Uric Acid Therapy Improves Clinical Outcome in Women With Acute Ischemic Stroke

Laura Llull, MD; Carlos Laredo, MSc; Arturo Renú, MD; Belén Pérez, PhD; Elisabet Vila, PhD; Víctor Obach, MD; Xabier Urra, MD, PhD; Anna Planas, PhD; Sergio Amaro, MD, PhD; Ángel Chamorro, MD, PhD

Background and Purpose—It is unknown whether women and men with acute ischemic stroke respond similar to an antioxidant regimen administered in combination with thrombolysis. Here, we investigated the independent effect of sex on the response to uric acid (UA) therapy in patients with acute stroke treated with alteplase.

Methods—In the Efficacy Study of Combined Treatment With Uric Acid and rtPA in Acute Ischemic Stroke (URICO-ICTUS) trial, 206 women and 205 men were randomized to UA 1000 mg or placebo. In this reanalysis of the trial, the primary outcome was the rate of excellent outcome at 90 days (modified Rankin Scale, 0–1, or 2, if premorbid score of 2) in women and men using regression models adjusted for confounders associated with sex. The interaction of UA levels by treatment on infarct growth was assessed in selected patients.

Results—Excellent outcome occurred in 47 of 111 (42%) women treated with UA, and 28 of 95 (29%) treated with placebo, and in 36 of 100 (36%) men treated with UA and 38 of 105 (34%) treated with placebo. Treatment and sex interacted significantly with excellent outcome (P=0.045). Thus, UA therapy doubled the effect of placebo to attain an excellent outcome in women (odd ratio [95% confidence interval], 2.088 [1.050–4.150]; P=0.036), but not in men (odd ratio [95% confidence interval], 0.999 [0.516–1.934]; P=0.997). The interactions between treatment and serum UA levels (P<0.001) or allantoin/UA ratio (P<0.001) on infarct growth were significant only in women.

Conclusions—In women with acute ischemic stroke treated with alteplase, the administration of UA reduced infarct growth in selected patients and was better than placebo to reach excellent outcome.

Clinical Trial Registration—URL: https://clinicaltrials.gov. Unique identifier: NCT00860366. (Stroke. 2015;46:2162-2167. DOI: 10.1161/STROKEAHA.115.009960.)

Key Words: allantoin ■ oxidative stress ■ sex ■ stroke ■ uric acid

The ischemic brain is particularly vulnerable to oxidative stress as the result of its high consumption of oxygen, its rich content of iron and unsaturated lipids, and its low antioxidant capacity.1 Uric acid (UA) is an end-product of the metabolism of purines and a potent antioxidant compound.2 Its multiple effects include scavenging of hydroxyl radicals, hydrogen peroxide, and peroxynitrite, suppression of the Fenton reaction, and limitation of lipid peroxidation and free radical induced damage to DNA.3,4 Previous studies showed that UA protected cultured neurons against excitotoxic death,5 improved the functional outcome, and reduced the amount of brain damage in experimental models of intraluminal,6 or thromboembolic transient brain ischemia in male rodents.6 Of note, UA formation in humans is accompanied by the emergence of free radicals,7,8 which may degrade the molecule to allantoin through nonenzymatic mechanisms.9 Thus, the resulting allantoin/UA (AL/UA) ratio is considered a reliable marker of the oxidative stress burden in patients with various conditions.10,11

Recently, a systematic review and meta-analysis of 8131 patients with acute ischemic stroke showed that the endogenous serum UA levels had a protective effect on neurological outcome as patients with good outcomes had a higher serum UA levels than those with poor outcome at follow-up.12 At the bedside, the Efficacy Study of Combined Treatment With Uric Acid and rtPA in Acute Ischemic Stroke (URICO-ICTUS) trial recently suggested that the addition of UA to thrombolytic therapy in patients with acute ischemic stroke improved the rate of excellent outcome compared with placebo in women, but not in men.13 However, the URICO-ICTUS trial did not evaluate whether the greater efficacy of UA therapy observed...
in women could have resulted from unreported confounders including the sex effect on stroke pathophysiology.14

In the early stages of the evaluation of neuroprotective therapies it is recommended to obtain evidence of the biological effect of the treatment under investigation.13 These biomarkers may include the effect of the drug in circulating levels of molecules, such as oxidative stress biomarkers, or in the longitudinal change in infarct volume between treatment groups.15 Infarct growth has been related to relevant clinical outcomes and may capture the effect of neuroprotective therapies, although further validation is required to be used as surrogate measure of clinical end points.16

Here, we reanalyzed the URICO-ICTUS data to test the independent effect of sex in the clinical response to UA therapy. Moreover, we analyzed the effect of therapy on circulating markers of oxidative stress and on infarct growth.

Methods
The URICO-ICTUS methodology has been described in detail previously.13,17 In short, the trial was approved by local institutional review boards, and written informed consent was obtained from the patients or from legally acceptable surrogates. URICO-ICTUS was overseen by a data safety monitoring board, and it was registered with ClinicalTrials.gov (NCT00860366).

URICO-ICTUS Primary Study Hypothesis and Patient Population
The primary hypothesis of the URICO-ICTUS trial was that treatment with UA 1000 mg would increase the rate of excellent outcome at 90 days compared with placebo in patients with acute ischemic stroke treated with alteplase within 4.5 hours of symptom onset. Excellent outcome was defined as a modified Rankin Scale (mRS) of 0 to 1, in patients with a premorbid mRS score <2, or a mRS of 2, in patients with a premorbid mRS score equal to 2. Additional eligibility criteria were age ≥18 years, baseline National Institutes of Health Stroke Scale (NIHSS) >6 and <25, and premorbid mRS of <2. Exclusion criteria were history of gouty arthritis or nephropathy, recent intake of allopurinol or lithium, or serum creatinine levels >1.5 mg/dL.

Study Design and Conduct
URICO-ICTUS was a multicenter, double-blind, phase 2b/3 trial, where 421 patients were randomly allocated (1:1) to receive UA or placebo infused intravenously in 90 minutes during the infusion of alteplase. Of those, 411 were finally included in the target population of the study. Six patients with stroke mimics and 4 patients who did not start the experimental medication were excluded adhering to recommendations of the International Conference on Harmonisation Topic E9 (CPMP/ICH/363/96), International Conference on Harmonisation Topic E9 guidelines.14 The study patients had a non–contrast computed tomography at baseline and at 36 hours and were admitted into stroke dedicated units. The vital signs and blood pressure were monitored at least for 24 hours, the neurological status was assessed with the NIHSS every 4 to 8 hours during the days of hospitalization, and the functional outcome was assessed with the mRS at 90 days by certified stroke neurologists. The qualifying stroke subtype was defined following the Trial of Org 10 172 in Acute Stroke Treatment criteria as large-artery atherosclerosis, cardioembolism, small-vessel occlusion, stroke of other determined cause, and stroke of undetermined cause.19 Vascular risk factors were defined according to usual definitions, as previously reported.15

Efficacy Outcomes
The primary outcome measure of the current reanalysis of the URICO-ICTUS data was the rate of excellent outcome at 90 days in separate analyses for women and men. Secondary analyses included the correlations in women and men between serum UA and AL/UA ratio with infarct growth as measured using multimodal brain imaging.

Advanced Brain Imaging and Serum Biomarkers
Multimodal brain imaging was performed in 46 participants admitted into 1 prespecified study site, and serial blood samples were collected in 43 of these patients (samples were not available in 3 subjects for technical reasons). The advanced brain imaging protocol included the assessment of the following parameters: (1) the Alberta Stroke Program Early CT Score (ASPECTS) on baseline non–contrast computed tomography;20 (2) the nonviable tissue volume on computed tomographic perfusion at the end of alteplase;21 (3) the infarct volume on diffusion-weighted imaging brain magnetic resonance imaging at 72 hours;22 (4) the arterial patency on computed tomographic angiography, MR angiography, or digital subtraction angiography at the end of thrombolysis, and at 72 hours; and (5) the collateral score on computed tomographic angiography (Methods in the online-only Data Supplement).23 Infarct growth was defined on brain imaging as the difference between 72-hour diffusion-weighted imaging infarct volume and baseline nonviable tissue volume on computed tomographic perfusion. Serum UA and allantoin were measured in blood samples collected before treatment onset, at 6 to 12 hours, at 48 hours, and at 90 days.24 The AL/UA ratio and the percent change of serum UA from baseline values were also calculated (Methods in the online-only Data Supplement). The ancillary imaging and biomarker data were analyzed by investigators blinded to clinical information.

Statistical Methods
The URICO-ICTUS target population was divided into women and men. Baseline demographic features, risk factors, baseline biological findings, stroke subtypes, neurological course, and radiological findings were compared between the 2 sex groups. Continuous variables were reported as mean±SD or median with interquartile ranges and were compared with the Student t test, 1-way ANOVA, ANCOVA, Mann–Whitney, or Kruskal–Wallis tests as appropriate. Correlations were assessed with Pearson or Spearman coefficients, and categorical variables were compared with the χ2 and Fisher exact tests. A treatment–sex interaction on the primary outcome was assessed in a model adjusted for treatment delay and the trial stratification factors (study site and baseline NIHSS score). Afterward, excellent outcome was estimated individually in women and men using separated binary logistic regression models adjusted for variables associated with sex and outcome in univariate analysis with a P value of <0.10. These variables were age, premorbid mRS, NIHSS score at randomization, stroke subtype, history of hypertension, dyslipidemia, coronary artery disease, smoking, alcohol intake, and creatinine on admission. The analyses were performed using SPSS Version 19.0 and the level of significance was established at the 0.05 level (2-sided).

Results
Main Characteristics of Women and Men in the URICO-ICTUS Trial
In this study, 206 women and 205 men had significant differences in demographics, risk factors, stroke subtypes, and clinical severity at baseline (Table 1). At 90 days, 75 (36.4%) women and 74 (36.1%) men achieved an excellent outcome (P=0.301).

UA Therapy Was More Effective in Women
Excellent outcome at 90 days was increased in women treated with UA compared with men treated with UA (Table 1). The interaction between treatment (UA or placebo) and sex had a significant effect on the rate of excellent outcome (P=0.045), indicating that the effect of therapy on outcome
Baseline biological findings

Table 1. Baseline Data, Clinical Course, and Primary Outcome in the URICO-ICTUS Target Population According to Sex (n=411 Patients)

<table>
<thead>
<tr>
<th></th>
<th>Females (n=206)</th>
<th>Males (n=205)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y, median (IQR)</td>
<td>79 (72–83)</td>
<td>72 (63–79)</td>
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<tr>
<td>Preadmission mRS, median (IQR)</td>
<td>0 (0–1)</td>
<td>0 (0–0)</td>
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<tr>
<td>Medical history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking (&lt;5 d cigarettes per last 6 mo), n (%)</td>
<td>20 (10)</td>
<td>51 (25)</td>
<td>0.000</td>
</tr>
<tr>
<td>Alcohol intake (&gt;60 g/d [male] or &gt;40 g/d [female]), n (%)</td>
<td>2 (1)</td>
<td>24 (12)</td>
<td>0.000</td>
</tr>
<tr>
<td>Hypertension (treated or &gt;140/90), n (%)</td>
<td>158 (77)</td>
<td>122 (60)</td>
<td>0.000</td>
</tr>
<tr>
<td>Diabetes mellitus (treated or &gt;110 mg/dL), n (%)</td>
<td>60 (29)</td>
<td>58 (28)</td>
<td>0.852</td>
</tr>
<tr>
<td>Dyslipidemia (treated or cholesterol &gt;220 mg/dL), n (%)</td>
<td>74 (36)</td>
<td>94 (46)</td>
<td>0.045</td>
</tr>
<tr>
<td>Atrial fibrillation, n (%)</td>
<td>60 (29)</td>
<td>30 (15)</td>
<td>0.000</td>
</tr>
<tr>
<td>Ischemic heart disease (MI or angina), n (%)</td>
<td>20 (10)</td>
<td>39 (19)</td>
<td>0.007</td>
</tr>
<tr>
<td>Peripheral vascular disease, n (%)</td>
<td>6 (3)</td>
<td>9 (4)</td>
<td>0.424</td>
</tr>
<tr>
<td>Previous stroke, n (%)</td>
<td>20 (10)</td>
<td>29 (14)</td>
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<tr>
<td>Baseline biological findings</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Mean blood pressure, mmHg, mean (SD)</td>
<td>129 (18)</td>
<td>126 (17)</td>
<td>0.105</td>
</tr>
<tr>
<td>Temperature, °C, median (IQR)</td>
<td>36 (36–37)</td>
<td>36 (36–37)</td>
<td>0.521</td>
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<tr>
<td>Glucose, mg/dL, median (IQR)</td>
<td>128 (111–161)</td>
<td>126 (108–150)</td>
<td>0.098</td>
</tr>
<tr>
<td>Creatinine, mg/dL, median (IQR)</td>
<td>0.8 (0.7–1.0)</td>
<td>1.0 (0.8–1.1)</td>
<td>0.000</td>
</tr>
<tr>
<td>Pre-rPA NIHSS score, median (IQR)</td>
<td>15 (9–19)</td>
<td>12 (9–17)</td>
<td>0.027</td>
</tr>
<tr>
<td>Screening NIHSS score, median (IQR)</td>
<td>15 (9–18)</td>
<td>12 (8–17)</td>
<td>0.026</td>
</tr>
<tr>
<td>Time to rPA infusion, min, median (IQR)</td>
<td>145 (100–180)</td>
<td>145 (105–182)</td>
<td>0.717</td>
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<tr>
<td>Time to uric acid or placebo, min, median (IQR)</td>
<td>180 (135–230)</td>
<td>180 (145–220)</td>
<td>0.997</td>
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<tr>
<td>Rescue endovascular treatment, n (%)</td>
<td>18 (9)</td>
<td>27 (13)</td>
<td>0.150</td>
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<td>TOAST classification</td>
<td></td>
<td></td>
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<tr>
<td>Atherothrombotic, n (%)</td>
<td>23 (11)</td>
<td>31 (15)</td>
<td></td>
</tr>
<tr>
<td>Cardioembolic, n (%)</td>
<td>98 (48)</td>
<td>84 (41)</td>
<td></td>
</tr>
<tr>
<td>Lacunar, n (%)</td>
<td>6 (3)</td>
<td>13 (6)</td>
<td></td>
</tr>
<tr>
<td>Undetermined cause, n (%)</td>
<td>77 (37)</td>
<td>67 (33)</td>
<td></td>
</tr>
<tr>
<td>Other cause, n (%)</td>
<td>2 (1)</td>
<td>19 (5)</td>
<td></td>
</tr>
</tbody>
</table>

Neurological course

Table 1. Continued

<table>
<thead>
<tr>
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<th>Females (n=206)</th>
<th>Males (n=205)</th>
<th>P Value</th>
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<td>NIHSS at day 5 or discharge, median (IQR)</td>
<td>11 (6–18)</td>
<td>9 (4–15)</td>
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<td>NIHSS at 24 h, median (IQR)</td>
<td>7 (2–16)</td>
<td>7 (2–14)</td>
<td>0.610</td>
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<td>NIHSS at 72 h, median (IQR)</td>
<td>4 (1–14)</td>
<td>5 (1–12)</td>
<td>0.869</td>
</tr>
<tr>
<td>NIHSS at day 5 or discharge, median (IQR)</td>
<td>3 (0–12)</td>
<td>4 (0–12)</td>
<td>0.679</td>
</tr>
</tbody>
</table>

(Continued)

Serum Biomarkers and Sex

UA and allantoin were highly correlated in serum at baseline (r=0.619; P<0.001), at 6 to 12 hours (r=0.769; P<0.001), at 48 hours (r=0.422; P=0.014), and at 90 days (r=0.681; P<0.001). Treatment and sex had a significant interaction with UA levels at 6 to 12 hours (P=0.006), serum allantoin at 6 to 12 hours (P=0.029) and at 48 hours (P=0.023); and the AL/UA ratio at 6 to 12 hours (P=0.034) and at 48 hours (P=0.028), respectively. The interactions remained significant after adjustment for the creatinine levels.

In untreated patients, serum UA declined at 6 to 12 hours (P=0.298), 48 hours (P=0.010), and 90 days (P=0.030) after stroke onset, and the serum UA levels were lower in women than in men (Figure 1A). After UA therapy both sex groups had increased serum UA levels at 6 to 12 hours (P<0.001; Figure 1B). Serum allantoin did not change over time in the placebo group and women had lower levels than men at 6 to 12 hours (P=0.040), and 48 hours (P=0.006), but not at 90 days (P=0.163; Figure 1C). Women and men showed a significant rise in allantoin levels at 6 to 12 hours (P<0.001), 48 hours (P<0.001), and at 90 days (P=0.012) of UA therapy (Figure 1D). The AL/UA ratio did not differ significantly between women and men in the placebo group, but women had a greater AL/UA ratio than men at 6 to 12 hours of UA therapy (P=0.021; Figure 2).

Infarct Growth and Sex

Advanced brain imaging was performed in a cohort of 46 patients (25 women and 21 men) that showed similar clinical differences according to sex. Therefore, multivariate predictor models were built to assess the effect of therapy in men and women separately. Thus, in discrete logistic regression models adjusted for variables associated with sex and outcome in univariate analysis, UA therapy was more effective than placebo in women (odd ratio [95% confidence interval], 2.088 [1.050–4.150]; P=0.036), but not in men (odd ratio [95% confidence interval], 0.999 [0.516–1.934]; P=0.997). The NIHSS score at study onset was the only additional independent factor associated with excellent outcome at 90 days in women (odd ratio [95% confidence interval], 0.844 [0.790–0.902]; P<0.001), and men (odd ratio [95% confidence interval], 0.821 [0.762–0.886]; P<0.001), in the multivariate models.
findings at baseline when compared with the whole population included in the URICO-ICTUS trial (data not shown). In this cohort, women and men also had similar brain imaging parameters at baseline and at follow-up (Table 2), and there was no significant interaction between age and sex with regard to infarct growth ($P=0.177$).

The interaction between treatment and sex with regard to infarct growth was not significant ($P=0.973$). However, UA therapy was better than placebo to reduce infarct growth in women (Mann–Whitney, $P=0.012$), and not in men (Mann–Whitney, $P=0.605$; Figure 3). Correspondingly, there was a significant interaction between treatment and UA levels on infarct growth in women ($P<0.001$), but not in men ($P=0.184$). Furthermore, infarct growth decreased in women with higher AL/UA ratio at 6 to 12 hours after UA treatment ($r=-0.849; P=0.004$), but this correlation was not significant in men (Pearson $r=-0.268; P=0.426$).

**Discussion**

The primary end point of this reanalysis of the URICO-ICTUS data showed that compared with placebo UA therapy doubled the rate of excellent outcome after acute ischemic stroke in women but not in men. Importantly, we identified a significant interaction between treatment (UA or placebo) and sex on the rate of excellent outcome and confirmed that this finding was also significant in multivariate models that accounted for the differences observed between women and men at study onset in demographics, risk factors, initial severity of stroke, and creatinine levels.

In the study, we did not find different rates of vessel recanalization after thrombolysis between women and men as previously suggested in some but not all cohorts. Furthermore, the effect of UA therapy on functional outcome was assessed in models adjusted for stroke subtype, age, initial severity of stroke, and for the variable effects of risk factors. Therefore, it is sensible to think that the clinical response to UA therapy was heightened in women compared with that in men and that the effect of the experimental drug was not biased by a sex-dependent susceptibility to thrombolytic therapy or by unbalanced effects of clinical variables.

The interaction between treatment and sex on infarct growth did not have sufficient statistical power but nevertheless the study found that infarct growth was decreased in women treated with UA, but not in men. In addition, UA therapy and the actual serum UA levels in treated patients had a significant interaction with infarct growth in women but not in men. Women, but not men, also showed reduced infarct growth...
women obtained greater clinical benefits after UA replenishment than men because they were in a greater need of antioxidants as the result of their lower constitutional levels of UA. We also believe that a lower UA-mediated antioxidant capacity in women could be a mechanism that might contribute to explain the more ominous course of stroke described in women than in men.14-31

It is increasingly recognized that stroke is a sexually dimorphic disease both experimentally and clinically, although these sex differences are still poorly understood.32 Experimental data suggest the existence of sex-dependent pathways in cell death after ischemic stroke.33 Namely, in males, cell death is triggered by poly (ADP-ribose) polymerase activation and nuclear translocation of apoptosis-inducing factor,34 whereas caspase activation is the major pathway involved in women after experimental brain ischemia.35 UA is a fundamental scavenger of peroxynitrite and it is well described that apoptosis is a typical consequence of low to moderate concentrations of peroxynitrite,4 whereas exposure of cells to higher concentrations of the oxidant has been associated with necrosis.35 In light of our findings, it can be speculated that sex-dependent mechanisms of cell death and their interaction with the antioxidant capacity were involved in the beneficial clinical response to UA therapy observed in women compared with men, but this possibility requires further study.

Limitations of the current study were the smaller cohort of patients who had multimodal brain imaging data to evaluate the course of the infarct, or serum samples collected for evaluation of the redox status using a larger battery of oxidative or antioxidant compounds other than UA and allantoin. However, it must be emphasized that UA is the most powerful extracellular antioxidant in human plasma,36 and the AL/UA ratio is considered a useful oxidative marker.9-11 Alternatively, other oxidative stress biomarkers have shown a more limited value.37 Strong points of the current study were its randomized, double blind design, and that careful measures were taken to minimize the risk of bias including the assessment of sex in multivariate models adjusted for relevant confounders. Although the ancillary tests were performed in a smaller cohort of individuals, the study showed that these patients were representative of the whole study cohort. In conclusion, this exploratory reanalysis of the URICO-ICTUS trial highlighted the clinical value of the administration of UA in women with acute ischemic stroke who received thrombolysis within 4.5 hours of clinical onset. Given the major practical implications that could be derived in the field of acute ischemic stroke, larger confirmatory studies will be urgently required to confirm these encouraging results.

**Sources of Funding**

The URICO-ICTUS trial was funded by the Institute of Health Carlos III (ISCIII) of the Spanish Ministry of Health (Grant No. EC07/90276), by the Spanish Ministry of Economy and Competence (RETICS-INVICTUS R012/0014), and by a private grant from Fundación Doctor Melchor Colet. The medication in the study was supplied by Grifols, Barcelona, Spain. We also thank the support of the Spanish Ministry of Economy and Competitiveness for grant to Dr Amaro (PI13/01268, funded as part of the Plan Nacional R+D+I and cofinanced by ISCIII-Subdirección General de Evaluación and by the Fondo Europeo de Desarrollo Regional).
Disclosures
None.

References
14. Luill et al
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Stroke. 2015;46:2162-2167; originally published online July 9, 2015;
doi: 10.1161/STROKEAHA.115.009960
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the
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Supplementary Methods

Liquid chromatography

For allantoin measurement samples were centrifuged (5 min; 12,000 g) and from the supernatant, 10 μl were injected into the HPLC system. Separation of allantoin was performed on a Synergi Hydro-RP C18 reversed-phase column (250mm ×4.6 mm I.D., 5 μm particle size) from Phenomenex (Torrance, CA, USA). Allantoin elution was at 4 minutes performed with potassium dihydrogen phosphate (pH 2.7; 10 mM): acetonitrile (85:15) and ultraviolet detection (235nm).

Advanced Brain imaging methods

The advanced brain imaging protocol was obtained at one predefined study site that reported the availability of the required imaging techniques during working hours. CTPerfusion maps were calculated by commercial software MIStar (Apollo Medical Imaging Technology, Melbourne, Australia). An absolute threshold of 2s was selected on the Delay Time map to obtain the Hypoperfusion Tissue volume on CTPerfusion and, inside this area, a relative threshold of 30% of the mean Cerebral Blood Flow value in the unaffected/contralateral hemisphere was used to obtain Non-Viable Tissue volumes. Tissue-At-Risk was obtained by subtracting the Non-Viable Tissue volume to the Hypoperfused Tissue volume. DWI lesion volumes were calculated by means of a semi-automated thresholding method to identify regions of interests with high DWI signal intensity (exceeding at least three standard deviations the intensity of the values in the contralateral hemisphere). Each 24 to 48 hours DWI was then co-registered to the corresponding CTPerfusion maps. Using Statistical Parametric Mapping (SPM8, Functional Imaging Laboratory, University College London, London, UK) the DWI image was automatically subjected to a sequence of two 3D registration procedures to match CTPerfusion maps using Time of Flight MRI as an intermediary. Once CTPerfusion and DWI were placed in register, images were analyzed pixel-wise to obtain the infarct volume change over time.

Arterial recanalization and assessment of collaterals

Full arterial recanalization indicated a TIMI 2 or 3 score on CT-angiography or MRangiography, and further classified as early onset if confirmed at the end of thrombolytic therapy, or late onset, if first confirmed at 72 hours. Patients with a brain infarct on MRI without arterial occlusion at baseline were also classified as early onset recanalization. Full arterial recanalization confirmed at seventy-two hours but not assessed at baseline was classified as uncertain onset and grouped with the late onset patients. Absence of recanalization defined a TIMI score of 0 or1 confirmed at seventytwo hours.

Collaterals were scored in CT-angiography according a validated grading system that ranged from 0 to 3 (0, absent collateral supply; 1, collateral supply filling <50%; 2, collateral supply filling >50% but <100% of the occluded arterial territory; and 3, 100% collateral supply).