Diabetes, No. (%)	9 (11)
Smoking, No. (%)	21 (25)
Dyslipidemia, No. (%)	30 (36)
Atrial fibrillation, No. (%)	27 (33)
Baseline NIHSS score, median (IQR)	14 (9-17)
Received tPA, No. (%)	77 (93)
Time to treatment, mean (SD), min	163 (47)
Time to 24-h MRI, median (IQR), h	25.9 (22.8-26.8)
Time to 90-d MRI, median (IQR), d	91 (88-96)
Baseline DWI volume, mean (SD), mL ^a	19.7 (28.7)
24-h DWI volume, mean (SD), mL	36.7 (42.8)
90-d FLAIR volume, mean (SD), mL	26.0 (37.2)

Abbreviations: DWI, diffusion-weighted imaging; FLAIR, fluid-attenuated inversion recovery; IQR, interquartile range; MRI, magnetic resonance imaging; NIHSS, National Institutes of Health Stroke Scale; tPA, tissue plasminogen activator.

^aThe sample size was 40 patients.

the relationship between infarct volume on 24-hour DWI and day 90 FLAIR was assessed using Pearson *r* and the Lin concordance coefficient (ρ_c).¹⁴ Linear regression on baseline, 24-hour, and day 90 infarct volumes was performed using data transformed to the fifth root to approximate a normal distribution (as judged by skewness, kurtosis, and visual inspection), and the distribution of residuals was checked. Vessel status was entered as a cofactor.

RESULTS

Imaging data were available for 83 patients, of whom 77 received intravenous thrombolysis (clinical characteristics are shown in the **Table**). The mean (SD) infarct volume was 36.7 (42.8) mL on 24-hour DWI. The DWI infarct volume at 24 hours had strong linear correlation with the day 90 FLAIR infarct volume ($r=0.98$; 95% confidence interval [CI], 0.97-0.99; Lin $\rho_c=0.937$) (**Figure 1A**). The variance (r^2) indicates that 96% of the variability in day 90 lesion volume is accounted for by the 24-hour DWI volume. The correlation of baseline DWI infarct volume to day 90 FLAIR infarct volume (assessable in 40 patients) was significantly lower ($r=0.93$; 95% CI, 0.87-0.96; Lin $\rho_c=0.850$) (Figure 1B).

Recanalization at 24 hours was assessable in 51 patients, of whom 24 (47%) had recanalization (baseline arterial imaging was lacking in 32 patients with no occlusion at follow-up). Arterial patency at 24 hours was assessable in all 83 patients, of whom 56 (67%) had patent major intracranial arteries. Linear regression analysis was performed after transformation of the data to approximate a normal distribution. Recanalization ($P=.008$) or arterial patency at 24 hours ($P=.001$) were significant cofactors with baseline infarct volume in determining 24-hour infarct volume (Figure 1C). In contrast, vessel status was not a significant cofactor with 24-hour infarct volume in predicting day 90 infarct volume (recanalization, $P=.28$; arterial patency at 24 hours, $P=.28$) (Figure 1D). Arterial patency at 24 hours was also associated with significantly reduced

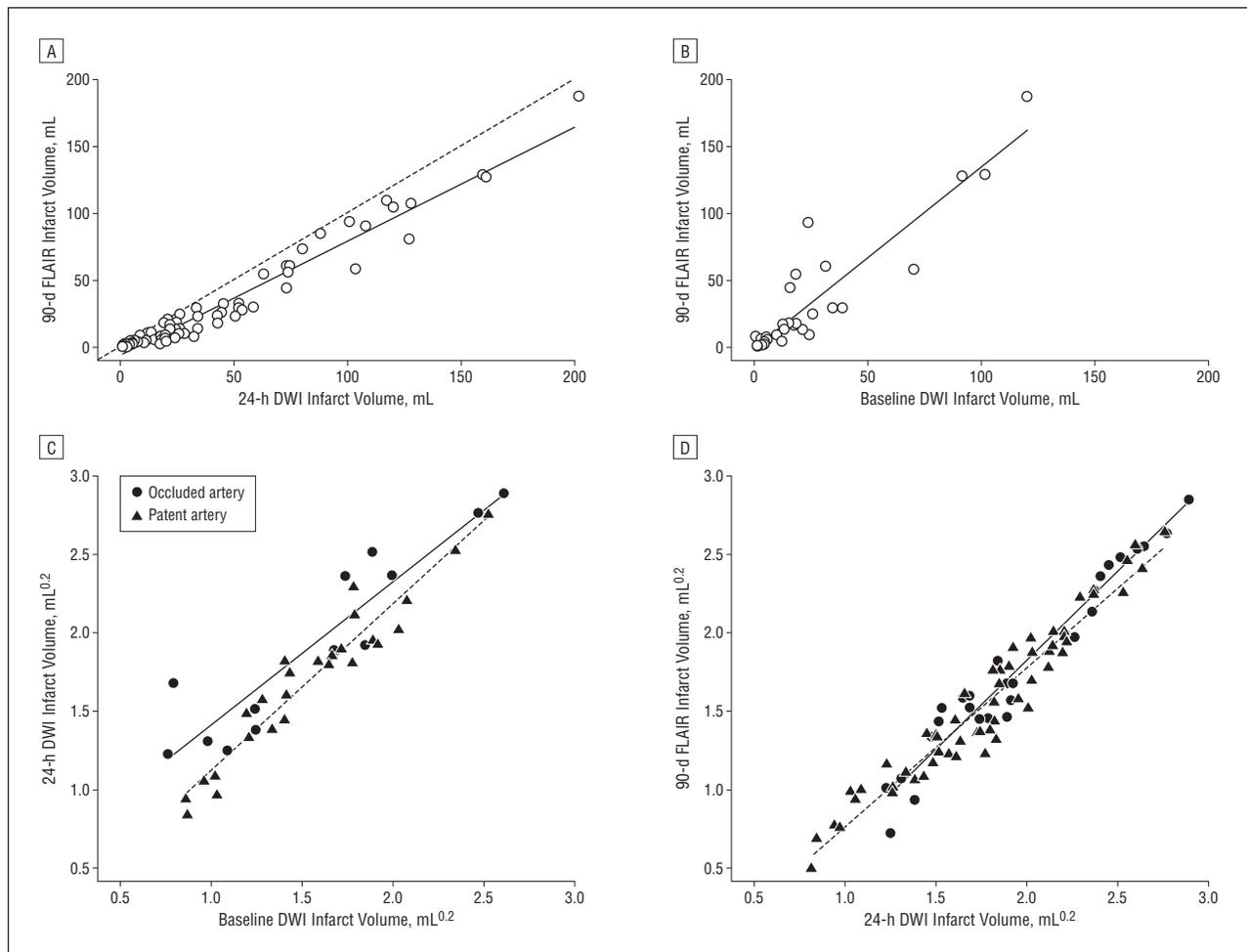


Figure 1. Scatterplots showing infarct volumes and the effect of vessel occlusion. A, The 24-hour diffusion-weighted imaging (DWI) and day 90 fluid-attenuated inversion recovery (FLAIR) infarct volume ($n=83$; $r=0.98$) with a regression line (solid) and a line of unity (dashed). Note that all day 90 infarct volumes are below the dashed line (ie, smaller than 24-hour DWI volume). B, Baseline DWI and day 90 infarct volume ($n=40$; $r=0.93$). Baseline and 24-hour infarct volume (C) and 24-hour and day 90 infarct volume (D) (transformed by the fifth root and stratified by arterial occlusion at 24 hours) with regression lines demonstrating that vessel occlusion at 24 hours is associated with significantly increased infarct volume from baseline to 24 hours ($P=.001$) but does not affect 24-hour to day 90 volume changes ($P=.28$).

absolute ($P=.04$) and relative ($P=.003$) infarct growth from baseline to 24 hours ($n=40$).

Infarct growth occurred between baseline and 24 hours (mean 15 mL absolute, 224% relative with 11 of 40 infarcts [28%] growing $>50\%$). In contrast, infarct volume at day 90 was consistently smaller than at 24 hours (mean [SD] volume reduction, 11 [8.3] mL; paired Wilcoxon $P<.001$) (Figure 1A). To reduce the risk that infarct atrophy had masked true growth in the territory of infarction subsequent to 24 hours, each patient's 24-hour and day 90 images were visually reviewed side by side. No anatomical regions that were unaffected at 24 hours were found to have been incorporated into the infarct at day 90.

Infarct volumes at 24 hours and 90 days were similarly predictive of functional outcome (day 90 modified Rankin Scale score, assessable in all 83 patients) in ordinal logistic regression. For the 24-hour DWI volume, the odds ratio for a good outcome was 0.78 (95% CI, 0.70-0.87; $P<.001$) per 10-mL increase in infarct volume; for the 90-day FLAIR volume, the odds ratio was 0.73 (95% CI, 0.64-0.83; $P<.001$) per 10-mL increase in infarct volume. This was independent of age and baseline National Institutes of Health Stroke Scale (NIHSS) score but

not 24-hour NIHSS score. Infarct growth at 24 hours also predicted functional outcome in multivariate ordinal logistic regression with age and baseline NIHSS score (odds ratio=0.6 per 10-mL growth; 95% CI, 0.42-0.85; $P=.005$) similarly to growth at day 90 (odds ratio=0.58 per 10-mL growth; 95% CI, 0.38-0.87; $P=.008$).

Interrater reliability was high for both DWI ROIs (Lin $\rho_c=0.998$) and FLAIR ROIs (Lin $\rho_c=0.982$). The median ROI volume discrepancy between raters was smaller for DWI than for FLAIR (1.4 mL [8%] vs 1.8 mL [17%], respectively; $P=.002$), and the Bland-Altman limits of agreement were narrower for DWI (-5.6 to 4.1 mL) (Figure 2A) compared with FLAIR (-10.2 to 6.4 mL) (Figure 2B). Preexisting infarcts and leukoaraiosis can create difficulties in accurately outlining FLAIR lesions (Figure 3) and may underlie the reduced interrater agreement compared with DWI.

COMMENT

Infarct volume at 24 hours is strongly correlated with that at 90 days. Although an increase in infarct volume is consistently reported beyond 24 hours (usually peaking

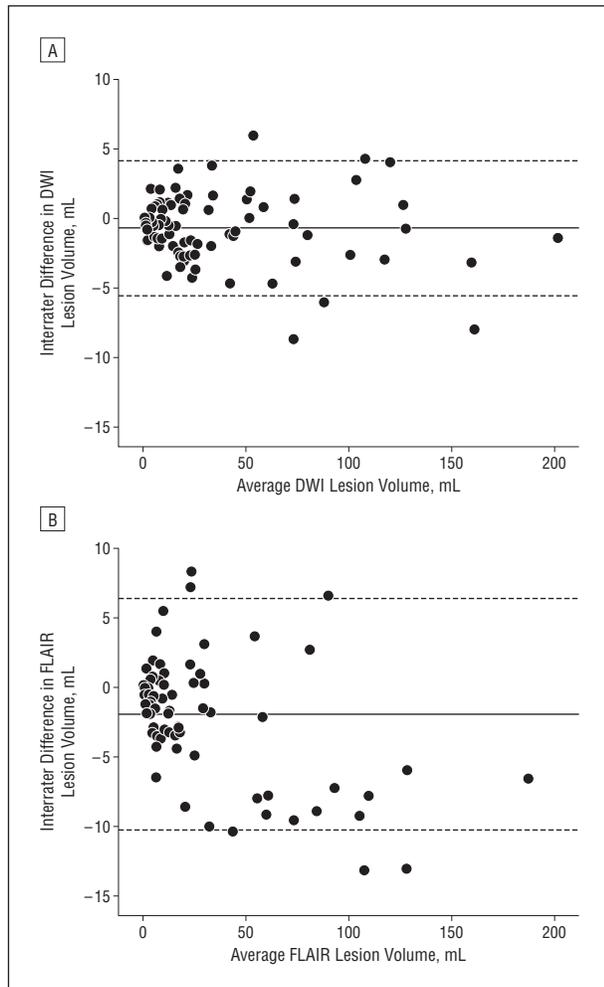


Figure 2. Bland-Altman plots with 95% prediction intervals for interrater agreement, showing 24-hour diffusion-weighted imaging (DWI) infarct volumes (A) and day 90 fluid-attenuated inversion recovery (FLAIR) infarct volumes (B).

around day 3-5),^{8,9} our data are consistent with this being largely due to edema rather than expansion of the infarcted territory. The highly predictable relationship between 24-hour and day 90 infarct volumes indicated by $r^2=0.96$ combined with the lack of visually apparent expansion in infarct topography suggest that the individual patient variability in infarct evolution occurs predominantly in the first 24 hours after stroke onset. Furthermore, the modifying effect of recanalization on infarct growth ceased to be significant beyond 24 hours, indicating that the treatment effect of reperfusion therapies is well captured at this time point.

The proportion of patients with favorable clinical outcome has been shown to reduce with increasing final infarct volume and with increasing infarct growth.^{2,3,15,16} In this study, the well-established effects of recanalization on reducing infarct growth and the relationship of infarct volume to clinical outcome remained significant when assessed at 24 hours. Infarct growth assessed at 24 hours was also similarly predictive of functional outcome compared with measurement at day 90. These effects were independent of baseline clinical predictors of outcome (age and NIHSS score). As reported elsewhere,¹⁵ the effect was not, however, independent of the 24-hour NIHSS score.

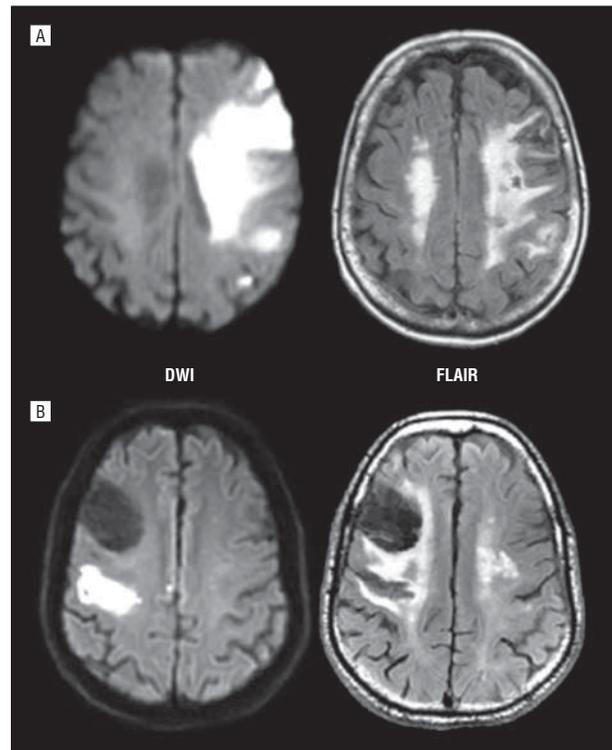


Figure 3. Comparison of 24-hour diffusion-weighted imaging (DWI) and day 90 fluid-attenuated inversion recovery (FLAIR) lesion delineation. Examples illustrate the greater difficulty in outlining FLAIR infarcts, especially in the presence of preexisting white matter disease (A) or infarction (B).

Assessing infarct volume using 24-hour DWI also appears to be more reproducible. This may relate to preexisting leukoaraiosis and infarcts complicating the interpretation of day 90 FLAIR images (Figure 3). The significant reduction in infarct volume between 24 hours and day 90 due to atrophy may also lead to the disappearance of small scattered DWI lesions because of a combination of greater conspicuity on DWI and possibly disappearance into interslice gaps. Additionally, DWI has the advantage of being a fast technique, less susceptible to motion degradation and therefore easier to obtain in ill or uncooperative patients.

A limitation of this study is that the vast majority of patients received tissue plasminogen activator. This could potentially have truncated the temporal course of infarct growth by inducing reperfusion. However, only a fraction of tissue plasminogen activator-treated patients do, in fact, have complete reperfusion.¹⁷ In this study, 27 patients (33%) had a persistent major vessel occlusion at 24 hours. The relationship between 24-hour DWI and day 90 FLAIR was not significantly different in these patients, although a small effect of recanalization cannot be excluded. This needs to be balanced against the very strong effect of recanalization observed in baseline to 24-hour infarct growth and the disadvantages of loss to follow-up incurred by delaying final infarct assessment. Our findings may be less applicable to the use of infarct growth as a surrogate for neuroprotection trials. If the postulated mechanism of action is prevention of secondary injury (eg, anti-inflammatory or antioxidant), the time course of infarct growth attenuation

might differ and warrant a slightly later assessment. Given the confounding effect of infarct atrophy to day 90, we cannot exclude that minor infarct growth may have occurred after 24 hours. However, the visual checking procedure and very high r^2 for the 24-hour to day 90 correlation gives reassurance that no major effect was missed. Another limitation of infarct growth as a surrogate for clinical outcome is variable eloquence of the affected brain region. This is difficult to quantitate but remains an important area for future investigation.

Using DWI at 24 hours is an attractive choice for assessing the surrogate end points of final infarct volume and infarct growth. The advantages of earlier assessment in expediting trials and reducing bias due to loss to follow-up may outweigh the effects of undetected minor infarct growth beyond 24 hours. Our data also indicate that routine 24-hour multimodal magnetic resonance imaging after thrombolysis, in addition to information on hemorrhagic transformation, provides useful prognostic information. This may be helpful in clinical practice when considering treatment direction and in discussing the likely prognosis.

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Author Contributions: Dr Campbell had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. **Study concept and design:** Campbell, Desmond, Levi, Donnan, Davis, and Parsons. **Acquisition of data:** Levi, Bladin, Hjort, Ashkanian, Sølling, Østergaard, and Parsons. **Analysis and interpretation of data:** Campbell, Tu, Christensen, Levi, and Parsons. **Drafting of the manuscript:** Campbell. **Critical revision of the manuscript for important intellectual content:** Tu, Christensen, Desmond, Levi, Bladin, Hjort, Ashkanian, Sølling, Donnan, Davis, Østergaard, and Parsons. **Statistical analysis:** Campbell and Christensen. **Obtained funding:** Levi, Donnan, Davis, Østergaard, and Parsons. **Administrative, technical, and material support:** Camp-

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REFERENCES

1. Albers GW, Thijs VN, Wechsler L, et al; DEFUSE Investigators. Magnetic resonance imaging profiles predict clinical response to early reperfusion: the Diffusion and Perfusion Imaging Evaluation for Understanding Stroke Evolution (DEFUSE) study. *Ann Neurol*. 2006;60(5):508-517.
2. Davis SM, Donnan GA, Parsons MW, et al; EPITHET Investigators. Effects of alteplase beyond 3 h after stroke in the Echoplanar Imaging Thrombolytic Evaluation Trial (EPITHET): a placebo-controlled randomised trial. *Lancet Neurol*. 2008;7(4):299-309.
3. Olivot JM, Mlynash M, Thijs VN, et al. Relationships between infarct growth, clinical outcome, and early recanalization in Diffusion and Perfusion Imaging for Understanding Stroke Evolution (DEFUSE). *Stroke*. 2008;39(8):2257-2263.
4. Zaro-Weber O, Moeller-Hartmann W, Heiss WD, Sobesky J. Maps of time to maximum and time to peak for mismatch definition in clinical stroke studies validated with positron emission tomography. *Stroke*. 2010;41(12):2817-2821.
5. Christensen S, Campbell BCV, Perez de la Ossa N, et al. Optimal perfusion thresholds for prediction of tissue destined for infarction in the combined EPITHET and DEFUSE dataset. *Stroke*. 2010;41:e297.
6. Olivot JM, Mlynash M, Thijs VN, et al. Optimal Tmax threshold for predicting penumbral tissue in acute stroke. *Stroke*. 2009;40(2):469-475.
7. Christensen S, Parsons MW, De Silva DA, et al. Testing the mismatch hypothesis in the randomized EPITHET data set: the effect of treatment, mismatch and their interaction on infarct growth. *Stroke*. 2010;41:e203.
8. Lansberg MG, O'Brien MW, Tong DC, Moseley ME, Albers GW. Evolution of cerebral infarct volume assessed by diffusion-weighted magnetic resonance imaging. *Arch Neurol*. 2001;58(4):613-617.
9. Gaudinski MR, Henning EC, Miracle A, Luby M, Warach S, Latour LL. Establishing final infarct volume: stroke lesion evolution past 30 days is insignificant. *Stroke*. 2008;39(10):2765-2768.
10. Chemmanam T, Campbell BCV, Christensen S, et al; EPITHET Investigators. Ischemic diffusion lesion reversal is uncommon and rarely alters perfusion-diffusion mismatch. *Neurology*. 2010;75(12):1040-1047.
11. Ebinger M, Christensen S, De Silva DA, et al; Echoplanar Imaging Thrombolytic Evaluation Trial Investigators. Expediting MRI-based proof-of-concept stroke trials using an earlier imaging end point. *Stroke*. 2009;40(4):1353-1358.
12. Parsons MW, Miteff F, Bateman GA, et al. Acute ischemic stroke: imaging-guided tenecteplase treatment in an extended time window. *Neurology*. 2009;72(10):915-921.
13. Hacke W, Albers G, Al-Rawi Y, et al; DIAS Study Group. The Desmoteplase in Acute Ischemic Stroke Trial (DIAS): a phase II MRI-based 9-hour window acute stroke thrombolysis trial with intravenous desmoteplase. *Stroke*. 2005;36(1):66-73.
14. Lin LI. A concordance correlation coefficient to evaluate reproducibility. *Biometrics*. 1989;45(1):255-268.
15. Barrett KM, Ding YH, Wagner DP, Kallmes DF, Johnston KC; ASAP Investigators. Change in diffusion-weighted imaging infarct volume predicts neurologic outcome at 90 days: results of the Acute Stroke Accurate Prediction (ASAP) trial serial imaging substudy. *Stroke*. 2009;40(7):2422-2427.
16. Parsons MW, Christensen S, McElduff P, et al; Echoplanar Imaging Thrombolytic Evaluation Trial (EPITHET) Investigators. Pretreatment diffusion- and perfusion-MR lesion volumes have a crucial influence on clinical response to stroke thrombolysis. *J Cereb Blood Flow Metab*. 2010;30(6):1214-1225.
17. Bhatia R, Hill MD, Shobha N, et al. Low rates of acute recanalization with intravenous recombinant tissue plasminogen activator in ischemic stroke: real-world experience and a call for action. *Stroke*. 2010;41(10):2254-2258.