Safety and Efficacy of Ultrasound-Enhanced Thrombolysis: A Comprehensive Review and Meta-Analysis of Randomized and Nonrandomized Studies

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Background and Purpose—Ultrasound-enhanced thrombolysis is a promising new approach to facilitate reperfusion therapies for acute ischemic stroke. So far, 3 different ultrasound technologies were used to increase the thrombolytic activity of tissue plasminogen activator (tPA), including transcranial Doppler (TCD), transcranial color-coded duplex (TCCD), and low-frequency ultrasound. We performed a meta-analysis to evaluate the safety and efficacy of ultrasound-enhanced thrombolysis compared to the current standard of care (intravenous tPA).

Subjects and Methods—Through Medline, Embase, and Cochrane database search, we identified and abstracted all studies of ultrasound-enhanced thrombolysis in acute cerebral ischemia. Principal investigators were contacted if data not available through peer-reviewed publication were needed. Symptomatic intracerebral hemorrhage (sICH) and recanalization rates were compared between tPA, tPA+TCD±microspheres (μS), tPA+TCCD±μS, and tPA+low-frequency ultrasound.

Results—A total of 6 randomized (n=224) and 3 nonrandomized (n=192) studies were identified. The rates of symptomatic intracerebral hemorrhage in randomized studies were as follows: tPA+TCD, 3.8% (95% CI, 0.0%–11.2%); tPA+TCCD, 11.1% (95% CI, 0.0%–28.9%); tPA+low-frequency ultrasound, 35.7% (95% CI, 16.2%–61.4%); and tPA alone, 2.9% (95% CI, 0.0%–8.4%). Complete recanalization rates were higher in patients receiving combination of TCD with tPA 37.2% (95% CI, 26.5%–47.9%) compared with patients treated with tPA alone 17.2% (95% CI, 9.5%–24.9%). In 8 trials of high-frequency (TCD/TCCD) ultrasound-enhanced thrombolysis, tPA+TCD/TCCD±μS was associated with a higher likelihood of complete recanalization (pooled OR, 2.99; 95% CI, 1.70–5.25; P<0.001) when compared to tPA alone. High-frequency ultrasound-enhanced thrombolysis was not associated with an increased risk of symptomatic intracerebral hemorrhage (pooled OR, 1.26; 95% CI, 0.44–3.60; P=0.67).

Conclusions—The present safety and signal-of-efficacy data of high-frequency ultrasound-enhanced thrombolysis should be taken into account in the design of future randomized controlled trials. (Stroke. 2010;41:280-287.)

Key Words: sonothrombolysis stroke tissue plasminogen activator transcranial color-coded duplex transcranial Doppler ultrasound-enhanced thrombolysis

Ultrasound-enhanced thrombolysis (UET) is a promising new approach to facilitate reperfusion therapies for acute ischemic stroke. So far, 3 different ultrasound technologies were used to increase the thrombolytic activity of tissue plasminogen activator (tPA) including transcranial Doppler (TCD), transcranial color-coded duplex (TCCD), and low-frequency ultrasound (LFUS). These technologies differ greatly in acoustic properties of the ultrasound beams, such as frequency, mechanical index, and the areas of the brain exposed to ultrasonic pressure waves. Although direct comparisons between technologies are not available, it is important to know the cumulative experience between centers worldwide.

Several groups now try gaseous microspheres (μS) that were first engineered as contrast agents for ultrasound imaging to further facilitate energy transfer from a mechanical wave to stagnant residual blood flow and thrombus structures. Although μS are currently approved for diagnostic purposes, experimental data suggest that they likely also possess therapeutic properties by potentiating UET and increasing the likelihood of recanalization. The clinical
development of this novel reperfusion technology is underway with safety trials being recently published.6,7,10

Therefore, we performed a meta-analysis to evaluate the safety and efficacy of sonothrombolysis compared to the current standard of care, ie, intravenous thrombolysis with tPA.

Subjects and Methods

Search Strategy

We sought all available previously published studies in which the efficacy and safety of UET were evaluated in humans in a randomized or nonrandomized fashion. Briefly, studies were identified by 2 independent observers (G.T. and A.V.A.) from PubMed, Embase, and Cochrane databases (search years January 1996–July 2008). In the first extraction step, we used the following search terms: “ultrasound-enhanced thrombolysis” (34 publications) OR “sonothrombolysis” (23 publications) OR “ultrasound AND thrombolysis” (877 publications) OR “ultrasound AND tissue plasminogen activator” (484 publications). In the second extraction step we added the following search terms: “stroke” and/or “cerebrovascular diseases.” We found 24 publications for “ultrasound-enhanced thrombolysis and stroke,” 17 for “ultrasound-enhanced thrombolysis and cerebrovascular diseases,” 20 for “sonothrombolysis AND stroke,” 19 for “sonothrombolysis AND cerebrovascular diseases,” 213 for “ultrasound AND thrombolysis AND stroke,” 172 for “ultrasound AND thrombolysis AND cerebrovascular diseases.” In the third extraction step we excluded studies including patients treated with intra-arterial tPA and intra-arterial infusion of μS microspheres using transfemoral insonation for delivering ultrasound in the vertebrobasilar system and treating patients with other thrombolytic agents (eg, tenecteplase) than alteplase, which is currently the only approved thrombolytic drug for the treatment of acute cerebral ischemia.

We also searched the bibliographies of all included studies and any relevant review articles for additional suitable studies. UET studies that were presented at the international stroke meetings and published in the form of abstracts in a peer-reviewed journal were also included in the present analysis. We carefully excluded articles that concerned patients already used in another article used from the same institution, except when the methods sections made it absolutely clear that the patients did not overlap. Potential disagreement between the 2 observers (G.T. and A.V.A.) regarding study identification was resolved by a third independent investigator (M.S.).

Data Extraction

Two independent reviewers (G.T. and A.V.A.) used a standardized form to extract available data. We contacted all authors of selected studies and asked to provide additional data when not available in the relevant publication. Unpublished data were obtained by all authors responding positively to our request.4–9,11,12 Main outcome variables were symptomatic intracerebral hemorrhage (sICH) for safety11 and recanalization rates for the signal of efficacy.18 We also evaluated the impact of UET on functional independence (defined as a modified Rankin Scale of 0 to 1)10 at 3 months after stroke onset in studies in which data were available to derive power calculations for a randomized phase III trial. We used the absolute numbers of these events as determined and reported by the investigators. Because some discrepancy between definitions of sICH and recanalization are expected between the studies, we limited these events to reported intracranial bleeding with neurological worsening (defined as an increase in the National Institutes of Health Stroke Scale score of ≥4 points in all selected studies) and complete recanalization on ultrasound or CT angiography or MR angiography.

Statistical Analyses

Statistical analyses were performed by 2 independent authors who were not involved in data collection (M.S and T.S.). First, the rates of sICH, complete recanalization, and functional independence were computed across the different ultrasound modalities used in the different studies of UET (tPA + TCD ≥ μS, tPA + TCCD ≥ μS, and tPA + LFUS) and in the subgroup of patients treated with intravenous thrombolysis (tPA alone). The adjusted Wald method, which provides the best coverage for binomial CI when samples are less than n = 150, was used for computation of 95% CI.20 Statistical comparisons between groups were performed using the χ² test and Fisher exact test as appropriate. The OR for sICH, complete recanalization, and functional independence at 3 months were calculated in all individual studies with available data comparing UET to systemic thrombolysis. The OR from separate studies were combined by fixed-effects meta-analysis according to the Mantel-Haenszel method, which is also valid for paired OR.21 Heterogeneity between studies was assessed by the Breslow-Day χ² test and I² statistic.22 The I² statistic describes the percentage of total variation across studies that is attributable to heterogeneity rather than chance. Compared with the classical Breslow-Day χ² test, its interpretation is more intuitive and the value does not depend on the number of studies. There is no simple categorization of values of I², although values > 75% are usually considered as meaning high heterogeneity.22

Results

A total of 6 randomized (224 patients)5,7,8,10–12 and 3 nonrandomized (192 patients)4,6,9 studies were identified. Eight of these studies were published as original articles in peer-reviewed journals,4–9,11,12 whereas 1 was presented at the 2007 International Stroke Conference and was published as an abstract in a peer-reviewed journal.10 We contacted corresponding authors of all 9 studies. Authors of 7 studies provided all requested data,4–9,11,12 1 provided partial data,10 and 1 did not reply.12 A total of 4 studies investigated UET using TCD,4–7 another 4 used TCCD8–11 as the preferred ultrasound modality, whereas the remaining 1 evaluated the impact of LFUS on sonothrombolysis.12 The design, the number of treated patients, the ultrasound modalities that were used, and the baseline characteristics of the patients included in these 9 UET studies are summarized in Table 1. The study of LFUS was a phase II, randomized, controlled trial that included acute stroke patients presenting within a 6-hour time window from stroke onset with a baseline stroke severity of >4 points on admission NIH Stroke Scale Score and with evidence of a proximal intracranial occlusion on brain MR angiography. Patients were randomized to either tPA plus LFUS (active treatment arm) or tPA alone (control treatment arm).12

All different definitions used for the assessment of UET safety (sICH) are presented in Table 2. The study of LFUS was terminated on the basis of the unacceptably high rate (36%) of sICH documented in the active treatment arm. The rates of complete recanalization and functional independence across individual studies and the different neuroimaging modalities used for the evaluation of complete recanalization are shown in Table 2.

The pooled rates of sICH, complete recanalization, and functional independence across the 3 different ultrasound modalities used in trials of UET (TCD ≥ μS, TCCD ≥ μS, LFUS) and in patients treated only with intravenous thrombolysis are presented in Table 3. The rates of sICH in randomized studies were as follows (Table 3): TCD 3.8% (95% CI, 0%–11.2%; n = 78), TCCD 11.1% (95% CI, 0%–28.9%; n = 27), LFUS 35.7% (95% CI, 16.2%–61.4%;
The rate of complete recanalization in patients treated with UET reached complete or partial recanalization.12 The rate of complete recanalization in the study of LFUS was higher compared to tPA alone (2.9% vs 0.0%–8.4%; n = 105). The rate of sICH in the study of LFUS was higher compared to tPA and tPA + TCD ± μS both in randomized studies (P < 0.001 and P = 0.002, respectively) and in combined trials (P < 0.001 and P < 0.001, respectively). The rate of sICH in combined trials of UET using TCCD was lower when compared to the LFUS trial (P = 0.014). There was significant heterogeneity between the 3 ultrasound modalities both in randomized (P = 0.002 by Fisher exact test) and combined trials (P < 0.001 by χ² test).

The following rates of complete recanalization were documented in randomized studies: TCD, 37.2% (95% CI, 26.5%–47.9%; n = 78); TCCD, 26.9% (95% CI, 9.9%–44.0%; n = 27); and tPA alone, 17.2% (95% CI, 9.5%–24.9%; n = 105). The rates of complete recanalization were not available in trials of LFUS, whereas the authors reported 29% of patients treated with UET using TCCD reached complete or partial recanalization.12 The rate of complete recanalization in patients treated with μS-potentiated sonothrombolysis (tPA + TCD/TCCD + μS) was higher (53.6%; 95% CI, 42.0%–64.9%) than in patients treated with the combination of tPA and TCD/TCCD (36.6%; 95% CI, 30.1%–43.7%; P = 0.014).

Given the fact that the only study of LFUS was discontinued for safety reasons (exceedingly high rate of sICH), and that in our primary analysis there was significant heterogeneity across studies assessing the safety of different ultrasound modalities in the field of UET, we performed a subgroup analysis based on the type of ultrasound including only trials using high-frequency ultrasound (HFUS, TCD, or TCCD) to derive power calculations regarding safety and efficacy that may be used in future phase III trials. Two trials using UET in their control arm (compared to μS-potential UET in the active arm) were not included in this analysis.7,9 There was no significant difference between high-frequency UET and intravenous thrombolysis with regard to the risk of sICH (Figure 1). The pooled analysis (n = 294) showed an increased risk of sICH with high-frequency sonothrombolysis compared to intravenous tPA (pooled OR, 1.26; 95% CI, 0.44–3.60; P = 0.67) without significant heterogeneity across studies (P = 0.48 by Breslow-Day test). There was a higher likelihood of complete recanalization with UET using TCD ± μS compared to tPA alone (OR, 3.70; 95% CI, 1.95–7.00; P < 0.0001; Figure 2). In sonothrombolysis trials using TCCD there was no significant difference in recanalization rate when compared to systemic thrombolysis (OR, 1.17; 95% CI, 0.32–4.34; P = 0.81; Figure 2). The pooled analysis of TCD and TCCD showed that UET was associated with a higher likelihood of complete recanalization (pooled OR, 2.99; 95% CI, 1.70–5.25; P = 0.0001; Figure 2) when compared to tPA alone without significant heterogeneity across studies (P = 0.45 by Breslow-Day test). We also documented a higher likelihood of 3-month functional independence with UET using TCD ± μS compared to tPA alone (OR, 1.88; 95% CI, 1.03–3.43; P = 0.04; Figure 3). A similar trend was documented in sonothrombolysis trials using TCCD (OR, 6.34; 95% CI, 0.64–58.01; P = 0.10; Figure 3). The pooled analysis of TCD and TCCD showed that UET was associated with a higher likelihood of functional independence (pooled OR, 2.09; 95% CI, 1.17–3.71; P = 0.01; Figure 3) when compared to tPA alone without significant heterogeneity across studies (P = 0.71 by Breslow-Day test).

Furthermore, we performed an additional subgroup analysis using data only from randomized studies of high-
<table>
<thead>
<tr>
<th>Study</th>
<th>Modality</th>
<th>Intervention, Active vs Control</th>
<th>siCH, n/N</th>
<th>Definition of siCH</th>
<th>Complete Recanalization, n/N*</th>
<th>Assessment of Recanalization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alexandrov et al (2004)</td>
<td>TCD</td>
<td>TCD + tPA</td>
<td>3/55 (6%)</td>
<td>Intracerebral hemorrhage on CT within 72 hours from symptom onset associated with an increase in NIHSS score of ≥4 points</td>
<td>20/55 (36%)</td>
<td>End of TPA infusion (1 hour after tPA bolus)/TCD</td>
</tr>
<tr>
<td>Alexandrov et al (2004)</td>
<td>TCD</td>
<td>TCD + tPA vs tPA</td>
<td>3/63 (5%) 3/63 (5%)</td>
<td>Intracerebral hemorrhage on CT within 72 hours from symptom onset associated with an increase in NIHSS score of ≥4 points</td>
<td>24/63 (38%) vs 8/63 (13%)</td>
<td>2 hours after TPA bolus/TCD</td>
</tr>
<tr>
<td>Molina et al (2006)</td>
<td>TCD</td>
<td>TCD + tPA + µS vs TCD + tPA</td>
<td>1/38 (3%) vs 1/37 (3%) vs 2/36 (6%)</td>
<td>Intracerebral hemorrhage on CT within 72 hours from symptom onset associated with an increase in NIHSS score of ≥4 points</td>
<td>21/38 (55.2%) vs 15/37 (40.5%) vs 8/36 (22.2%)</td>
<td>2 hours after TPA bolus/TCD</td>
</tr>
<tr>
<td>Alexandrov et al (2008)</td>
<td>TCD</td>
<td>TCD + tPA vs US + tPA</td>
<td>0/12 (0%) vs 0/3 (0%)</td>
<td>Intracerebral hemorrhage on CT within 72 hours from symptom onset associated with an increase in NIHSS score of ≥4 points</td>
<td>5/12 (42%) vs 0/3 (0%)</td>
<td>2 hours after TPA-bolus/TCD</td>
</tr>
<tr>
<td>Eggers et al (2003)</td>
<td>TCCD</td>
<td>TCCD + tPA</td>
<td>2/11 (18%) vs 0/14 (0%)</td>
<td>Intracerebral hemorrhage on CT within 72 hours from symptom onset associated with an increase in NIHSS score of ≥4 points</td>
<td>3/11 (27%) vs 3/14 (21%) partial recanalization 2/11 (18 %) vs 0 (0%)</td>
<td>1 hour after TPA bolus/TCCD</td>
</tr>
<tr>
<td>Perren et al (2008)</td>
<td>TCCD</td>
<td>TCCD + tPA + µS vs TCCD + tPA</td>
<td>1/11 (9%) vs 1/15 (7%)</td>
<td>Intracerebral hemorrhage on CT within 72 hours from symptom onset associated with an increase in NIHSS score of ≥4 points</td>
<td>7/11 (64%) vs 8/15 (53%)</td>
<td>End of TPA infusion (1 hour after tPA bolus)/TCCD</td>
</tr>
<tr>
<td>Larrue et al (2007)</td>
<td>TCCD</td>
<td>TCCD + tPA + µS vs tPA</td>
<td>0/9 (0%) vs 0/11 (0%)</td>
<td>Any hemorrhage on MRI within 24–36 hours from symptom onset associated with an increase in NIHSS score of ≥4 points</td>
<td>4/8 (50%) vs 5/10 (50%)</td>
<td>4–8 hours after symptom onset/CTA or MRA</td>
</tr>
<tr>
<td>Eggers et al (2008)</td>
<td>TCCD</td>
<td>TCCD + tPA</td>
<td>1/7 (14%) vs 0/5 (0%)</td>
<td>Intracerebral hemorrhage on CT within 72 hours from symptom onset associated with an increase in NIHSS score of ≥4 points</td>
<td>Complete recanalization 0/7 (0%) vs 0/5 (0%) partial recanalization 4/7 (57%) vs 2/5 (40%)</td>
<td>1 hour after TPA bolus/TCCD</td>
</tr>
<tr>
<td>Daffertshofer et al (2005)</td>
<td>LFTUS</td>
<td>LFTUS + tPA vs tPA</td>
<td>5/14 (36%) vs 0/12 (0%)</td>
<td>Any hemorrhage on CT within 3 days to 7 days from symptom onset associated with an increase in NIHSS score of ≥4 points</td>
<td>Complete or partial recanalization 4/14 (29%) vs 6/12 (50%)</td>
<td>US†</td>
</tr>
</tbody>
</table>

*The numbers of patients correspond to the column of the study design.
†Initial assessments were performed with brain MRI but were also confirmed using brain CT in all cases.
‡The authors of the study do not specify which ultrasound modality was used for the assessment of recanalization.
frequency UET. Sonothrombolysis with TCD (1 randomized trial) was associated with similar safety (OR, 1.00; 95% CI, 0.19–5.15) and higher likelihood of complete recanalization (OR, 4.23; 95% CI, 1.72–10.40) when compared to intravenous thrombolysis (OR, 1.17; 95% CI, 0.32–4.34) in comparison to intravenous tPA. Sonothrombolysis with TCCD (3 trials) was not associated with a higher likelihood of sICH (OR, 4.73; 95% CI, 0.49–45.89) and complete recanalization (OR, 1.17; 95% CI, 0.32–4.34) in comparison to intravenous tPA. Finally, we reanalyzed our data set after including the recently (July 2009) published results of Transcranial Ultrasound in Clinical SONothrombolysis (TUCSON) trial, a phase II, international, multicenter, randomized, controlled trial evaluating the safety and efficacy of μS-potentiated sonothrombolysis in comparison to tPA μS alone. This additional pooled analysis (n=329) confirmed that was was no excess in the risk of sICH with UET using HFUS compared to intravenous tPA (pooled OR, 1.50; 95% CI, 0.57–3.98; P=0.41) without significant heterogeneity across studies (P=0.54 by Breslow-Day test). Moreover, sonothrombolysis with HFUS was associated with a higher likelihood of complete recanalization (pooled OR, 2.94; 95% CI, 1.74–4.97; P<0.001) when compared to tPA alone without significant heterogeneity across studies (P=0.62 by Breslow-Day test). Interestingly, we also documented that.

### Table 3. sICH, Complete Recanalization, and Functional Independence Rates Among Different US Modalities in Randomized and Nonrandomized Studies of Ultrasound-Enhanced Thrombolysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>tPA</th>
<th>tPA+TCD+μS§</th>
<th>tPA+TCCD+μS¶</th>
<th>tPA+LFUS</th>
</tr>
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<tr>
<td><strong>Randomized studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N of patients</td>
<td>105</td>
<td>78</td>
<td>27</td>
<td>14</td>
</tr>
<tr>
<td>sICH (95% CI)*</td>
<td>2.9% (95% CI, 0.0–8.4%)</td>
<td>3.8% (95% CI, 0.0–11.2%)</td>
<td>11.1% (95% CI, 0.0–28.9%)</td>
<td>35.7% (95% CI, 16.2%–61.4%)</td>
</tr>
<tr>
<td>Complete recanalization (95% CI)</td>
<td>17.2% (95% CI, 9.5%–24.9%)</td>
<td>37.2% (95% CI, 26.5%–47.9%)</td>
<td>26.9% (95% CI, 9.9%–44.0%)</td>
<td>NA</td>
</tr>
<tr>
<td>Functional independence (mRS 0–1)*</td>
<td>20.6% (95% CI, 12.6%–31.8%)</td>
<td>40.7% (95% CI, 29.1%–53.4%)</td>
<td>22.2% (95% CI, 6.4%–47.6%)</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Randomized and nonrandomized studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N of patients</td>
<td>141</td>
<td>208</td>
<td>53</td>
<td>14</td>
</tr>
<tr>
<td>sICH (95% CI)*</td>
<td>3.5% (95% CI, 0.0–8.3%)</td>
<td>3.8% (95% CI, 0.7–7.5%)</td>
<td>9.7% (95% CI, 0.0–20.7%)</td>
<td>35.7% (95% CI, 16.2%–61.4%)</td>
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<tr>
<td>Complete recanalization (95% CI)</td>
<td>18.8% (95% CI, 12.0%–25.5%)</td>
<td>40.9% (95% CI, 34.2%–47.6%)</td>
<td>42.3% (95% CI, 28.9%–55.7%)</td>
<td>NA</td>
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<tr>
<td>Functional independence (mRS 0–1)*</td>
<td>24.0% (95% CI, 16.8%–33.1%)</td>
<td>40.3% (95% CI, 32.4%–48.8%)</td>
<td>22.2% (95% CI, 6.4%–47.6%)</td>
<td>NA</td>
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</tbody>
</table>

mRS indicates modified Rankin Scale Score.

*Adjusted Wald.
†P=0.002 vs TCD (Fisher exact test); ‡P=0.001 vs tPA (Fisher exact test); †P=0.097 (Fisher exact test). There was significant heterogeneity between the studies using different ultrasound modalities (P=0.002 by Fisher exact test).
§All patients randomized in the study of Alexandrov et al (2008) were included in this group (tPA+TCD+μS) because patients were randomized to receive either tPA+TCD+μS or tPA+TCD.
¶All patients included in the study of Perren et al (2008) were included in this group (tPA+TCCD+μS) because patients were treated either with tPA+TCCD+μS or tPA+TCCD.

Figure 1. Comparison of sonothrombolysis (using TCD or TCCD) with intravenous thrombolysis (tPA alone) for the risk of sICH. The OR were calculated using data from 5 studies. Data from 1 study without control subjects and 2 trials comparing the safety of microsphere-potentiated sonothrombolysis (tPA+TCD/TCCD+μS) against sonothrombolysis (tPA+TCD/TCCD) were not included in the analyses. Heterogeneity: the probability value corresponds to the Breslow-Day test.
higher rates of complete recanalization were achieved in patients treated with the combination of tPA, HFUS, and $\mu$S (54.3%; 50/92) in comparison to the combination of tPA and HFUS (36.6%; 70/191; $P < 0.005$).

**Discussion**

Our analysis showed the safety of sonothrombolysis with HFUS. In contrast the rate of sICH was higher in the single UET trial using LFUS compared to sonothrombolysis with HFUS or systemic thrombolysis alone. In addition, our findings indicate that UET with HFUS coupled with or without $\mu$S is associated with a nearly 3-fold increased likelihood of complete recanalization and 2-fold higher likelihood of functional independence at 3 months.

In experimental models using lower frequencies (20 kHz–MHz), tPA-mediated clot degradation was as much as 50% more efficient when ultrasound was added. Although the underlying mechanisms are not fully understood, it is speculated that ultrasound accelerates enzymatic fibrinolysis primarily through nonthermal mechanisms by increasing transport of drug molecules into the clot. Mechanical effects of ultrasound such as radiation force and acoustic cavitation also have been implicated in UET. Consequently, it has been postulated that the use of therapeutic LFUS in combination with intravenous thrombolytic therapy might be a feasible, safe, and potentially effective acute stroke treatment option. This hypothesis was tested in the TRanscranial low-frequency Ultrasound-Mediated thrombolysis in Brain Ischemia (TRUMBI) trial, a multicenter, randomized, controlled

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Sonothrombolysis + tPA Events</th>
<th>tPA alone Events</th>
<th>Weight</th>
<th>Odds Ratio MH, Fixed, 95% CI</th>
<th>Odds Ratio MH, Fixed, 95% CI</th>
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<tbody>
<tr>
<td>1.1 Sonothrombolysis TCD</td>
<td>Alexanderov AV 2004 22</td>
<td>53</td>
<td>14</td>
<td>49</td>
<td>51.3% 1.77 [0.78, 4.05]</td>
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<tr>
<td></td>
<td>Molina 2006 30</td>
<td>75</td>
<td>9</td>
<td>36</td>
<td>44.0% 2.80 [0.93, 8.94]</td>
</tr>
<tr>
<td></td>
<td>Subtotal (95% CI) 120</td>
<td>85</td>
<td>9</td>
<td>36</td>
<td>44.0% 2.80 [0.93, 8.94]</td>
</tr>
<tr>
<td></td>
<td>Total events 52</td>
<td>23</td>
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<tr>
<td></td>
<td>Heterogeneity: $ Chi^2 = 0.04, df = 1 (P = 0.85), I^2 = 0%</td>
<td>Test for overall effect: $ Z = 2.05 ( P = 0.04)</td>
<td></td>
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</tr>
<tr>
<td>1.2 Sonothrombolysis TCCD</td>
<td>Eggers 2003 3</td>
<td>11</td>
<td>0</td>
<td>14</td>
<td>1.9% 1.12 [0.55, 2.60]</td>
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<tr>
<td></td>
<td>Eggers 2006 1</td>
<td>7</td>
<td>0</td>
<td>5</td>
<td>2.2% 2.54 [0.93, 7.57]</td>
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<tr>
<td></td>
<td>Subtotal (95% CI) 4</td>
<td>19</td>
<td>2</td>
<td>17</td>
<td>4.2% 6.34 [0.69, 50.0]</td>
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<tr>
<td></td>
<td>Total events 0</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>Heterogeneity: $ Chi^2 = 0.44, df = 1 (P = 0.51), I^2 = 0%</td>
<td>Test for overall effect: $ Z = 1.63 ( P = 0.10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (95% CI) 145</td>
<td>104</td>
<td>100.0%</td>
<td>2.09 [1.17, 3.71]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total events 56</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Heterogeneity: $ Chi^2 = 1.40, df = 3 (P = 0.71), I^2 = 0%</td>
<td>Test for overall effect: $ Z = 2.50 (P = 0.01)</td>
<td></td>
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</tbody>
</table>

Figure 2. Comparison of sonothrombolysis (using TCD or TCCD) with intravenous thrombolysis (tPA alone) for the likelihood of complete recanalization. OR were calculated using data from 5 studies. Data from 1 study without control subjects and 2 trials comparing the safety of microsphere-potentiated sonothrombolysis (tPA + TCD/TCCD + $\mu$S) against sonothrombolysis (tPA + TCD/TCCD) were not included in the analyses. Heterogeneity: the probability value corresponds to the Breslow-Day test.

Figure 3. Comparison of sonothrombolysis (using TCD or TCCD) with intravenous thrombolysis (tPA alone) for the likelihood of functional independence (defined as a modified Rankin Scale of 0 to 1). OR were calculated using data from 4 studies. Data from 1 study without control subjects and 2 trials comparing the safety of microsphere-potentiated sonothrombolysis (tPA + TCD/TCCD + $\mu$S) against sonothrombolysis (tPA + TCD/TCCD) were not included in the analyses. Also, the functional outcome at 3 months was unavailable in 1 study that was also excluded from the present analyses. Heterogeneity: the probability value corresponds to the Breslow-Day test.
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Opening of the blood–brain barrier.2,3 The recent findings of sound. The occurrence of subarachnoid hemorrhage particularly suggest differential bioeffects of LFUS and diagnostic ultrasound. The occurrence of subarachnoid hemorrhage particularly underlines the hypothesis that LFUS may have some mechanical disrupting small vessels or may be causing vasodilation and opening of the blood–brain barrier.2,3 The recent findings of Reinhard et al27 showing that abnormal permeability of the human blood–brain barrier can be induced by wide-field low-frequency insonation are in line with the former hypothesis and also indicate that the observed excessive bleeding rate with low-frequency sonothrombolysis might be attributable to primary blood–brain barrier disruption by ultrasound.

The association of UET with nearly 3-fold higher likelihood of complete recanalization compared to intravenous tPA is particularly encouraging. Clinical improvement and functional independence after stroke usually occur after arterial recanalization. A recent meta-analysis showed that recanalization is associated with a 4- to 5-fold increase in the odds of a good final functional outcome and a 4- to 5-fold reduction in the odds of death.18 These results lend strong support to the hypothesis that recanalization is the mechanism of how systemic tPA increases the likelihood of recovery from stroke and the use of recanalization as a surrogate end point in phase II trials of reperfusion agents in acute ischemic stroke. The signal of efficacy in terms of recanalization with sonothrombolysis at diagnostic ultrasound frequencies also led to higher likelihood of functional independence in our secondary analyses. On the basis of the rates of functional independence at 3 months documented in the pooled analysis (38% in the combined TCD and TCCD group [56/146] and 22% in the intravenous tPA group [23/104]), 170 patients per group will be needed for such a study to be able to demonstrate the effect of thrombolysis enhanced by diagnostic frequency ultrasonography on the outcome of stroke, with a 2-sided alpha level of 0.05 and 90% power.

Certain potential limitations need to be addressed. First, the limited number of patients included, particularly in sonothrombolysis studies using TCCD, should be acknowledged because the limited power may account for the non-significant trend toward higher functional independence and recanalization rates with this ultrasound modality. Second, the limited power was the main reason that we decided to analyze together studies of UET with and without the addition of μS. Moreover, the higher rate of recanalization documented in the combined group of tPA, HFUS, and μS in comparison to tPA with or without ultrasound should be interpreted with caution given the small sample size even after the addition of TUCSON data. Third, different time points were used for the assessment of recanalization across different studies, whereas the duration of insonation varied between 60 and 120 minutes and may account for potential disparities in the documented recanalization rates. Additionally, one of the randomized studies using TCCD plus tPA vs tPA included only subjects with proximal middle cerebral main stem occlusion without residual flow.11 Given the fact that in this situation recanalization is more difficult to achieve than in branch occlusions, the lower rate of complete recanalization using TCCD compared to TCD does not necessarily mean that TCCD is less powerful than TCD. Interestingly, the rate of partial recanalization in the former study was higher in the group receiving sonothrombolysis compared to the group treated with intravenous tPA alone.11 Finally, publication bias cannot be excluded because certain negative trials may not have been published even in form of an abstract and therefore were not included in the present meta-analysis.

Conclusion
In conclusion, sonothrombolysis with HFUS appears to be safe, leading to higher rates of complete recanalization when compared to systemic thrombolysis. The present safety and signal-of-efficacy data should be taken into account in the design of randomized controlled trials evaluating UET.

Disclosures
Dr Tsivgoulis, Dr Ribo, Dr Rubiera, Dr Saqqur, Dr Molina, and Dr Alexandrov have participated in CLOTBUST or TUCSON or analyzed and presented data on behalf of CLOTBUST or TUCSON investigators.

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Safety and Efficacy of Ultrasound-Enhanced Thrombolysis: A Comprehensive Review and Meta-Analysis of Randomized and Nonrandomized Studies
Georgios Tsvigoulis, Jürgen Eggers, Marc Ribo, Fabienne Perren, Maher Saqqur, Marta Rubiera, Theodoros N. Sergentanis, Konstantinos Vadikolias, Vincent Larrue, Carlos A. Molina and Andrei V. Alexandrov

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