Relationships Between Infarct Growth, Clinical Outcome, and Early Recanalization in Diffusion and Perfusion Imaging for Understanding Stroke Evolution (DEFUSE)

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Background and Purpose—The purpose of this study was to determine the relationships between ischemic lesion growth, recanalization, and clinical response in stroke patients with and without a perfusion/diffusion mismatch.

Methods—DEFUSE is an open label multicenter study in which 74 consecutive acute stroke patients were treated with intravenous tPA 3 to 6 hours after stroke onset. Magnetic resonance imaging (MRI) scans were obtained before, 3 to 6 hours after, and 30 days after treatment. Lesion growth was defined as the difference between the final infarct volume (30 day FLAIR) and the baseline diffusion lesion. Baseline MRI profiles were used to categorize 44 patients into Mismatch versus Absence of Mismatch subgroups. Early recanalization was assessed in 28 patients with an initial vessel lesion on magnetic resonance angiography. Infarct growth was compared based on whether a favorable clinical response (FCR) occurred and whether early recanalization was achieved.

Results—in the Mismatch subgroup, FCR was associated with less infarct growth (P=0.03) and early recanalization was predictive of both FCR (odds ratio: 22, P=0.047) and reduced infarct growth (P=0.024). There was no significant relationship between recanalization, infarct growth, and clinical outcome in the Absence of Mismatch subgroup. A threshold of < 7 cc of growth had the highest sensitivity and specificity for predicting a FCR in Mismatch patients (odds ratio: 65, P=0.015, sensitivity 82%, specificity 75%).

Conclusion—in contrast to Absence of Mismatch patients, significant associations between recanalization, reduced infarct growth, and favorable clinical response were documented in patients with a perfusion/diffusion mismatch who were treated with tPA within 3 to 6 hours after stroke onset. These findings support the Mismatch hypothesis but require validation in a larger study. (Stroke. 2008;39:2257-2263.)

Key Words: acute cerebral infarction ■ magnetic resonance imaging ■ diffusion-weighted imaging ■ thrombolysis

Early recanalization is the aim of thrombolytic treatment for acute ischemic stroke. Early recanalization may prevent the extent of irreversible infarction by the reversal of the ischemic penumbra. After more than 10 years, intravenous tissue plasminogen activator (tPA) administration remains the only approved pharmacological therapy. Patient selection for tPA is typically determined on clinical and CT scan–based criteria. More recently, multimodal MRI has received considerable attention as a method for assessment of acute ischemic stroke: Diffusion weighted imaging (DWI) demonstrates the presence of acute ischemic injury within minutes; magnetic resonance angiography (MRA) reveals vessel obstructions, and perfusion-weighted imaging (PWI) provides an estimate of cerebral hemodynamics. The perfusion/diffusion mismatch has been proposed as a surrogate for potentially salvageable ischemic tissue. Several groups have hypothesized that the presence of a mismatch on baseline MRI could facilitate the selection of patients who are likely to benefit from thrombolytic therapy beyond the current 3-hour time window. The Diffusion and perfusion imaging Evaluation For Understanding Stroke Evolution (DEFUSE) study demonstrated that patients with a perfusion/diffusion mismatch were likely to have a favorable outcome in association with early recanalization after treatment with IV tPA 3 to 6 hours after symptom. Multimodal MRI can also estimate infarct expansion by comparing the early ischemic lesion on DWI to the final infarct volume assessed by fluid attenuated inversion recov-
ery (FLAIR) sequence or T2 weighted images. It has been hypothesized that infarct expansion may be an appropriate surrogate marker of clinical outcome for phase 2 acute stroke trials. Surrogacy requires specific relationships between the surrogate and the primary end point. For the DEFUSE study this could be interpreted as: (1) A statistically significant relationship between a reduced infarct growth and a favorable clinical response; (2) An increased rate of favorable clinical response and reduced infarct growth in patients with early recanalization; (3) The mismatch hypothesis predicts that these relationships will occur in patients with a mismatch but not in the absence of mismatch.

In this preplanned substudy of DEFUSE, we investigated the relationships between infarct growth and clinical outcome as well as associations between early recanalization and lesion growth according to the baseline MRI profile.

Methods

Patients and Methods

The inclusion criteria, study design, and primary results of the DEFUSE trial have been previously reported. Briefly, patients with an acute ischemic stroke with National Institute of Health Stroke Scale score (NIHSS) greater than 5 were treated with IV tPA 0.9 mg/kg 3 to 6 hours after symptom onset. Participating patients underwent an MRI of the brain before tPA treatment as well as 3 to 6 hours and 1 month afterward. Neurological deficits were evaluated before tPA therapy, at the time of the 2nd (3 to 6 hours after tPA) MRI scan, and at 30 and 90 days. A favorable clinical response (FCR) was defined as a NIHSS of 0 to 1, or ≥8 points of improvement between baseline and 30 days.

MRA Protocol

The MRI protocol has been previously reported. Briefly, sequences obtained at baseline and 3 to 6 hours after tPA treatment included DWI, PWI, and 3-dimensional time-of-flight MRA (3-D TOF) of the circle of Willis. Regions of interest (ROI) were identified on DWI using a semi-automated thresholding algorithm. PWI maps were generated using a previously described technique. T-map maps were generated by deconvolution of the tissue concentration over time curve using an arterial input function from the contralateral supraclinoid internal carotid artery (ICA), the middle cerebral artery (MCA), the anterior cerebral artery (ACA), and the posterior cerebral artery (PCA). A numeric scale was used with 1=normal, 2=decreased flow, 3=occluded for the terminal ICA and the first branch of the MCA (M1). A different rating scale was used for the ACA (A1), PCA (P1), and second branch of the MCA (M2): 1=normal; 2=abnormal. Recanalization was assessed only among patients with initial vessel lesion. The rate of recanalization was defined as complete (occlusion or reduced flow to normal), partial (occlusion to reduced flow), and no recanalization (no change of vessel lesion). Recanalization was dichotomized into partial and complete recanalization versus no recanalization for the analyses performed in the current study. The “symptomatic vessel” was identified for all patients with a baseline MRA lesion. This was defined as the vessel that corresponded with the patient’s acute DWI lesion.

The MRA rating scale as well as the assessment by a second reader differs slightly from the approach previously reported. These changes in the MRA reading protocol resulted in 4 patients who were initially scored as having no MRA lesion being reclassified as follows: 3 with MCA M2 lesions and 1 with a PCA lesion. All 4 of these patients had partial recanalization, and 2 of these had a mismatch MRI profile. In addition, 1 mismatch patient who was initially classified as having no recanalization was reclassified as having a complete recanalization.

Study Plan, Statistical Analysis

This is a 2-part study with incremental inclusion criteria (Figure 1). The goal was to include as many patients as possible at each step to accurately explore the relationships assessed.

Part 1: Investigation of the Relationship Between Clinical Outcome and Infarct Growth and Mismatch Pattern

Inclusion Criteria

1. A technically adequate baseline DWI and 30-day FLAIR;
2. A quantifiable lesion related to acute stroke on initial DWI or final FLAIR.

For general characteristics, groups were compared by t test or nonparametric Mann–Whitney Test, proportions were compared by Chi-square and Fisher’s exact test according to the distribution and sample size of the observed data. Accordingly, mean±SD or median interquartile range (IQR) was estimated for each group.

Infarct growth according to the occurrence of FCR was examined in all eligible patients, then in Mismatch and in Absence of Mismatch groups using Mann–Whitney Test. The infarct growth cutoff that could predict a FCR with the greatest sensitivity and specificity was defined by receiver operator characteristic curve (ROC Curve) in Mismatch patients. We then calculated the odds ratio (OR) of achieving a FCR according to this infarct growth cutoff adjusted for age, sex, baseline NIHSS, DWI volume, and MRI profile using logistic regression. We also investigated the interaction between infarct growth, mismatch pattern, and FCR by logistic regression.

Part 2: Investigation of the Relationship Between Early Recanalization, Infarct Growth and Clinical Outcome and Mismatch Pattern

Inclusion Criteria

1. Meets inclusion criteria for Part 1 of the study;
2. An MRA lesion (occlusion or reduced flow) in a symptomatic vessel on the baseline scan.

Infarct growth according to the occurrence of early recanalization was examined in all eligible patients, then in Mismatch and in Absence of Mismatch groups using Mann–Whitney test. We calculated the OR of achieving a FCR according to the occurrence of recanalization adjusted...
Interactions between recanalization, infarct growth threshold, and FCR were assessed with logistic regression.

All statistical tests were 2-tailed at $\alpha<0.05$ for statistical significance. Data were analyzed using SPSS version 15.0 (SPSS Inc).

Results

Part 1: Infarct Growth and Clinical Outcome

Patient Selection and General Characteristics

Seventy-four patients were enrolled in DEFUSE. Of these, 24 did not meet the inclusion criteria for the Part 1 study group: 19 did not have an interpretable 30 day FLAIR examination (9 refused, 8 died, 2 were technically inadequate), and 5 patients had no lesion on the initial DWI, PWI, and MRA as well as no new lesion on the 30-day FLAIR. The included patients were younger (age $67\pm15$ versus $77\pm12$, $P=0.008$), had a smaller initial DWI lesion volume ($18.2\pm22.5$ cc versus $37\pm56.3$ cc, $P=0.043$), and were treated earlier ($318\pm34$ minutes versus $336\pm37$ minutes, $P=0.038$). The included patients were less likely to have an unfavorable outcome, mRS 4 to 6 at 3 months, 30% versus 58.3%, $P=0.019$.

Included patients were dichotomized into 2 groups according to their baseline MRI profile: 46% (23/50) had a “Mismatch” and 42% (21/50) had the “Absence of Mismatch” profile (12 Small and 9 No Mismatch). Six patients did not have a technically adequate baseline PWI and were excluded from analyses performed in Mismatch and Absence of Mismatch groups.

General characteristics are presented in the Table. The patients in the Absence of Mismatch group had a significantly different distribution of stroke subtypes ($P=0.009$), a shorter time to first MRI ($P=0.02$), a smaller initial PWI volume ($P=0.0001$), and a smaller final infarct lesion volume on FLAIR ($P=0.033$).

The rate of FCR was not statistically different between the 2 groups: 48% (11/23) in mismatch versus 43% (9/21) in the absence of mismatch group, $P=0.741$. The median (IQR) infarct growth was not statistically different between the 2 groups, however there was a trend toward more infarct growth in Mismatch patients: Mismatch $+$5.
cc (+1, +30) and Absence of Mismatch +1 cc (−1, +10), \( P=0.095 \).

**Infarct Growth and Clinical Outcome: All Patients**
The mean infarct growth observed among all 50 patients in the study was 11.6 ± 24 cc, range: −40, +106. Forty-six percent (23/50) had a FCR. These patients had less infarct growth compared to the patients who did not have a FCR: median (IQR) infarct growth: +2 cc (−2, +6) versus +9 cc (−1, +31), \( P=0.038 \) (Figure 2a).

**Baseline MRI Profile Subgroup**
Mismatch patients with a FCR had a significantly less infarct growth compared to mismatch patients without a FCR, median (IQR): +2 cc (−2, +6) versus +9 cc (−1, +31), \( P=0.004 \). This relationship was not observed in the Absence of Mismatch group, median (IQR): +1 cc (−1, +5) (n=9) versus +2 cc (0, +15) (n=12), \( P=0.464 \).

**Infarct Growth Threshold and Odds of Favorable Clinical Response**
The Mismatch profile was not significantly associated with FCR, \( P=0.127 \). ROC curve analysis identified a threshold of 7 cc of infarct growth to have the highest sensitivity (82%) and specificity (75%) for predicting FCR in the Mismatch subgroup. We found a significantly increased rate of FCR in patients with a mismatch and an infarct growth 7 cc, \( P=0.013 \).

The odds ratio for achieving a FCR in patients with infarct growth <7 cc compared with ≥7 cc was assessed after adjustment for age, sex, initial DWI volume, baseline NIHSS, and baseline MRI profile. Among all patients (n=50) the odds ratio was 9.3 (95% CI: 2.0 to 44.3, \( P=0.005 \)). The OR in the Mismatch group was 64.9 (95% CI: 1.8 to 1863, \( P=0.015 \)). No relationship was found in the Absence of Mismatch subgroup; \( P=0.107 \).
Part 2: Early Recanalization, Infarct Growth, and Clinical Outcome

Patients Selection and Characteristics
Nineteen of the 50 patients included in Part 1 did not meet the Part 2 inclusion criteria: 8 did not have a technically adequate initial or follow-up MRA, and 11 did not have a symptomatic MRA lesion on the baseline scan (Figure 1). As in Part 1, patients were dichotomized according to their baseline MRI profile into the Mismatch (n=18) and the Absence of Mismatch (n=10: 5 Small lesion profile +5 No Mismatch profile). Three patients did not have a technically adequate baseline PWI (these patients were excluded from analyses performed in Mismatch and Absence of Mismatch groups). There were no statistically significant differences between the Mismatch and the Absence of Mismatch groups regarding age, sex, initial DWI volume, time-to-treatment, and baseline NIHSS. The time between symptom onset and MRI was shorter in the Absence of Mismatch subgroup compared to the Mismatch group, p=0.045.

The location of the initial symptomatic MRA lesion in the Mismatch patients were: Combined (ICA+MCA)=4, M1 segment of the MCA=12, M2 segment of MCA=2. In the Absence of Mismatch patients the MRA lesions were in the ICA=1, Combined (ICA+MCA)=4, M1 segment of the MCA=3, M2 segment of MCA=1, P2 part of PCA=1. For the patients with unknown baseline MRI profile: Combined ICA+MCA=1, M1 segment of the MCA=2. The rate of recanalization was of 50% in both Mismatch (9/18) and Absence of Mismatch (5/10) groups. Median (IQR) infarct growth was not statistically different between the 2 groups: Mismatch +5 cc (+1, +16) versus Absence of Mismatch +5 cc (0, +28) P=0.906.

Infarct Growth and Early Recanalization
Forty-five percent (14/31) of the Part 2 study group experienced early recanalization. These patients had significantly less infarct growth compared to those who did not recanalize, median (IQR): +3 cc (0, +9; n=14), versus +20 cc (+2, +41; n=17), P=0.047 (Figure 2b).

Baseline MRI Profile
Mismatch patients who had early recanalization had significantly less infarct growth compared to those who did not recanalize, median (IQR): +1 cc (-3, +5) versus +28 cc (+11, +50), P=0.024. This difference was not observed in the Absence of Mismatch group, +6 cc (+2, +17) versus +2 cc (+1, +29), P=0.548.

Among the Mismatch patients in Part 2 (n=18), there was a similar relationship between infarct growth and FCR as reported in Part 1 of the study: infarct growth was significantly less in the mismatch patients with favorable clinical outcome (P=0.031). In addition, infarct growth <7 cc increased the odds of a FCR (OR 22, 95% CI: 0.92 to 535.5, P=0.052).

Odds of Favorable Clinical Response According to Early Recanalization and Infarct Growth
The odds of a FCR for patients with early recanalization versus no early recanalization were adjusted for age, sex, baseline NIHSS, and MRI profile. Among all 31 patients included in Part 2, early recanalization was not associated
with a FCR (OR 3.3, 95% CI: 0.5 to 13.3, P=0.20); however, infarct growth <7 cc was (OR: 11.6, 95% CI: 1.7, 77.8, P=0.012). We found a significant interaction between early recanalization, infarct growth <7 cc, and a FCR (P=0.026). Early recanalization was associated with a significant increase in the odds of a FCR among mismatch patients (OR 20, 95% CI: 1.01 to 397, P=0.047). There was no association between early recanalization and a FCR in the Absence of Mismatch group P=1.

Discussion
This study confirms previous observations that infarct growth is strongly associated with clinical outcomes in acute stroke patients. In addition, our results provide unique data demonstrating that early recanalization is associated with both reduced infarct growth and better clinical outcomes in Mismatch patients, but not in the Absence of Mismatch. This differential effect in Mismatch versus Absence of Mismatch patients is potentially of considerable clinical significance.

The “mismatch hypothesis” predicts that patients with a PWI/DWI mismatch are more likely to benefit from recanalization therapies, such as intravenous tPA, compared with patients who do not have a mismatch pattern. Clinical data to support the mismatch hypothesis, however, have been limited, and a recent systematic review concluded that “there are no meaningful data on clinical outcomes in patients with or without mismatch in the presence of thrombolysis.”

Our findings provide a strong indication that a safe treatment that leads to an increase in the rate of early recanalization is more likely to produce beneficial effects on both ischemic lesion evolution and clinical outcomes, if it is tested in mismatch patients.

ROC analysis defined a threshold of <7 cc of infarct growth to be the highest sensitivity and specificity for predicting FCR. In addition, there was a significant interaction between mismatch pattern, infarct growth, and favorable outcome. Mismatch patients with minimal or no infarct growth were likely to have a FCR, whereas Mismatch patients with infarct growth ≥7 cc were unlikely to have a good clinical response. The variable that is most likely mediating this relationship is recanalization. Several studies have previously demonstrated that tPA-associated early recanalization correlates with improved clinical outcomes.

Our results clarify that Mismatch patients appear to be the main mediators of this effect. Among Mismatch patients in our study, recanalization was clearly associated with both a reduction in infarct growth and FCR. This relationship was not present in patients who did not have a mismatch; for these patients there was no evidence of a relationship between recanalization and either infarct growth or clinical response. These results are supported by the findings of previous studies. In 2001, Schellinger and al found that tPA-induced or spontaneous early recanalization was associated with reduced ischemic lesion volumes as assessed by MRI at day 5. The relationship between infarct expansion and thrombolysis was reported in another prospective cohort of patients treated by IV tPA within 6 hours of onset of hemispheric ischemic stroke. These patients were compared to a historic cohort not treated with tPA. The thrombolytic group had significantly reduced infarct growth and a trend was found toward a better rate of clinical recovery. Analyses restricted to Mismatch patients demonstrated a statistically significant benefit for clinical outcomes. These findings support the mismatch theory.

The mismatch theory assumes that the initial PWI lesion reflects dysfunctional but not irreversibly injured tissue. Mismatch patients who do not have early recanalization are likely to experience lesion growth and less likely to have desirable clinical outcomes. These associations should be substantially reduced or lost if recanalization occurs; infarct growth is diminished because of salvage of dysfunctional, but still viable, tissue. For the Absence of Mismatch patients the initial DWI lesion will be similar in size or smaller than the PWI lesion. The size of the initial lesion (larger of the DWI or PWI volume) should correlate with clinical outcome regardless of recanalization. Lesion growth is expected to be absent or modest irrespective to recanalization. The DEFUSE data presented in this analysis support these predictions from the mismatch hypothesis.

Study Limitations
Only 50 of the 74 DEFUSE patients were eligible for Part 1 of this study; the primary reason for exclusion was failure to obtain the 30 day MRI scan (typically because of death before 30 days or refusal of the 30 day scan, which was often because of severe disability). Therefore, the patients who were excluded were more likely to have unfavorable outcomes than the included patients. This may be a source of bias, as these patients had larger initial DWI lesion volumes than the included patients and potentially may have had different infarct growth patterns. Therefore, the results of this study should not be generalized to all stroke patients. Obtaining a follow-up MRI at an earlier time point should be considered to reduce this potential bias in future trials.

An additional 19 patients were not eligible for the analyses in Part 2 of the study. The most common reason for exclusion was that a symptomatic MRA lesion could not be visualized on the baseline scan. In these patients, either spontaneous early recanalization had already occurred or the vascular occlusion was below the resolution of the MRA. Therefore, data from Part 2 apply only to patients who have a visible MRA lesion in the 3- to 6-hour time period. These patients are likely the most appropriate target group for a recanalization therapy in this time window.

Despite the very limited sample size in Part 2 of the study, the same relationships between infarct growth, mismatch pattern, and clinical outcome that were found in Part 1 of the trial were confirmed in Part 2. However, independent confirmation of these findings in other patient samples is required because of the limited sample size and the patient exclusions mentioned above.

Finally, DEFUSE is an exploratory pilot study in which all patients were treated with IV tPA. Hence, it is not possible to separate the effects of spontaneous versus “tPA induced” recanalization. A randomized trial is required to determine the risks and benefits of tPA therapy in patients with various MRI profiles. The ability to confirm which patients had early
recanalization (within 3 to 6 hours after tPA) is a key strength of the DEFUSE study design. Most previous studies did not assess recanalization until at least 24 hours after therapy—a time frame in which recanalization likely has more limited beneficial effects. In future trials, continuous transcranial ultrasound monitoring could provide a more accurate information regarding the benefits of recanalization over time. 27

In summary, our results demonstrate that reduced infarct growth, likely mediated by early recanalization, is significantly associated with improved clinical outcome in mismatch patients. These findings support the mismatch hypothesis as well as the role of infarct growth as a suitable biomarker for clinical outcomes in mismatch patients.

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