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Expediting MRI-Based Proof-of-Concept Stroke Trials Using an Earlier Imaging End Point

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Background and Purpose—Before Phase III trials of acute stroke therapies, proof-of-concept MRI trials are increasingly used to gauge the likelihood of success. Given that animal models use infarct volume as the end point, Phase II trials have aimed to translate the findings using infarct growth. These trials could be expedited if subacute diffusion-weighted imaging lesion volume replaced late T2-weighted lesion volume as the primary end point.

Methods—in the Echoplanar Imaging Thrombolytic Evaluation Trial, patients with acute ischemic stroke presenting within 3 to 6 hours were randomized to tissue plasminogen activator or placebo. We assessed correlations between acute (Day 1), subacute (Day 3 to 5) as well as late (Day 90) lesion volumes and clinical outcome (National Institutes of Health Stroke Scale). We compared lesion growth between placebo- and tissue plasminogen activator-treated patients.

Results—All 3 scans were performed in 72 of 101 patients (32 tissue plasminogen activator, 40 placebo). Median time to subacute imaging was 3 days (interquartile range, 2 to 4) and 90 days (interquartile range, 90 to 95) for the late scan. Increase in lesion volume from acute to subacute scans was smaller in the tissue plasminogen activator group compared with the placebo group (6.77 mL; interquartile range, 2.30 to 49.10; versus 30.00 mL; interquartile range, 7.19 to 85.93; P=0.03). Subsequent shrinkage did not reveal significant treatment effects. Correlation coefficient between acute and late lesion volumes was 0.81 (P<0.01). Subacute and late lesion volumes were strongly correlated (rho=0.94, P<0.01). Correlation coefficient for acute, subacute, and late lesion volume and late National Institutes of Health Stroke Scale score was 0.64 (P<0.01), 0.81 (P<0.01), and 0.77 (P<0.01), respectively.

Conclusions—These findings suggest that subacute imaging at Day 3 after thrombolysis is an appropriate imaging end point for proof-of-concept MRI-based stroke treatment trials and can replace later MRI measurements. (Stroke. 2009;40:1353-1358.)

Key Words: MRI ■ plasminogen activator ■ stroke ■ timing ■ tissue plasminogen activator

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linical end points in acute stroke studies are traditionally assessed on Day 90, because the bulk of neurological recovery has occurred by this time point.1,2 One of the biggest challenges in stroke research is the translation of findings from animal models to human studies. Although clinical outcome is the most important end point in Phase III stroke studies, surrogate measures derived from imaging techniques can facilitate study design.3–7 The most commonly used imaging end point is infarct growth, ideally assessed with serial MRI. This surrogate has been chosen because it facilitates translation from preclinical models, in which infarct volume is traditionally used as the primary end point. Before pivotal Phase III trials of acute stroke therapies, proof-of-concept studies using this end point can be used to estimate the likelihood of success of putative therapeutic interventions.8 At present, however, the optimal time point for outcome imaging is unknown.

Previous studies indicate that lesions grow during the acute and subacute periods up to 3 to 4 days before gradually decreasing in size.9–15 To date, most studies have used a late scan performed concomitantly with clinical assessments. Typically, final infarct volume is measured on a T2-weighted image between Days 30 and 90.10,16,17 This can be problematic because patients may be lost to follow-up or die before these time points.

Surrogate MRI stroke trials could be expedited if subacute diffusion-weighted imaging (DWI) lesion volume could be used to replace late T2-weighted lesion volume as the primary end point. We used the data set of the Echoplanar...
Imaging Thrombolytic Evaluation Trial (EPITHET) to test the hypotheses that subacute lesion volumes are sufficient to demonstrate treatment effects and that the relationship between early lesion evolution and clinical outcome was at least equivalent to the relationship between later imaging and clinical outcome. In this substudy, we did not aim to demonstrate the efficacy of tissue plasminogen activator (tPA) in the 3- to 6-hour time window. Rather, we investigated whether relevant imaging information is lost when completing the last scan at 3 to 5 days after stroke onset rather than reliance on a scan at 90 days.

Methods
EPITHET was a prospective, double-blind, multicenter trial with acute hemispheric stroke patients randomized to placebo or tPA 3 to 6 hours after symptom onset. Informed consent was obtained from all subjects. Human research and ethics committees approved study protocols and informed consent procedures at all recruiting sites. Methodological details have been reported previously. In brief, patients had acute MRI and clinical assessment through the National Institutes of Health Stroke Scale (NIHSS) performed before treatment on Day 1 and repeated Days 3 to 5 (subacute) and day 90 (late) after acute stroke. A baseline screening noncontrast CT scan was used to exclude patients with acute hemorrhage and major early ischemic change of more than one third of the territory of the middle cerebral artery.

Lesion Measurement
All MRI scans were read at the coordinating center by investigators blinded to treatment assignment and clinical outcomes but not to time point. DWI at baseline and Days 3 to 5 and T2-weighted lesions at Day 90 were assessed by 2 independent raters who used standard planimetric software (Analyze 7.0; Biomedical Imaging Resource, Mayo Clinic, Rochester, Minn). The mean DWI and T2-weighted lesion volumes from the 2 raters were used for subsequent analysis.

Statistical Analysis
Only patients who had all 3 scans were included in this subgroup analysis. Correlations between lesion volumes (on acute and subacute DWI and late T2) were assessed. Correlations between lesion volumes and clinical scores (NIHSS) were assessed. The difference in the median lesion volume changes between tPA- and placebo-treated patients was assessed using the nonparametric Mann–Whitney U test. We fitted an analysis of covariance model that allowed for an interaction between treatment group and the earlier scans in the association with the late scan, ie, late scan = acute/subacute scan + treatment group + acute/subacute scan*treatment group. Data were analyzed using SPSS statistical software Version 15.0. P<0.05 was deemed significant for all analyses.

Results
Patients
Of 101 patients enrolled into EPITHET, one withdrew consent after randomization to placebo and before treatment. Of the remaining 100 patients, 91 had a subacute scan, whereas 72 patients (40 placebo, 32 tPA) had imaging and clinical outcome assessment at all 3 time points. There were 20 deaths before day 90 (13 tPA, 7 placebo) of whom 7 died before the subacute scan (6 tPA, one placebo). Two patients did not have subacute MRI for other reasons. Overall, 7 patients were lost to follow-up and one patient refused clinical assessment on Day 90. Six further patients did not receive all planned scans for other reasons. Baseline characteristics of the included patients are shown in the Table. There were no significant baseline differences between the placebo group and tPA-treated patients. Comparison of baseline characteristics of patients included and excluded revealed no statistical differences except for a higher prevalence of diabetes among patients excluded from this substudy (17% of the patients included versus 36% of the patients excluded, P=0.04; Supplemental Table I, available online at http://stroke.ahajournals.org).

Lesion Measurement
Results correlated well between raters for baseline DWI lesion volume ($r^2=0.97$), Day 3 to 5 DWI lesion volume ($r^2=0.98$), and Day 90 T2-weighted lesion volume ($r^2=0.99$). Median

### Table. Baseline Characteristics of Patients Included in This Substudy

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All (n=72)</th>
<th>Placebo (n=40)</th>
<th>tPA (n=32)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, years (SD)</td>
<td>71 (14)</td>
<td>71 (13)</td>
<td>71 (14)</td>
<td>0.966</td>
</tr>
<tr>
<td>Male sex, no. of patients (%)</td>
<td>34 (47)</td>
<td>19 (48)</td>
<td>19 (59)</td>
<td>0.315</td>
</tr>
<tr>
<td>Hypertension, no. of patients (%)</td>
<td>50 (69)</td>
<td>25 (63)</td>
<td>25 (78)</td>
<td>0.152</td>
</tr>
<tr>
<td>Diabetes mellitus, no. of patients (%)</td>
<td>12 (17)</td>
<td>7 (18)</td>
<td>5 (16)</td>
<td>0.832</td>
</tr>
<tr>
<td>Hyperlipidemia, no. of patients (%)</td>
<td>27 (38)</td>
<td>14 (35)</td>
<td>13 (41)</td>
<td>0.624</td>
</tr>
<tr>
<td>Atrial fibrillation, no. of patients (%)</td>
<td>26 (36)</td>
<td>13 (33)</td>
<td>13 (41)</td>
<td>0.476</td>
</tr>
<tr>
<td>Current/past smoker, no. of patients (%)</td>
<td>42 (58)</td>
<td>16 (40)</td>
<td>11 (34)</td>
<td>0.624</td>
</tr>
<tr>
<td>Median NIHSS at presentation (IQR)</td>
<td>12 (9)</td>
<td>10 (9)</td>
<td>14 (18)</td>
<td>0.343</td>
</tr>
<tr>
<td>Median time from stroke onset to emergency department presentation, minutes (IQR)</td>
<td>136 (79)</td>
<td>121 (75)</td>
<td>146 (88)</td>
<td>0.489</td>
</tr>
<tr>
<td>Median time from stroke onset to MRI scan, minutes (IQR)</td>
<td>263 (78)</td>
<td>245 (89)</td>
<td>270 (51)</td>
<td>0.266</td>
</tr>
<tr>
<td>Median time from stroke onset to treatment, minutes (IQR)</td>
<td>308 (69)</td>
<td>309 (80)</td>
<td>307 (48)</td>
<td>0.751</td>
</tr>
<tr>
<td>Median time to subacute imaging, days (IQR)</td>
<td>3 (2)</td>
<td>72 (50)</td>
<td>72 (30)</td>
<td>0.799</td>
</tr>
<tr>
<td>Median times to late imaging, days (IQR)</td>
<td>90 (5)</td>
<td>92 (4)</td>
<td>91 (8)</td>
<td>0.487</td>
</tr>
<tr>
<td>DWI lesion volume on Day 1, mL (IQR)</td>
<td>20 (33)</td>
<td>21 (33)</td>
<td>14 (32)</td>
<td>0.951</td>
</tr>
</tbody>
</table>

Data are mean (SD), no. (%), or median (range).
difference in DWI lesion volume between raters was 0.5 mL (interquartile range [IQR], −2.1 mL to 3.6 mL). Median difference in T2 lesion volume was 0.0 mL (IQR, −3.1 mL to 3.6 mL).

**Lesion Evolution**

Median time to the subacute scan was 3 days (IQR, 2 to 4) and 90 days to the late scan (IQR, 90 to 95). Median acute DWI lesion volume was 19.56 mL (IQR, 8.46 to 41.36), median subacute DWI lesion volume was 45.15 mL (IQR, 15.87 to 125.10), and median late T2-weighted lesion volume 23.01 mL (IQR, 5.39 to 81.91).

**Placebo Versus Tissue Plasminogen Activator**

In the placebo patients, median lesion volume was 20.51 mL (IQR, 8.17 to 41.65) on the acute scan, 61.15 mL (IQR, 17.55 to 143.14) on the subacute scan, and 46.43 mL (IQR, 10.15 to 117.73) on the late scan. In patients treated with tPA, median lesion volume was initially 14.37 mL (IQR, 8.69 to 40.18), grew to 27.74 mL (IQR, 11.79 to 78.87), and shrunk subsequently to 7.62 mL (IQR, 3.79 to 56.62; Figure 1). Thus, acute to subacute lesion growth was smaller in tPA- compared with placebo-treated patients. However, reduction in lesion volume between subacute and late imaging did not differ significantly between placebo- and tPA-treated patients.

From acute to late imaging, the median lesion growth was 15.25 mL (IQR, −2.61 to 59.19) in the placebo group and −1.80 mL (IQR, −6.33 to 3.77) in tPA-treated patients (P=0.002).

From acute to subacute imaging, median lesion volume growth was 30.00 mL (IQR, 7.19 to 85.93) in the placebo group and 6.77 mL (IQR, 2.30 to 49.10) in tPA-treated patients (P=0.032). In both groups, lesion volume decreased between subacute and late imaging without significant differences (placebo: median, −9.92 mL; IQR, −28.23 to 3.66 versus tPA: −11.73 mL; IQR, −43.00 to −3.98; P=0.371).

**Relationship Between Lesion Volumes at Different Time Points**

Lesion volumes acutely and subacutely correlated with final infarct volume on Day 90. Overall, Spearman’s correlation between acute DWI and late T2 was 0.81 (P<0.01; n=72). Separated by treatment group, Spearman’s correlation between acute DWI and late T2 was 0.76 (P<0.01; n=40) for placebo-treated patients and 0.86 for patients treated with tPA (P<0.01; n=32). Analysis of covariance revealed that the main effects of both acute DWI lesion volume and treatment group on late DWI volume were statistically significant (P<0.01). The tPA group was associated with a 27.9-mL smaller volume on Day 90 relative to the control group (95% CI, 8.6 to 47.2). The interaction between group and acute DWI lesion volume was not statistically significant (P=0.68).

Overall, Spearman’s correlation between subacute DWI and T2 was 0.94 (P<0.01; n=72). This indicated less variance in lesion volume from subacute to late imaging compared with lesion volume from acute to late imaging. Spearman’s correlation between subacute DWI and T2 was 0.97 (P<0.01; n=40) for placebo-treated patients and 0.83 for patients treated with tPA (P<0.01; n=32). Analysis of covariance analysis revealed that the main effect of subacute lesion volume was statistically significant (P<0.01), whereas the effect of treatment group was not (P=0.068). The interaction between treatment group and subacute DWI lesion volume was not statistically significant (P=0.77; Figure 2).

**Relationship Between Lesion Volume and Clinical Outcome**

Subacute lesion volume correlated tightly with final neurological outcome measured with NIHSS. Spearman’s correla-
tion coefficient of lesion volume and outcome NIHSS score was 0.64 for acute, 0.81 for subacute, and 0.77 for late scanning (*P*<0.01; Supplemental Figure I, available online at http://stroke.ahajournals.org).

**Discussion**

This substudy of EPITHET shows that ischemic lesion volume measured by MRI at 3 days after stroke onset is a suitable imaging surrogate end point. Our results suggest that imaging patients with stroke 90 days after symptom onset does not provide additional information over that obtained from subacute MRI data. This is an important finding given the logistic and additional financial difficulties associated with imaging patients with stroke at later time points. For example, in EPITHET, 30% of patients were unable to have a Day 90 MRI, which meant that their outcomes were based on the last observation carried forward method using the subacute MRI time point. In contrast, only 2 patients in

**Figure 2.** Scatterplots of lesion volumes. Spearman’s correlation between acute DWI (A) and late T2 was 0.81 (*P*<0.01; *n*=72). Spearman’s correlation between subacute DWI (B) and T2 was 0.94 (*P*<0.01; *n*=72). Circles indicate patients treated with tPA (Spearman’s correlation between acute and late lesion volume 0.86, *P*<0.01; *n*=32; Spearman’s correlation between subacute and late lesion volume 0.83, *P*<0.01; *n*=32). Black triangles symbolize patients who received placebo (Spearman’s correlation between acute and late lesion volume 0.76, *P*<0.01; *n*=40; Spearman’s correlation between subacute and late lesion volume 0.97, *P*<0.01; *n*=40).
addition to those who died within the first days were not able to undergo subacute MRI, indicating good feasibility of imaging at this time point. Adopting a study design based on subacute imaging would help to eliminate the need to use the last observation carried forward method in the future.

Lesion Measurement
To standardize image analysis and eliminate interobserver variability, all MRI scans were read at the coordinating center by 2 investigators. Thus far, investigator judgment is needed for delineation of lesion boundaries. In theory, fully automated software to measure lesion volumes may be able to reduced sources of errors. However, no such software has been validated sufficiently. Quantitative volumes by interrater measurements can provide consistent and repeatable results.\(^5\)\(^,\)\(^18\) There was good agreement between lesion volumes measured by the 2 raters and the mean of lesion volumes obtained by the 2 raters was used for further analyses.

Lesion Evolution
The results of this study suggest that shortening Phase II MRI-based surrogate end point studies of thrombolytic agents from 3 months to 3 days could expedite and economize research progress without loss of essential information. Our study indicates that the treatment effect of tPA on lesion growth was observed between the acute and subacute time points. The subsequent decrease in lesion volume after subacute MRI did not differ significantly between treatment groups, suggesting that the effect of reperfusion therapy is ideally assessed at earlier time points. The excellent correlation indicated that the decrease from subacute to late imaging was predictable. The infarct dynamics related to the acute stroke and its treatment had manifested itself by imaging on Day 3. The natural evolution of cerebral infarct volume in the placebo group in our study was consistent with a pattern of initial lesion growth and subsequent shrinkage previously described.\(^4\)\(^,\)\(^6\)\(^,\)\(^-\)\(^15\) This shrinkage is likely to reflect a combination of resolution of edema and later gliosis. Although the correlation between acute and late lesion volumes was similar to the correlation between subacute and late lesion volume in patients treated with tPA (0.86 versus 0.83, respectively), these correlations differed in patients who received placebo. The evolution of lesion volumes from the acute to the subacute scan was variable and less predictable in the placebo group (Spearman’s rho = 0.76). However, the correlation between subacute and late lesion volume was very strong in patients who received placebo (Spearman’s rho = 0.97). The fact that this correlation was stronger than the one observed in tPA-treated patients may be attributable to the greater lesion volumes and therefore reduced risk of measurement errors in the placebo group.

Relationship Between Lesion Volume and Clinical Outcome
Correlations between clinical scores and lesion volume were also in accordance with most previously reported studies, although this has become a recent source of controversy.\(^4\)\(^-\)\(^9\)\(^,\)\(^19\) Correlations of subacute and late lesion volume with clinical outcome were similar, indicating that there is no disadvantage in using the earlier time point as a predictor of clinical outcome. Thus, subacute lesion volume can predict the neurological deficit on Day 90 at least as well as the scan on Day 90. Furthermore, an outcome scan within the first week would also cover safety aspects such as hemorrhagic transformation occurring most often within 48 hours after therapy.\(^20\)

Limitations
The results of this study cannot be generalized to all MRI-based stroke research. Treatment with tPA aims at recanalization ideally assessed within hours.\(^21\) Effects on final infarct volume of thrombolytic-induced reperfusion are expected to occur early and later lesion evolution appears to be unrelated to thrombolytic effects. This may not be the case with neuroprotective drugs, which may potentially require later scanning times.

Previous studies of DWI lesion evolution have demonstrated that diffusion restriction is relatively stable from 36 hours to 2 weeks after onset.\(^15\) Our subacute scans were completed over a relatively wide time interval (IQR, 2 to 4 days). Additional intermediate time points before and after the subacute scan were not planned.

The increase in lesion volume at the subacute time is due not only to an increase in the extent of irreversibly infarcted tissue, but also to swelling caused by an uptake of water into the tissue secondary to both ionic and vasogenic edema.\(^22\) However, swelling does not necessarily result in infarction.\(^23\) The volume analysis performed in this study did not allow for determination of the relative contributions of swelling versus infarction. Patients who received tPA are more likely to have reperfused at an early stage and are therefore less likely to have severe infarct swelling. Thus, the apparent greater increase in infarct volume in the placebo group may be due to an excess of tissue swelling. In general, smaller lesions swell less. Although there was no significant difference in lesion size on Day 1, the smaller lesions of tPA-treated patients could have been expected to have less increase in infarct volume than the placebo-treated patients.

An acknowledged limitation of this analysis was the selective nature of the trial in general and in particular the exclusion of nonsurvivors in this substudy. Of the 1224 patients screened within 6 hours, only 101 were enrolled in EPITHET.\(^16\) Only patients with comparable information on lesion volume on all 3 points in time were included in this particular substudy. This likely explains why we found a significant difference in lesion growth attenuation with tPA in this substudy, whereas in the full EPITHET trial, previously only trends had been found that did not achieve formal statistical significance.\(^16\) The design of this analysis therefore includes a bias in favor of a tPA treatment effect. The exclusion of the excess deaths related to tPA meant that these patients could not be studied at the 3 time points. Important adverse clinical effects may have been missed. For example, almost twice as many patients who received tPA did not have the Day 90 scan and this was mostly because they had died, a substantial excess compared with placebo. By focusing on imaging surrogates, we can only refer to those patients who survived and were able to complete scanning at all 3 time points.
points. However, this substudy was not designed to prove the efficacy of tPA beyond 3 hours but to demonstrate the appropriateness of an earlier imaging end point in imaging based trials, which cannot replace clinical outcomes.

Summary
Our findings suggest completing an outcome scan as early as 3 days after thrombolysis is appropriate for proof-of-concept stroke treatment trials using infarct growth as the surrogate end point. Reperfusion and recanalization should be evaluated within hours after treatment. This may lead to more uniform results and a decrease in lost data. These factors are to be considered in the design of future MRI-based acute stroke studies.

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Disclosures
S.M.D. is a member of the Servier, Novo Nordisk, and Sanofi Aventis Advisory Boards. G.A.D. is a member of the Boehringer Ingeheim, PAION, Servier, and Sanofi Aventis Advisory Boards.

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