Optimizing thrombolysis in acute ischaemic stroke
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APPENDIX A

A RANDOMISED CONTROLLED TRIAL OF ANTIPLATELET THERAPY IN COMBINATION WITH RT-PA THROMBOLYSIS IN ISCHAEMIC STROKE: RATIONALE AND DESIGN OF THE ARTIS TRIAL

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ABSTRACT

Background
Thrombolysis with intravenous rt-PA is currently the only approved acute therapy for ischaemic stroke. Reocclusion after initial recanalization occurs in up to 34% in patients treated with rt-PA, probably caused by platelet activation. In acute myocardial infarction, the combination of thrombolysis and antiplatelet therapy leads to a greater reduction of mortality compared to thrombolysis alone. In patients with acute ischaemic stroke, several studies showed that patients already on antiplatelet treatment prior to thrombolysis had an equal or even better outcome compared to patients without prior antiplatelet treatment, despite an increased risk of intracerebral haemorrhage. Based on the fear of intracerebral haemorrhage, current international guidelines recommend to postpone antiplatelet therapy until 24 hours after thrombolysis. Remarkably, prior use of antiplatelet therapy is not a contra-indication for thrombolysis. We hypothesize that antiplatelet therapy in combination with rt-PA thrombolysis will improve outcome by enhancing fibrinolysis and preventing reocclusion.

Methods
ARTIS is a randomised multicenter controlled trial with blind endpoint assessment. Our objective is to investigate whether immediate addition of aspirin to rt-PA thrombolysis improves functional outcome in ischaemic stroke. Patients with acute ischaemic stroke eligible for rt-PA thrombolysis are randomised to 300 mg aspirin within 1.5 hours after start of thrombolysis or standard care, in which antiplatelet therapy is started 24 hours after thrombolysis. Primary outcome is poor functional health at 3 months follow-up (modified Rankin scale score 3–6).

Discussion
This is the first clinical trial investigating the combination of rt-PA and aspirin by means of a simple and cheap adjustment of current antiplatelet regimen. We expect the net benefit of improved functional outcome will overcome the possible slightly increased risk of intracerebral haemorrhage.
BACKGROUND

Stroke is an important cause of death and acquired disability in industrial world. In the large majority of ischaemic strokes, cerebral arteries become occluded either by a cardiac embolus or by thrombus formation in atherosclerotic vessel walls. The process of thrombus formation is initiated by two separate but interacting mechanisms: fibrin formation and platelet activation. Current standard treatment in acute ischaemic stroke with intravenous recombinant tissue plasminogen activator (rt-PA) aims at breaking down the fibrin clot in order to restore recanalisation of the occluded artery. Rt-PA treatment results in an absolute 10% reduction of poor outcome compared to placebo. However, benefit from this treatment rapidly declines over time following symptom onset. The number needed to treat to have 1 patient with favourable outcome is 4.5 if treatment is started within 90 minutes after symptom onset as compared to controls, while this number increases to 14.1 if treatment is started between 3 to 4.5 hours after symptom onset.

Within this current time window, early recanalization is strongly associated with improved functional outcome and reduced mortality. Unfortunately, recanalization rates with intravenous rt-PA are only modest. In a pooled analysis of 14 intravenous thrombolysis studies, overal recanalization rate was 46%, partly due to reocclusion. In continuous transcranial Doppler monitoring studies, reocclusion occurred in 20-34% of rt-PA treated patients at a mean time of 65 minutes after start of treatment. Moreover, these studies showed that reocclusion accounted for two thirds of the observed clinical deterioration after initial improvement. A recent study confirmed the association between reocclusion and clinical deterioration and showed that early reocclusion is highly predictive of long-term poor outcome.

Reocclusion after initial recanalisation is probably initiated by increased platelet activation. Thrombolytic therapy strongly activates the coagulation cascade leading to formation of thrombin, a potent platelet activator. This haemostatic activation is maximal at 2 hours after initiation of rt-PA treatment. Exposure of the lipid core of a disintegrating thrombus also leads to increased platelet ag-
aggregation. These activated platelets induce secretion of plasminogen activator inhibitor-1, which has been shown to be the responsible factor in rt-PA resistance to lysis in platelet rich arterial thrombi.\textsuperscript{11}

Among all antiplatelet drugs, aspirin (acetylsalicylic acid) is the most widely used drug. Inhibition of platelet aggregation by aspirin is caused by the irreversible acetylation of cyclo-oxygenase 1 and inhibition of prostaglandin thromboxane A2. Aspirin has a rapid onset of action resulting in substantial elimination of activated platelets.

In myocardial infarction, large clinical trials have shown that adding aspirin to thrombolysis prevents reocclusion thereby improving outcome considerably. The results of the second International Study of Infarct Survival Trial (ISIS-2) showed that mortality was reduced by 42\% if patients were treated with streptokinase in combination with aspirin while mortality was reduced by only 25\% if patients were treated with streptokinase alone.\textsuperscript{12} Aspirin is therefore the standard adjunctive treatment in acute myocardial infarction.

In acute ischaemic stroke, the Multicenter Acute Stroke Trial-Italy duplicated the design of the ISIS 2 and found an absolute risk reduction of 12\% for disability in patients treated with the combination of streptokinase and aspirin as compared streptokinase alone. This overall net benefit overcame an excess mortality rate which was observed in the combination group. Symptomatic intracerebral haemorrhages (SICH) largely contributed to this increased mortality rate.\textsuperscript{13} Meta-analysis of the streptokinase trials confirmed a positive effect on functional outcome with concomitant use of aspirin that compensated for higher mortality rates.\textsuperscript{14} Besides the high dosage of streptokinase used in these trials, its non-fibrin-selectivity is nowadays generally held responsible for the high number of SICH observed in this study.

The addition of aspirin to rt-PA, which is a more fibrin-selective thrombolytic agent, has never been investigated prospectively. In the protocol of the Neurological Institute of Neurological Disorders and Stroke (NINDS) Trial the use of antiplatelet agents was postponed for 24 hours after rt-PA treatment to prevent possible bleeding complications.\textsuperscript{15} However, the protocol did allow enrolling pa-
patients already on antiplatelet drugs. Current guidelines adopted these trial criteria for fear of SICH.\textsuperscript{16}

Subgroup analysis of the NINDS trial reveals that patients with prior use of aspirin had a better outcome, with lower frequencies of clinical deterioration and the same SICH rate as compared to patients with no aspirin use.\textsuperscript{17} Regarding the association between clinical deterioration and the occurrence of vessel reocclusion in ischaemic stroke\textsuperscript{6,8} and the lower incidence of clinical deterioration in patients with antiplatelet pre-treatment,\textsuperscript{17} one might suggest that previous antiplatelet therapy prevents reocclusion. This hypothesis is supported by the observation from recent prospective cohort studies, which confirm this favourable outcome after thrombolysis in patients with prior use of antiplatelet drugs.\textsuperscript{18,19}

Based on all these observations we hypothesize that immediate addition of antiplatelet therapy to rt-PA in acute ischaemic stroke improves outcome by enhancing clot lysis and preventing reocclusion after initial recanalisation.

METHODS AND DESIGN

Study design and objective
The Antiplatelet therapy in combination with Recombinant t-PA Thrombolys in Ischaemic Stroke (ARTIS) trial is a multicenter, prospective open label, randomised controlled trial with blind endpoint assessment (PROBE design). We compare direct addition of 300 mg aspirin to intravenous rt-PA thrombolysis for ischaemic stroke versus standard thrombolysis care, in which antiplatelet drugs are usually administered 24 hours after rt-PA. All participating centres are experienced in thrombolytic treatment for acute stroke.

The primary objective of the ARTIS trial is to investigate whether the addition of aspirin to standard rt-PA thrombolysis reduces poor outcome in acute ischaemic stroke. Poor outcome is defined as death or dependency assessed by the modified Rankin scale (mRs, score 3–6) at 3 months follow-up.
Enrolment procedures
The study population consists of acute ischaemic stroke patients who are treated with intravenous (IV) thrombolysis with rt-PA. Patients $\geq 18$ years of age can be enrolled. Patients will be asked for written informed consent. The trial itself has no other firm exclusion criteria than those established by the judgment of the individual treating physician using local protocols for IV rt-PA treatment. When the patient has a diminished decision-making capacity as result of the stroke (e.g. aphasia), informed consent will be obtained from a representative of the patient. Exclusion of these patients would lead to a selective patient sample. Patients are also excluded if they have:

- known antiplatelet therapy in the previous 5 days (in case of uncertainty the patient may be included);
- known thrombocytopenia or thrombocyte count $< 100 \times 10^9/l$;
- known contra-indications to acetylsalicylic acid treatment;
- known anticogualant therapy in the previous 5 days;
- known legal incompetence of the patient prior to this stroke.

Randomisation
The randomisation procedure will be computer- and web based, using permuted blocks. Randomisation will be stratified by centre, age ($\leq 60$ years, $>60$ years), gender and the time between symptom onset and time of rt-PA bolus ($< 2$ hours, $2–3$ hours, $>3$ hours).

Intervention
In order to prevent delay of start of thrombolytic treatment, informed consent and randomisation procedures will be performed as soon as continuous infusion of rt-PA (0.9 mg/kg) has started after bolus administration (10% of total dose) Patients allocated to the active group will receive 300mg aspirin (Aspégic®) as lysine salt intravenous as bolus. Since there is a peak in platelet activation after 2 hours after initiation of rt-PA thrombolysis, aspirin will be administered within 1.5 hours after the rt-PA bolus. Patient and treating physician are not blinded for treatment allocation.
We choose to apply aspirin intravenously for two reasons. First, onset of action has to be as soon as possible as reocclusion starts to occur soon after rt-PA administration.\textsuperscript{6,7} Intravenous aspirin leads to faster platelet suppression than oral aspirin, which results in a widely varying uptake.\textsuperscript{20} Aspirin may be given simultaneous with the rt-PA continuous infusion, preferably through a different intravenous line. In case of only one intravenous access, rt-PA infusion has to be shortly interrupted in order to administer aspirin through this line with saline flushing before and afterwards. Second, intravenously administration enables patients having swallowing difficulties caused by their stroke to be included. Exclusion of this subgroup would make the trial prone to inclusion bias.

Investigational medicinal product
Aspirin intravenous is registered in the Netherlands as Aspégic\textsuperscript{®} (Sanofi-Aventis). Thrombocyte aggregation is irreversibly reduced by this calcium-ureum-salt, causing longer coagulations times. Aspirin use may lead to gastrointestinal reactions. However, due to the single use adverse reactions caused by the trial medication are expected to be limited.

Alteplase\textsuperscript{®} (Boehringer Ingelheim GmbH) is essential and important co-medicine in the ARTIS trial. Interaction of Aspégic\textsuperscript{®} with rt-PA is unknown, although rt-PA treatment might increase the risk of intracerebral haemorrhage in aspirin pre-treated stroke patients. The characteristics of rt-PA may therefore influence our results even though rt-PA itself is not under investigation.

Recommendations concerning rt-PA treatment
Patients will receive rt-PA treatment in both groups according to local protocols at participating centres. Recommendations of rt-PA treatment concerning hypertension and thrombocyte count are based on standard international guidelines.\textsuperscript{16} Blood pressure should not be lowered with medication prior to rt-PA treatment. If during rt-PA administration blood pressure rises above 180 mm Hg systolic or 105 mm Hg diastolic it is recommended to administer 10 mg labetalol intravenous within 1–2 minutes. This should be repeated every 10–20 minutes until blood pressure is below 180 mm Hg systolic or below 105 mm Hg diastolic.
150 mg labetalol is the maximum dose in 24 hours. During this treatment blood pressure should be measured every 15 minutes. If the blood pressure does not respond to labetalol, iv nitroprusside 0.5–10 μg/kg/minute should be added, with continuous blood pressure monitoring. In case the diastolic blood pressure is above 140 mmHg nitroprusside should be administered immediately as stated above. Thrombocyte count is not necessary before starting rt-PA treatment unless a patient is known with thrombocytopenia. Deviations from these recommendations are not regarded as protocol violations, but will be registered.

Concomitant medication and secondary prophylaxis
All medication used before the stroke may be continued, except anticoagulance. Standard secondary prophylaxis is recommended according to the following scheme:

- aspirin 300 mg - once/daily - 24 hours after rt-PA for 14 days
- aspirin 100 mg - once/daily - 14 days after rt-PA
- simvastatine 40 mg - once/daily - 0–24 hours after rt-PA
- dipyridamole 200 mg - twice/daily - 24 hours after rt-PA

Additional anti-diabetic or antihypertensive medication may be started as regarded appropriate by the treating physician.

Endpoints
The primary endpoint is poor functional health at 3 months defined as dependency or death (mRs score 3–6).

The secondary endpoints are:

- complications within 48 hours after randomisation including the occurrence of symptomatic intracranial haemorrhage (SICH) and serious systemic bleeding. SICH is defined as CT-documented haemorrhage and a clinical deterioration leading to 4 or more points increase on the National Institutes of Health Stroke Scale (NIHSS) as compared to the lowest score on the NIHSS since admission. Serious systemic bleeding is defined as a potentially life threatening bleeding which requires immediate medical intervention;
• neurological symptoms quantified by the NIHSS 7–10 days after randomisation or at discharge if the patient is discharged within 7 days;
• mortality at 3 months;
• disability at 3 months assessed by the AMC Linear Disability Scale;
• functional health at 3 months non-dichotomized (ordinal mRs);
• causes of poor outcome.

Data collection
At baseline following patient characteristics are collected at each participating site: age, sex, ethnicity, medical history, pre-stroke medication, pre-stroke mRs score, blood pressure, Glasgow Coma Scale (GCS), National Institutes of Health Stroke Scale (NIHSS), time of symptom onset, time of administration of rt-PA bolus and (if applicable) trial medication, thrombocyte count and coagulation-International Normalized Ratio. Baseline CT scans will be collected from participating centres and assessed blindly at the coordinating centre for hyperdens middle cerebral artery sign, early ischaemic changes and degree of leukoariosis by two independent blinded neuroradiologists.

At follow-up, neurological deficits are assessed by the NIHSS at 7–10 days or at discharge, if this is before 7 days. Clinical deterioration, defined as a 4 or more points increase on the NIHSS, requires a CT scan and registration as (serious) adverse event including possible cause by each participating site. This CT scan will be assessed at the coordinating centre as well.

Primary endpoint will be assessed by a blind research nurse from the clinical trial office of the coordinating centre, who will score the mRs by telephone using a structured interview. To increase the interobserver reliability the number of research nurses will be limited to a maximum of three. Disability will be assessed by the same research nurse during the same telephone interview using the Amsterdam Linear Disability Scale.22 Appendix B lists all data collection forms. In patients with a poor outcome at three months, a central adjudication committee composed of the investigators of the coordinating centre, judges whether this poor outcome is attributed to the initial ischaemic stroke, reported adverse event or other causes.
Safety reporting
All adverse event will be recorded. In case of serious adverse events (SAE), the principal investigator will be notified by email or telephone within 24 hours. The principal investigator subsequently reports SAE to the Data Safety Monitoring Board (DSMB). This is an independent committee of trial experts, who will focus on both safety monitoring and analysis of effectiveness on unblinded data. The DSMB will perform ongoing safety surveillances, especially with regard to the occurrence of serious adverse events in terms of SICH and serious systemic bleeding within 48 hours. The DSMB can recommend the Steering Committee of the ARTIS trial to terminate the trial when there is clear and substantial evidence of harm. All SAE will be reported to the central medical ethics committee according to their requirements as well.

Trial size
Based on our own experience in the stroke unit cohort and the results of the rt-PA thrombolysis trials and the SITS-MOST registry it is expected that 50% of the patients with an ischaemic stroke treated with rt-PA thrombolysis will have a poor outcome.1,23 We aim to reduce this percentage by 10%, which is a relative risk reduction of 20%.

A two group $\chi^2$ test with a 0.05 two-sided significance level will have 80% power to detect the difference between the control group proportion of 0.50 and an experimental group proportion of 0.40 (odds ratio of 0.667) when the sample size in each group is 400 (total trial size 800). With this sample size, a two-sided 95% confidence interval for the difference between the proportions will extend 0.069 from the observed difference in proportions.

Statistical analyses
Baseline characteristics will be summarized using descriptive statistics. The main analysis of this trial consists of a single comparison between the trial medication groups of the primary outcome after three months (dichotomized Rankin score). The analysis will be based on the intention-to-treat principle. The effect size will be expressed in a relative risk (RR) estimates and absolute risk reduction (ARR). Additionally the primary outcome will be analysed using multivariate logistic re-
gression, adjusting (if necessary) for clinically relevant baseline imbalances. The differences between NIHSS, ALDS scores and non-dichotomized mRs will be analyzed using the two-group \( t \)-test, the Mann Whitney U test, linear regression and ordinal logistic regression, when appropriate. The remaining secondary outcomes will be analysed using 2x2 tables and logistic regression. In all analyses, statistical uncertainty will be quantified by 95% confidence intervals.

Interim analysis
Besides interim analyses on the safety data the DSMB will also perform an unblinded interim analysis on the primary outcome to assess the strength of the efficacy data when half of the patients are enrolled. The DSMB will also check the assumptions for sample size calculations. The analysis will be performed by an independent statistician of the Academic Medical Center Clinical Research Unit, who is not involved in managing the trial. The DSMB can recommend the Steering Committee of the ARTIS trial to

- adjust the sample size;
- early terminate the study when there is clear and substantial evidence of benefit;
- early terminate the study in case the data suggests no benefit or in case accrual rates are too low to provide adequate statistical power for identifying the primary endpoint.

Predefined subgroup-analysis
With respect to the primary outcome a predefined subgroup-analyses will be performed:

- thrombolysis <2 hours versus 2–3 hours versus >3 hours from symptom onset. Effectiveness of thrombolysis declines over time following symptom onset probably caused by an increase in clot stability. Regarding clot dissolution and reocclusion the beneficial effect of adding antiplatelet therapy might therefore be different over time. The risk of bleeding can also change over time.²
- administration of trial medication within 1 hour versus between 1–1.5 hours from rt-PA bolus. Reocclusion occurs at a median time of
65 minutes after start of rt-PA treatment. Administration of aspirin in the first hour after the start of rt-PA treatment is therefore expected to result in better outcome.

- based on ethnicity differences: whites versus blacks, whites versus Hindu’s, whites versus blacks and Hindu’s, Hindu’s versus the other ethnic groups. Previous studies on thrombolytic therapy in acute myocardial infarction suggest that racial differences do exist with an increased thrombolytic effect in blacks accompanied by an increased risk of bleeding complications. The beneficial or detrimental effect of the addition of antiplatelet therapy to thrombolysis might therefore differ among different ethnic groups.\textsuperscript{24-26}

Subgroup analyses consist will consist of a simple comparison of these different groups on primary and secondary endpoints.

Ethical considerations
The ARTIS study will be conducted according to the principles of the Declaration of Helsinki (version of 2004) and in accordance with the Medical Research Involving Human Subjects Act (WMO) and other guidelines, regulations and Acts. The Medical Ethics Committee of the Academic Medical Center approved the protocol before start of the trial. Data management, monitoring and reporting of the study will be performed in accordance with the ICH GCP guidelines. Approval by the local medical ethical review board is required for each participating centre before start of inclusion. The AMC Medical Research BV has an insurance, which is in accordance with the legal requirements in The Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of June 23, 2003). This insurance provides cover for damage to research subjects through injury or death caused by the trial.

Publication policy
The trial results will be published by the coordinating investigator on behalf of the ARTIS study group. Members of the ARTIS study group will then be listed at the end of the article.
DISCUSSION

We present the protocol of a randomised controlled clinical trial to investigate the efficacy of direct addition of 300 mg aspirin to rt-PA thrombolysis in acute ischaemic stroke. In accordance with thrombolysis in myocardial infarction, in which the combination of acute aspirin and thrombolysis improves outcome considerably, we hypothesize that immediate platelet inhibition will improve outcome in acute ischaemic stroke by enhancing thrombolysis and preventing reocclusion after initial recanalisation. As far as we know, this is the first clinical trial investigating the efficacy of direct addition of aspirin to intravenous rt-PA for acute ischaemic stroke.

A major safety concern in this trial refers to the occurrence of SICH. Subgroup-analysis of patients receiving APT within 24 hours after rt-PA thrombolysis in the first European Cooperative Acute Stroke Study (ECASS-I) showed a slight trend towards increased mortality from all causes (including SICH). This risk is now explained by the higher rt-PA dose (1.1mg/kg) used in this trial since there was no increased risk in ECASS-II where the currently standard dosage of 0.9 mg/kg rt-PA was used.1

Several cohort studies could not find a significant association between pre-treatment with antiplatelet agents and SICH.27-30 Other prospective observational studies observed even a net benefit in favourable outcome after 3 months in patients using antiplatelet drugs prior to rt-PA thrombolysis, despite a strong relationship between this antiplatelet therapy and SICH.18-19 Recent results from the large SITS-MOST registry of more than 6,000 stroke patients treated with intravenous rt-PA confirmed the increased risk of SICH in patients with antiplatelet pre-treatment.31 Previous use of aspirin had an odds ratio of 1.58 (95% CI: 1.04–2.39) of SICH per Safe Implementation of Thrombolysis in Stroke-Monitoring Study definition, a remote parenchymal haemorrhage type 2 on the 22–36 hours follow-up imaging scans after the start of thrombolysis treatment. The clinical relevance of these SICH remains to be determined since independency and mortality within 3 months were not associated with previous aspirin use in this registry. Although prior antiplatelet therapy is a contra-indication in this protocol,
we are aware of the possible increased risk of SICH due to the combination of rt-PA and aspirin. Therefore, the DSMB will continuously monitor serious adverse events in relation to efficacy outcome measures.

ARTIS is a randomised controlled trial investigating the efficacy of the acute addition of aspirin to intravenous rt-PA thrombolysis in patients with acute ischaemic stroke. ARTIS will answer a highly relevant question in acute stroke care by means of a simple adjustment of current antiplatelet regimen with regard to rt-PA thrombolysis. A condensed version of the protocol has been published in Cerebrovascular Diseases.32

The ARTIS trial has started at the end of 2008. Thirty-seven centres are actively randomising patients. As of May 10th 2010, 361 of the 800 patients have been included so far. This trial is set up in the Netherlands. However, other centres - also from foreign countries - experienced in thrombolysis are invited to participate as well. The principle investigator can be contacted by e-mail.

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TRIAL ORGANISATION

Executive committee: R.J. de Haan, Y.B. Roos (principal investigator), J. Stam, M. Vermeulen and S.M. Zinkstok (trial coordinator).
Steering committee (constituted of selected investigators of each randomising centre): S.L.M. Bakker, H.P. Bienfait, A.E. Boon, S.F.T.M. de Bruijn, C.L. Franke, B.P.W. Jansen, K. Keizer, H. Kerkhoff, V.I.H. Kwa, P. Portegies, T.C. van der Ree, W.J. Schuiling, M.C. Visser, H.B. van der Worp) and the members of the executive committee.
Data Safety Monitoring Board: M.H. Prins (epidemiology), M. Limburg (neurology) and R.J.G. Peters (cardiology).
Trial coordinating centre: Clinical Trial Office Neurology, Department of Neurology, Academic Medical Center, Amsterdam, the Netherlands.
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REFERENCES

12. ISIS-2 (Second International Study of Infarct Survival) Collaborative Group. Randomised trial of intravenous streptokinase, oral aspirin, both, or neither among 17,187

