MR CLEAN-Multicenter Randomized Clinical trial of Endovascular treatment for Acute ischemic stroke in the Netherlands.

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MR CLEAN - PROTOCOL FOR A MULTICENTER RANDOMIZED CLINICAL TRIAL OF ENDOVASCULAR TREATMENT FOR ACUTE ISCHEMIC STROKE IN THE NETHERLANDS (NTR1804/ISRCTN10888758)

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The study is designed, and will be conducted, analyzed, and interpreted by the investigators independently of all sponsors.

SUMMARY

RATIONALE AND AIM

Intra-arterial treatment increases the likelihood of recanalization in patients with acute ischemic stroke caused by proximal intracranial arterial occlusion. The purpose of the Multicenter Randomized Clinical trial of Endovascular treatment for Acute ischemic stroke in the Netherlands (MR CLEAN) is to assess the safety and effect on functional outcome of intra-arterial treatment in these patients.

DESIGN

MR CLEAN is a pragmatic phase III multicenter randomized clinical trial with blinded outcome assessment. The intervention contrast is intra-arterial treatment versus no intra-arterial treatment.

STUDY POPULATION

Patients should have a clinical diagnosis of acute ischemic stroke, MRI[1] or CT ruling out intracerebral hemorrhage, a score on the National Institutes of Health Stroke Scale (NIHSS) of 2 points or more, a relevant intracranial arterial occlusion, demonstrated by neuro-imaging and the possibility to start endovascular treatment within 6 hours after stroke onset.

INTERVENTION

Endovascular treatment may consist of intra-arterial thrombolysis with urokinase or alteplase, mechanical treatment or both. Mechanical treatment refers to retraction or aspiration of the thrombus with a catheter guided device, or stenting. The exact choice of endovascular treatment modality for each patient is left to the discretion of the local investigator and treating physicians. The steering committee will provide recommendations and guidelines for treatment and selection of patients in the study. Background medical management is delivered according to national standards and guidelines. It may include intravenous alteplase within the first 4.5 hours after onset.

MAIN STUDY OUTCOMES

The primary outcome is the score on the modified Rankin scale (mRS) 90 days after inclusion in the study. Secondary outcomes are the NIHSS score at 24 hours, vessel patency at 24 hours and infarct size at day 5-7 and the occurrence of major bleeding.

The randomization will be stratified for use of intravenous alteplase, planned treatment modality (intra-arterial thrombolysis, mechanical thrombectomy or both) and center. We will estimate the effect of treatment by means of the ordinal logistic regression (shift analysis), which considers the whole range of the mRS. In total, 500 patients will be included.

[1] All abbreviations are listed in Table 1.

BURDEN AND RISKS ASSOCIATED WITH PARTICIPATION.

All patients that participate in the trial will undergo a second CTA within 24 hours after admission and a CT scan at day 5-7. All patients will have a telephone interview at three months. Patients who are randomized for intra-arterial treatment sometimes need sedation or anesthesia, and intubation during the procedure. Finally, endovascular treatment is associated with increased risk of intra-cerebral hemorrhage.

DISCUSSION

MR CLEAN is a pragmatic trial. Inclusion of patients will take 4 years, and starts early in 2010.

Key words: alteplase, endovascular treatment, acute ischemic stroke, randomized controlled trial

1. INTRODUCTION AND RATIONALE

In Western Europe and the US, the annual incidence of ischemic stroke is 1-2 per 1000.[1, 2] Half of all patients with stroke die or remain severely handicapped. Stroke is one of the major causes of death and the first cause of dependency in the western world. Treatment with intravenous alteplase, aiming at early reperfusion has been proven effective for these patients, when they are treated within 4.5 hours, and when there are no contra-indications.[3-5] The absolute reduction in the chance of poor outcome in patients treated with i.v. alteplase within 3 hours from onset amounts to 10%; the number needed to treat is 10.[6] For the patients treated within 3 to 4.5 hours, this effect was reduced to 7%, for a number needed to treat of 14.[4]

In general, the number of patients eligible for treatment with intravenous alteplase is limited because of the restricted time window. In about 25% of the patients with acute anterior circulation ischemic stroke, symptoms are caused by a proximal occlusion of one of the major intracranial arteries, i.e. the distal intracranial internal carotid artery, the proximal segments of the middle cerebral artery and the anterior cerebral artery.[7, 8] The likelihood of a proximal occlusion increases with severity of the neurological deficit at presentation.[9-11] The effect in these patients with a symptomatic intracranial arterial occlusion is limited. Treatment with i.v. alteplase leads to recanalization in up to 33% of treated patients only.[12] In those without recanalization, outcome is generally poor.[13, 14]

1.1 EFFECTIVENESS OF INTRA-ARTERIAL THROMBOLYSIS

Four randomized clinical trials that assessed the effectiveness of intra-arterial thrombolysis in patients with acute ischemic stroke due to intracranial occlusion have been reported (Table 2). The PROACT I and PROACT II were randomized controlled trials that compared intra-arterial thrombolysis by means of pro-urokinase with treatment with heparin in patients with angiographically demonstrated M1 of M2 segment occlusion, within 6 hours from onset of symptoms. Recanalization rates were high and recanalization occurred more often in the active treatment group than in controls.[15, 16] The results of the PROACT-II study indicated an improved functional outcome on day 90; 40% of the patients had a score on the modified Rankin Scale (mRS) of less than 3). In total, 11% to 15% of the patients in the PROACT studies had a symptomatic intracranial hemorrhage after treatment with intra-arterial thrombolysis. A third randomized clinical trial from Japan (MELT) compared intra-arterial urokinase with standard treatment (but not intravenous alteplase) within 6 hours from onset was terminated prematurely. For a secondary endpoint, (mRS >2 at 90 days, a significant effect was observed.[17] The results of these trials have to be interpreted with care and cannot be extrapolated to the current clinical situation. In the three trials, intravenous alteplase was not an option, neither as pre-treatment nor as part of the control treatment. In MELT, mechanical treatment was allowed, but not in PROACT I and II. MELT was an open label randomized trial with blind outcome assessment, which implies that the treatment effect could have been known at the time the decision was made to prematurely stop the trial. In PROACT I and II the control treatment included intravenous heparin, which can now be considered obsolete.[18] Finally, a fourth, Italian RCT (SYNTHESIS) compared treatment with intra-arterial alteplase with intravenous alteplase. The trial started in 2004, and was stopped after 50 patients had been included. More patients with intra-arterial treatment than patients with intravenous treatment showed recanalization and good outcome, but the effect was not statistically significant.[19] Several non-randomized studies with historical controls,[20] or controls in other centers,[21] suggested a benefit of intra-arterial thrombolysis.

1.2 SAFETY OF INTRAVENOUS AND INTRA-ARTERIAL THROMBOLYSIS.

The combination of intravenous and intra-arterial rt-PA was compared with intravenous placebo followed by intra-arterial alteplase in the EMS Bridging trial (Table 3). The risk of hemorrhagic transformation was increased in patients who received the combination treatment.[22] In two observational studies, patients were treated within 5 hours from onset of symptoms. The intravenous dose was adjusted to 0.6 mg/kg, with a maximum of 60 mg. The incidence of hemorrhages was not larger than in studies of treatment with intravenous thrombolysis only.[23, 24] Similar results have been reported by others.[25-27] In several case-series, endovascular treatment with low dose i.a. alteplase was preceded by full dose i.v. alteplase (i.e. 0.9 mg/kg). Risks for sICH ranged from 0 to 13%.[28-32] These studies suggest that in patients who have been treated in this way, recanalization rates can be high, without unacceptable high risks of complications (Table 3).

1.3 SAFETY AND EFFECTIVENESS OF MECHANICAL TREATMENT

Mechanical treatment is a promising technique, either as a secondary in patients who do not respond quickly to intra-arterial thrombolytic treatment, or in patients for whom thrombolytic agents are contra-indicated. The MERCI device is a retractor device. Several studies of the effect of treatment with the MERCI device have been published (Table 4).[33-36] The rates of recanalization were similar to observed rates in the intra-arterial thrombolytic treatment studies. However, these studies were not randomized, but used historical controls derived from the NINDS RTPA stroke trial. The MERCI device has been approved by the FDA.

Several case series describing the treatment of symptomatic occlusion with other devices, such as the PENUMBRA device and the EKossonic SV have been published.[37-39] Other approaches to mechanical treatment include use of aspiration devices, such as the PENUMBRA device, which has also recently been approved for treatment of acute ischemic stroke by the FDA, and stents, such as the EV3 stent, and the Wingspan stent.[39-41] The Wingspan stent itself is FDA approved for elective treatment of intracranial arterial stenosis but not for treatment of acute ischemic stroke.

The results of these uncontrolled studies of mechanical thrombectomy are difficult to compare, because of differences in case mix, pretreatment and patient selection, severity of intracranial occlusions, and definitions of revascularization. However, these studies suggest that in experienced hands, mechanical thrombectomy could be safe and may lead to substantial recanalization rates. A recent AHA guideline therefore stated that "….Although the Concentric MERCI device can be useful for extraction of intra-arterial thrombi in appropriately selected patients, the utility of the device in improving outcomes after stroke remains unclear. " and "The usefulness of other endovascular devices is not yet established, but they may be beneficial."[18]

1.4 NEEDED: A RANDOMIZED CLINICAL TRIAL OF ENDOVASCULAR TREATMENT IN ACUTE ISCHEMIC STROKE

We conclude from this overview that intra-arterial treatment, either with a thrombolytic agent or by mechanical means is able to recanalize acutely occluded cerebral arteries in selected patients, within reasonable safety margins. Whether this is the case in an unselected sample of patients with acute ischemic stroke caused by occlusion is likely, but unproven. A randomized clinical trial addressing the question whether intra-arterial treatment improves neurological outcome in patients with a relevant occlusion in the intracranial proximal anterior circulation is therefore needed.

For the trial results to be generalizable and representative of what is state of the art approach in intra-arterial treatment, the trial design should accommodate the possibility to use local fibrinolytics and or mechanical

thrombectomy devices, for a broad range of patients with acute ischemic stroke caused by a proximal thrombo-embolic occlusion of one of the intracranial arteries belonging to the anterior circulation.

Important subgroups to whom this question of effectiveness and safety applies are patients who have been treated unsuccessfully with intravenous thrombolysis, patients who can be treated within 6 hours, but do not meet the time-window requirements for intravenous thrombolysis, and patients with contra-indications for intravenous and/or intra-arterial thrombolytic treatment (thrombectomy only). To answer this question, we initiated a large multicenter pragmatic trial of intra-arterial treatment (by means of alteplase and or mechanical treatment) versus standard medical treatment, in patients with acute ischemic stroke of less than 6 hours of onset, the MR CLEAN study.

The trial applies the grey area principle: when a patient's clinical profile meets inclusion and exclusion criteria, and according to investigator and treating physician there is sufficient uncertainty concerning the question whether the patient should receive intra-arterial treatment, the patient is eligible for inclusion in the trial.

2. OBJECTIVES

The primary objective of this study is to estimate the effect of endovascular treatment on overall functional outcome after acute ischemic stroke of less than six hour duration, in patients with a symptomatic occlusion. The secondary objectives are to assess the safety of endovascular treatment with regard to the occurrence of hemorrhagic and ischemic complications, the efficacy with regard to obtaining recanalization, and to evaluate predictors of recanalization, including imaging aspects and hemostatic parameters. Moreover, we want to assess the safety and efficacy of different types of endovascular treatment (i.e. mechanical treatment, intra-arterial thrombolysis) different combinations of treatment (i.e. with intravenous alteplase) and different timings of treatment. Tertiary objectives are to carry out case studies of implementation strategies and loco-regional solutions for barriers to the delivery of endovascular treatment for acute ischemic stroke and to collect data for cost-effectiveness analysis of endovascular treatment compared with standard treatment.

3. STUDY DESIGN

This is a multicenter clinical trial with randomized treatment allocation, open label treatment and blinded endpoint evaluation (PROBE design).

The intervention contrast is endovascular treatment (alteplase or urokinase, and/or mechanical treatment) versus no endovascular treatment. The treatment is provided in addition to best medical management, including intravenous thrombolysis.

The study will run in at least 10 large hospitals in the Netherlands for a period of five years (4 years of patient inclusion), and starts in 2010.

4. STUDY POPULATION

4.1 POPULATION

Patients over 18 years old, with acute ischemic stroke, a symptomatic anterior proximal artery occlusion which can be treated within 6 hours after stroke onset are eligible for participation in this trial.

4.2 INCLUSION AND EXCLUSION CRITERIA

INCLUSION CRITERIA

- A clinical diagnosis of acute stroke, with a deficit on the NIH stroke scale of 2 points or more.
- CT or MRI scan ruling out intracranial hemorrhage.
- Intracranial arterial occlusion of the distal intracranial carotid artery or middle (M1/M2) or anterior (A1/A2) cerebral artery, demonstrated with CTA, MRA or DSA.
- The possibility to start treatment within 6 hours from onset.
- Informed consent given.
- Age 18 or over.

GENERAL EXCLUSION CRITERIA

- Arterial blood pressure > 185/110 mmHg.
- Blood glucose < 2.7 or > 22.2 mmol/L.
- Intravenous treatment with thrombolytic therapy in a dose exceeding 0.9 mg/kg alteplase or 90 mg.
- Intravenous treatment with thrombolytic therapy despite contra-indications, i.e. major surgery, gastrointestinal bleeding or urinary tract bleeding within the previous 2 weeks, or arterial puncture at a non-compressible site within the previous 7 days.
- Cerebral infarction in the distribution of the relevant occluded artery in the previous 6 weeks.

SPECIFIC EXCLUSION CRITERIA FOR INTENDED MECHANICAL THROMBECTOMY

- Laboratory evidence of coagulation abnormalities, i.e. platelet count <40 x 10^9/L, APTT>50 sec or INR >3.0.

SPECIFIC EXCLUSION CRITERIA FOR INTENDED INTRA-ARTERIAL THROMBOLYSIS

- History of intracerebral hemorrhage.
- Severe head injury (contusion) in the previous 4 weeks.
- Clinical or laboratory evidence of coagulation abnormalities, i.e. platelet count <90 x 10^9/L, APTT>50 sec or INR >1.7. Current treatment with oral thrombin antagonists, such as argatroban and dabigatran or treatment with oral selective Factor Xa inhibitors, such as rivaroxaban.

4.3 PARTICIPATING CENTERS AND CENTER ELIGIBILITY

To be fully eligible for participation in the trial and to include patients in the trial, centers should meet the following minimum criteria:

- The center should have experience in conducting acute stroke trials.
- The intervention team should have ample experience with endovascular interventions for cerebrovascular disease (carotid stenting or aneurysm coiling), peripheral artery disease, or coronary artery disease.
- At least one member of the intervention team should have sufficient experience with intra-arterial thrombolysis.

In order to include and randomize patients who may be treated with mechanical thrombectomy centers should meet the following additional criteria:

- The intervention team should make use of one or more of the devices that have been approved by the trial steering committee. Other devices are not allowed into the trial.
- At least one member of the intervention team should have sufficient experience with the particular device.

Sufficient experience is defined as the completion of at least 5 full procedures with het particular device. Procedures that have been carried out by two team members (for example, in a training setting) do count. Procedures do not need to be successful, nor uncomplicated. Procedures consisting of mechanical thrombectomy combined with intra-arterial thrombolysis count for both.

Centers that do not comply with the third criterion may participate in the registration phase of the study. In these centers, patients cannot be included and randomized. However, patients who undergo intra-arterial treatment will be registered and data will be processed. The center can apply for a status as fully participating center when criterion 3 is met.

Note that patients may only be included in the trial when the intervention team that will actually treat the patient includes at least one member with sufficient experience when one of the treating interventionists. For this reason, the possibility of treatment by an interventionist with sufficient experience is listed as an inclusion criterion.

Training sessions for intra-arterial thrombolysis and mechanical treatment will be held, draft guidelines and recommendations for endovascular procedures and general management of included patients, on paper and on video will be issued by the steering committee and distributed among participating centers.

Three centers will be added as 'randomization centers'. These centers do not have an intervention team, but are in close range to an approved and active MR-CLEAN participating center with a neurointerventional team. If a patient presents in a randomization center, who is eligible for inclusion in the MR CLEAN trial, the neurologist will contact the closest MR CLEAN center with an active interventional team. If the interventional team is available, the neurologist will proceed to randomize the patient. Either the patient will be included in the treatment arm and will be transferred to the center with the interventional team or the patient will be assigned to the standard arm and will remain at the randomization center. Follow up will be monitored in this center until discharge. [2]

4.4 SAMPLE SIZE

The effect of the intervention on the primary outcome (the mRS, a 7-point ordered categorical scale) will be assessed with ordinal logistic regression. We assume a moderate effect of 10% absolute increase in the cumulative proportion of patients with mRS 0-3 in the intervention group, compared with controls. We based the distribution of outcome categories on the results of the PROACT II trial.[15] Figure 2 shows the expected

[2] One of the randomization centers (Reinier de Graaf) has the available resources to treat patients with intra-arterial therapy (equipment and supporting staff) but there is a shortage of neurointerventionalist. Instead of transferring the patient to the closest MR CLEAN center (HAGA) the members of the neurointerventional team will visit the Reinier de Graaf hospital.

distributions of mRS categories. A total study size of 500 patients (2x250 pts) provides a power (1-beta) of 82% at a significance level of 0.05, taking into account 10% cross over rate. [42]

This sample size is also sufficient to assess the effect of the intervention on secondary endpoints: Analysis of a meaningful reduction on NIH stroke scale at one week of 3-4 points (Cohen's d=0.33) would require a sample of 400 patients, assuming that at 24-48 hours mean NIH would be 12, with a standard deviation of 10. A doubling of the recanalization rate from 30% to 60% would require 126 patients to achieve a power of 0.90.

Simulation studies have indicated that ordinal logistic regression is a more powerful method for analysis of trials with ordered categorical outcome data, and have proven its robustness against violations of the proportional odds assumption.[43]

5.TREATMENT OF SUBJECTS

5.1 INVESTIGATIONAL TREATMENT

Endovascular treatment will consist of arterial catheterization with a microcatheter to the level of occlusion and delivery of a thrombolytic agent and/ or mechanical thrombectomy.

Both alteplase and urokinase for intra-arterial thrombolysis is allowed into the trial, a dose of 1 mg alteplase is considered to be equivalent to 10.000-15.000 U urokinase.[44]

Mechanical treatment may consist of mechanical thrombectomy, aspiration, or stenting. Specific recommendations with regards to procedures and devices will be issued regularly by the trial steering committee.

The steering committee will make recommendations for dosages of thrombolytic agents, procedures, and for devices that will be allowed in the trial based on proposals by the executive committee or local investigators. The requirements for a device being allowed for use in the trial are: documented evidence of safety in experienced hands, and recanalization rates that are similar to rates with other mechanical devices. Devices that are currently allowed in the trial are listed in Appendix 4. Evidence on safety or efficacy of particular devices from other studies, or recommendations made by the DMC on the basis of monitoring results, or both, may lead the steering committee to decide to discontinue allowance into the trial of a particular device. Proposals will be prepared by the executive committee, and should be approved by a majority of the steering committee.

6.METHODS

6.1 STUDY OUTCOMES

PRIMARY OUTCOME

The primary outcome is the score on the modified Rankin scale at 90 days (Table 5a).

SECONDARY OUTCOMES

IMAGING PARAMETERS

- Vessel recanalization at 24 hours after treatment, assessed by CTA or MRA. The criteria for recanalization on CTA or MRA are based on the Arterial Occlusive Lesion (AOL) scale, and the Clot Burden Score, proposed by Puetz et al (Table 5c)[46].
- Infarct size assessed by CT on day 5-7, using standard methods, including manual tracing of the infarct perimeter and semi-automated pixel thresholding.[47, 48] Infarct size at day 5-7 will be compared with plain CT and perfusion CT results (if available) at baseline.
- CTA or MRA at 24 hours will be compared with baseline vessel imaging data, to estimate the recanalization rate. Perfusion CT at baseline is optional, but available at most centers. Clinical parameters

CLINICAL PARAMETERS

- NIHSS [49], including NIH supplemental motor score,[50] at 24 hours.
- NIHSS at 1 week or at discharge.

FUNCTIONAL OUTCOME

- Score on the EQ5D at 90 days,[51]
- Barthel index at 90 days.[52]

The 90-days follow-up will be conducted by telephone interview, through the central trial office.

SAFETY PARAMETERS

Safety is an issue of concern, as the experience with the intervention, overall, and within the participating centers, is limited. Safety parameters include hemorrhagic complications, and short term outcome (mortality, Barthel index and NIHSS at 24 hours and at one week or discharge). As we will make use of web-based data-entry, these data will be available on short notice.

The primary safety parameter will be neurologic deterioration within 24 hours from inclusion in the study. Neurological deterioration is defined as any decline in NIHSS of more 4 points or more. In these patients, urgent brain CT is mandatory. This serious adverse event will be further classified as due to intracranial hemorrhage, ischemia or other (undetermined) cause. A full list of serious adverse events is provided in section 7.1 Adverse events.

If the local investigator or other member of the team at a trial centre has a concern about the outcome of their trial procedures, they should inform the MR CLEAN trial office, which will organize a blinded assessment of the relevant outcome events. This will be submitted by the central office to the chairman of the data monitoring committee, who may recommend further action, such as suspending randomization at the centre. Similarly, the database manager at the trial office will monitor outcome events and if there are three consecutive deaths or three consecutive serious adverse events at a single centre within 30 days of treatment in the same arm of the study, then assessment of the events will be triggered. A cumulative death rate of more than 50% or a

cumulative serious adverse event rate exceeding 20% over 10 cases during hospital admission would also trigger careful assessment of the relevant outcome events.

DATA FOR COST-EFFECTIVENESS ANALYSIS

A limited amount of health and medical costs data will be collected, in a piggy-back fashion i.e. during the registration of other data, and will include length of stay. Measurement of health care costs will be based on international and national guidelines.[58, 59] Analysis of these data however, and the modeling for the cost-effectiveness study is outside the scope of the current study. Therefore a separate substudy on cost-effectiveness will be carried out (see 10.2 substudies and the separate substudy protocol for more detailed information).

6.2 RANDOMIZATION

The randomization procedure will be computer- and web-based, using permuted blocks. Back-up by telephone will be provided. Randomization is allowed when the occlusion has been established by CTA, MRA, DSA or TCD. Selection of patients for randomization follows the grey area principle. Randomization will be stratified for center, use of intravenous alteplase, planned treatment modality (mechanical thrombectomy or not) and stroke severity, (NIHSS >14 or not).

Patients with contra-indications for intravenous thrombolysis are allowed into the trial. This concerns patients who cannot be treated within 4.5 hours from onset, but only in the 4.5 to 6 hour interval, and patients with either major surgery, gastrointestinal bleeding or urinary tract bleeding within the previous 2 weeks, or arterial puncture at a non-compressible site within the previous 7 days.

Patients with exclusion criteria for intra-arterial thrombolysis are also allowed into the trial. They can be included and randomized for endovascular treatment or no endovascular treatment. Treatment with mechanical thrombectomy is allowed, but intra-arterial thrombolysis is not allowed in these patients. The treating physicians are free to change the actual mode of treatment during the procedure, as long as they comply with the treatment-specific exclusion criteria and recommended devices.

6.3 BLINDING

It will not be possible to view the treatment allocation before the patient is registered in the study database, nor will it be possible to remove the patient from the study base after treatment assignment has become known. Both patient and treating physician will be aware of the treatment assignment. Information on outcome at three months will be assessed through standardized forms and procedures. Assessment of outcome on the modified Rankin scale will be based on this information, by assessors who are blind to the treatment allocation. Results of neuroimaging will be also assessed in a blinded manner. Information on treatment allocation will be kept separate from the main study database. The steering committee will be kept unaware of the results of interim analyses of efficacy and safety. The trial statistician will combine data on treatment allocation with the clinical data in order to report to the data monitoring committee (DMC).

6.4 STUDY PROCEDURES

BASELINE DATA OBTAINED AT ADMISSION

Clinical data, neuro-imaging data and procedure-related data that might be related to treatment effect or to adverse events caused by the intervention, as well as several stroke risk factors, in order to illustrate the representativeness of the study population will be recorded (Table 6).

INCLUSION AND RANDOMIZATION

A minimal amount of data has to be entered before randomization, as randomization will be stratified. The trial office will be notified when a new patient is entered into the web-based database. A treatment allocation will be provided by the web-based computer system. Personal data will be sent to the trial office separately, through scrambled email.

FOLLOW-UP DATA

At 24 hours, a clinical examination including NIH stroke scale assessment will be carried out. Also, all patients will undergo CTA or MRA imaging. At day 5-7 all patients will undergo CT or MRI. Raw data will be forwarded to the trial office for blind evaluation. At 1 week, clinical status, NIH stroke scale score and adverse events will be reported as well as discharge destination, in order to enable the trial office to conduct the final 3-month follow-up by telephone interview.

The standardized telephone interview will include a short questionnaire based on the three simple questions, assessment of modified Rankin Scale, Barthel Index, and Euroqol5D.[51-53, 60-62].

WITHDRAWAL

Patients can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study or stop the allocated intervention for urgent medical reasons. Every attempt will be made to complete follow-up in these patients.

6.5 TRIAL ORGANIZATION

The steering committee will make decisions regarding continuation of the trial and protocol changes. Decisions will be prepared by the executive committee. The chairman of the steering committee will be advised by the independent data monitoring and safety committee, see section 7.2 Safety and data monitoring committee. The steering committee consists of the local investigators from each center, a neurologist and a neuro-interventionist, and the executive committee. The executive committee consists of the six co-principal investigators, and study-coordinator. The post of study-coordinator will be taken by each of three junior-researchers, taking turns. The study-coordinator is responsible for running the trial on a day-to-day basis, and will report to the executive committee. The executive committee will meet on a bi-monthly basis. The steering committee will meet at least annually. The steering committee meeting is chaired by the principal investigator (DD). Other important committees are the neuro-imaging assessment committee, the functional outcome adjudication committee and the serious adverse event adjudication committee.

The trial office is located in Rotterdam, Erasmus MC University Medical Center. The neuro-imaging assessment unit is located in AMC, Amsterdam.

7.SAFETY REPORTING.

7.1 ADVERSE EVENTS

Adverse events are undesirable experiences occurring to a subject during the study, whether or not they are considered to be related to the experimental treatment. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

A serious adverse event is any untoward medical occurrence or effect that can cause mortality, is life-threatening, requires prolonged hospitalization, or results in persistent significant disability.

Expected serious adverse events are neurologic deterioration, symptomatic intracranial hemorrhage, extracranial hemorrhage, technical complications or vascular damage at the target lesion such as perforation or dissection and mortality in the first week of stroke, aspiration pneumonia, allergic reaction towards contrast fluid, death from any cause within the study period.

A cumulative log will be kept of all serious adverse events, for review by the DMC.

7.2 SAFETY AND DATA MONITORING COMMITTEE

In order to increase the safety of the intervention, the trial will be monitored by an independent data monitoring committee (DMC). The data monitoring committee will be chaired by a neurologist, and include a neuro-interventionist and an independent methodologist/statistician. The DMC will meet frequently and assess the occurrence of unwanted effects by center and by procedure. During the period of intake to the study, interim analyses of mortality and of any other information that is available on major endpoints (including serious adverse events believed to be due to treatment) will be supplied, in strict confidence, to the chairman of the Data Monitoring Committee, along with any other analyses that the Committee may request. In the light of these analyses, the Data Monitoring Committee will advise the chairman of the Steering Committee if, in their view, the randomized comparisons in MR CLEAN have provided both (i) "proof beyond reasonable doubt" that for all, or for some specific types of patients, one particular treatment is clearly indicated or clearly contraindicated in terms of a net difference in outcome, and (ii) evidence that might reasonably be expected to influence materially patient management. Appropriate criteria of proof beyond reasonable doubt cannot be specified precisely, but a difference of at least 3 standard deviations in an interim analysis of a major endpoint may be needed to justify halting, or modifying, the study prematurely. This criterion has the practical advantage that the number of interim analyses is of little importance.

8. STATISTICAL ANALYSES

Baseline characteristics will be summarized by means of simple descriptive statistics. The main analysis of this trial consists of a single comparison between the trial treatment groups of the primary outcome after 90 days. The analysis will be based on the intention-to-treat principle. The primary effect parameter should take the whole range of the modified Rankin scale (mRS) into account and is defined as the relative risk for improvement on the mRS estimated as an odds ratio with ordinal logistic regression.[63]

Multivariable regression analysis will be used to adjust for chance imbalances in main prognostic variables between intervention and control group, such as age, stroke severity (NIHSS), time since onset, previous stroke, atrial fibrillation and diabetes mellitus. Secondary effect parameters will be the improvement according to the classical dichotomizations of the modified Rankin scale at 0-1 vs 2-6 and 0-2 vs 3-6, the presence of vessel patency on CTA, MRA or DSA at 24 hours, and the score on the NIHSS at 24 hours and 1 week or discharge.

With regard to the range of secondary outcome parameters we will use simple 2x2 tables, two-group t-tests, Mann-Whitney tests, and multivariable linear and logistic regression models, where appropriate. In all analyses, statistical uncertainty will be quantified by means of 95% confidence intervals. Subgroup analyses will be carried out to estimate the effect intra-arterial thrombolysis, mechanical treatment and combination therapy. Although the size of this study will not allow for precise estimates of treatment effect in subgroups, we will assess heterogeneity of effects, and analyze consistency of effects on secondary outcomes.

9.ETHICAL CONSIDERATIONS, ACCESS TO APPROPRIATE TREATMENT

9.1 REGULATION STATEMENT

The trial will be conducted in accordance with the principles of the Declaration of Helsinki, as amendended by the World Medical Association General Assembly in October 2008, and with the guidelines for Good Clinical Practice.

9.2 RECRUITMENT AND CONSENT

Written informed consent will be obtained from all patients and a copy must be retained by the randomizing centre. All patients will be provided with a written explanation of the study. Because of the urgent character of the treatment informed consent is subdivided in an informed consent form with a short description of the procedure for immediate use, and an extensive version which will be discussed with the patient after the procedure.

After approval by the patient or his/her legal representative, the patient's treating physician will inform a study physician of the presence of a patient with acute ischemic stroke who is potentially eligible for the present study. The study physician will inform the patient orally and in writing and will obtain his/her written informed consent. In case the patient is legally incompetent, for example because of aphasia or anosognosia, written informed consent will be obtained from a legal representative. Because the study physicians are also involved in the clinical care of patients with acute ischemic stroke, it appears inevitable that in some occasions the study physician will also be the patient's treating physician.

As this is an acute stroke trial with a narrow time window for the start of treatment, and because of time-consuming study-related procedures after informed consent will be obtained, the time for consideration of participation in the trial will be limited to the first few hours after stroke onset. Even within this time frame, a decision on participation should preferably be taken as soon as possible, at least within 30 minutes.

Especially patients with large cortical infarcts may not be able to judge the pros and cons of participation in the trial sufficiently, most often because of aphasia. As aphasia is present in approximately 25% of the patients with acute stroke and because patients with large cortical infarcts may benefit most from intra-arterial treatment on theoretical grounds, it may be considered inappropriate to exclude these patients from the trial. Incapacitated patients in the control group are treated according to current standards and participation in the trial does therefore not carry a risk; the burden caused by the additional investigations is considered minimal.

9.3 ACCESS TO APPROPRIATE TREATMENT.

The design of MR CLEAN allows that all patients with an indication for intravenous alteplase will be treated accordingly. All patients will be managed according to local stroke care protocols, which have to be in agreement with national guidelines.

9.4 RADIATION EXPOSURE

All participants in the trial will undergo CTA at 24-28 hours to assess recanalization after treatment. Radiation exposure for CTA is an attributable 3.5-3.6 mSV (milliSievert). At day 5-7 all patients will undergo a CT scan to assess infarct size. Radiation exposure for this CT scan is an attributable 2.1-2.3 mSV.

For CTA patients need to be injected with contrast fluid. We believe the indication for this CTA justifies the extra contrast load.

10. ADMINISTRATIVE ASPECTS AND PUBLICATION

10.1 PRIVACY

All included patients will be assigned a unique number. Name and address will be stored separately from the study data. Consent with participation in the study will be asked from all patients after presenting them with standard written forms. The information describes the purpose of the study, interventions, potential hazards and benefits and the procedures for recording of clinical information and three month follow up.

10.2 SUBSTUDIES

Substudies will be carried out on the role of hemostatic factors as effect modifiers in endovascular treatment (van Oostenbrugge). The hematological substudy is called: SMARTIS (the study of haemostatic markers in intra arterial treatment for acute ischemic stroke). We refer to a separate study protocol for this. A second substudy will be carried out to investigate the role of MRI (DWI sequence). This sequence is conducted to identify the infarct core (see separate substudy protocol: DIANE; ("Value of Diffusion-Weighted MRI for Selection of Patients for IA Treatment for Acute Ischemic Stroke in the NEtherlands"). Furthermore we will carry out a third substudy on long term follow-up and cost-effectiveness of endovascular treatment (Roos) . For this study the follow-up will be extended to two years. We refer to a separate substudy protocol for a detailed description ('CLOT-MR CLEAN: Cost-effectiveness analyses and LOng Term follow-up in patients randomised in a multicenter randomized clinical trial of endovascular treatment for acute ischemic stroke in The Netherlands').

A substudy on clinical and radiological predictors of recanalization (Majoie) and functional outcome after treatment (Dippel) as well as interobserver and validation studies of rapid reperfusion scores will be carried out. The MR CLEAN investigators share a positive attitude towards the conductance of substudies in general. Proposals for substudies will be discussed within the executive committee and decisions will be made by the steering committee.

10.3 PUBLICATION POLICY

The writing committee for the main publication consists of members of the executive committee. Publication of the main study results, substudies described in this protocol and of future substudies will be on behalf of the MR CLEAN investigators. All investigators will have the opportunity to read and comment on a manuscript before it will be submitted for publication.

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Tables

TABLE 1. ABBREVIATIONS

Abbreviation	Description
ALDS	Academic Medical Center Linear Disability Scale
ASPECTS	Alberta Stroke Program Early CT score
BI	Barthel index
CT	Computed tomography
CTA	Computed tomography angiography
DMC	Data Monitoring Committee
DWI	Diffusion Weighted Imaging
EQ5D	EuroQol 5 dimensions scale
GCS	Glasgow Coma Score
ICH	Intracerebral hemorrhage
MI	Myocardial infarction
MRA	Magnetic Resonance Angiography
MRI	Magnetic Resonance Imaging
mRS	Modified Rankin Scale
NIHSS	National Institutes of Health Stroke Scale
PAD	Peripheral arterial disease
TICI	Thrombolysis in Cerebral infarction
TICS	Telephone Interview for Cognitive Status
TIMI	Thrombolysis in Myocardial Infarction

TABLE 2: RANDOMIZED CLINICAL TRIALS OF INTRA-ARTERIAL THROMBOLYSIS

Study	Intervention-contrast	N	Recanalization	Poor outcome mRS >2	ARR	RR
PROACT I [15]	IA r-pro-UK + heparin vs heparin	42	15/26 vs 2/14 58% vs 14%	18/26 vs 11/14	9%	0.9 (0.6-1.3)
PROACT II[16]	IA r-pro-UK + heparin vs heparin	180	66% vs 18%	73/121 vs 44/59	14%	0.8 (0.7-1.0)
MELT[17]	Ia urokinase +/- mechanical thrombectomy vs control	114	53% vs (n.r.)*	29/57 vs 35/57	11%	0.8 (0.6 – 1.2)
SYNTHESIS[19]	IA alteplase w/wo MERCI vs I.V. alteplase, both 0.9 mg/kg	54	NR	52% vs72%	19%	0.7 (0.5 -1.1)

ARR=absolute risk reduction; RR= relative risk; mRS= modified Rankin Scale score. Recanalization: TIMI 2+3

TABLE 3 CONTROLLED STUDIES AND CASE SERIES OF I.V. + I.A. ALTEPLASE

Study or author	Intervention (dose, mode)	Design	NIHSS	N	SICH (%)	Recanalization (N,%)	Poor outcome mRS >2 (N,(%)
EMS [22]	I.V. alteplase 0.6 mg/kg followed by i.a. alteplase vs i.a. alteplase alone	RCT	-	35	6% 11%	9/11 (82%) 5/10 (50%)	NR
Wolfe[27]	I.V. alteplase 0.6 mg/kg followed by i.a. alteplase vs i.a. alteplase alone	Cohort	-	96	12%	27/41 (66%) 33/55 (60%)	22/41 (54%) 35/55 (64%)
Ernst[25]	I.V. alteplase 0.6 mg/kg followed by i.a. alteplase	Cohort		16	5%	11/16 (69%)	6/16 (38%)
Suarez[26]	I.V. alteplase 0.6 mg/kg followed by i.a. alteplase / urokinase	Cohort		45	0%	18/24 (67%)	5/24 (21%)
Hill[29]	I.V. alteplase 0.9 mg/kg followed by i.a. alteplase	Cohort		6	0%	3/6 (50%)	NR
Shaltoni[28]	I.V. alteplase 0.9 mg/kg followed by i.a. thrombolytics	Cohort		69	6%	50/69 (73%)	45%
Burns[30]	I.v. alteplase 0.9 mg/kg followed by i.a. reteplase w/wo mechanical thrombectomy	Cohort		33	12%	24/33 (73%)	NR
Keris[64]	IA alteplase 25mg followed by i.v. alteplase 25 mg versus no thrombolysis	RCT		45	0% 0%	6/12 (50%) NR	2/12(17%) 22/33 (67%)
IMS I[65]	I.V. alteplase 0.6 mg/kg followed by angiography and endovascular treatment (ia alteplase, heparin) when indicated	Cohort		80	6.3%	35/62 (56%)	46/80 (58%)
IMS II[24]	I.V. alteplase 0.6 mg/kg followed by angiography and endovascular treatment (ia alteplase, heparin and i.a. ultrasonography (MicroLysUS device)	Cohort		81	9.9%	33/55 (60%)	44/81 (54%)
Sohn[31]	I.v. alteplase, followed by i.a. urokinase and/or mechanical thrombectomy	Cohort	14	157	12.7%	NR	NR
Kim[32]	I.v. alteplase followed by urokinase	Cohort	16	18	5.6%	16/18 (89%)	9/18 (50%)

TABLE 4 STUDIES OF MECHANICAL TREATMENT IN ACUTE ISCHEMIC STROKE

Study	Device/ Intervention	Therapeutic Window	NIHSS	N	Recanalization (TIMI2,3) (N,%)[3]	Symptomatic haemorrhages (N,%)	Poor outcome (mRS >2) (N,%)
MERCI –II[33]	MERCI retriever, i.a. alteplase	8 hrs	20	141	69 (46%)	11 (7%)	94/130 (72%)
Multi MERCI final[34]	MERCI retriever, i.a. alteplase, preceded by i.v. alteplase[4]	8 h	19	164	112 (68%)	16 (10%)	102/160 (68%)
Devlin[35]	MERCI retriever, i.a. alteplase, preceded by i.v. alteplase	8h	18	25	14 (56%)	1 (4%)	19 (76%)
Kim[36]	MERCI retriever, i.a. alteplase, preceded by i.v. alteplase	8h	21	24	12 (50%)	2 (8%)	18 (75%)
Bose[37]	PENUMBRA device	8h	23	21	21 (93%)	2 (10%)	?
McDougall[38]	PENUMBRA device	8h	18	125	102 (82%)	14 (11%)	94 (75%)
Zaidat[40]	Wingspan-Neuroform stents w or w/o i.a. or i.v. alteplase	8 h	18	9	8 (89%)	0	3 (33%)
Brekenfeld[41]	Wingspan stents after failed i.v. treatment w or w/o i.v. or i.a. alteplase	8 hrs	14	12	11 (92%)	0	9 (75%)

[3] Recanalization and outcome rates, but not SICH rates were recalculated according to intent to treat principle, assuming that not-reported patients (i.e. patients without intervention) had no recanalization and poor outcome.

[4] In most studies, patients treated within 3 hrs received i.v. alteplase, and a small percentage was treated with i.a. thrombolytic.

TABLE 5A. MODIFIED RANKIN SCALE[66]

Category	Short description	Long description
0	No symptoms	No symptoms
1	Symptoms, no disability	Minor symptoms that do not interfere with lifestyle
2	Slight disability	Slight disability, symptoms that lead to some restriction in lifestyle, but do not interfere with the patient's capacity to look after himself.
3	Moderate disability	Moderate disability, symptoms that significantly restrict lifestyle and prevent totally independent existence
4	Moderately severe disability	Moderately severe disability, symptoms that clearly prevent independent existence though not needing constant attention
5	Severe disability	Severe disability, totally dependent patient requiring constant attention day and night.
6	Dead	Death

TABLE 5B. MODIFED THROMBOLYSIS IN CEREBRAL INFARCTION (TICI) SCALE.[45]

Grade	Short description	Long description
0		No perfusion.
1		Antegrade reperfusion past the initial occlusion, but limited distal branch filling with little or slow distal reperfusion.
2a		Antegrade reperfusion of less than half of the previously occluded target artery ischemic territory (eg, in 1 major division of the MCA and its territory).
2b		Antegrade reperfusion of more than half of the previously occluded target artery ischemic territory (eg, in 2 major divisions of the MCA and their territories).
3		Complete antegrade reperfusion of the previously occluded target artery ischemic territory, with absence of visualized occlusion in all distal branches.

TABLE 5C CLOT BURDEN SCORE FOR CTA AND MRA[46]

Absence of contrast opafication at	Score	
Supraclinoid internal carotid artery	2	
Proximal M1	2	
Distal M1	2	
Infraclinoid internal carotid artery	1	
A1 branch	1	
M2 branch 1	1	
M2 branch 2	1	
Total score: 10 – Sum	Sum	

TABLE 5D ARTERIAL OCCLUSIVE LESION SCALE

AOL Grades	Definitions
Grade 0	Complete occlusion of the target artery.
Grade 1	Incomplete occlusion of the partial local recanalization at the target artery with no distal flow.
Grade 2	Incomplete occlusion of the partial local recanalization at the target artery with any distal flow.
Grade 3	Complete recanalization and restoration of the target artery with any distal flow.

TABLE 6A. SCHEDULE OF STUDY ACTIVITIES

	Inclusion	24 hrs	day 5-7	3 months
Clinical evaluation	x	x	X	x
NIHSS	x	x	X	
Laboratory	x	x		x
Neuroimaging	x	x	X	
Barthel index			X	x
Modified Rankin Scale				x
EQ5D				x

TABLE 6B. LIST OF STUDY DATA

Screening	
Inclusion criteria	
Exclusion criteria	

Baseline	
Demographics	Age, gender
Clinical	NIHSS, NIHSS supplemental motor score, pre-stroke mRS, blood pressure, GCS, weight, height, body temperature.
Medical history	Previous stroke, previous MI, PAD, diabetes mellitus
Medication	Antiplatelet agents, coumarines, heparin(oids), oral thrombin antagonists and oral factor Xa inhibitors., statines
Vascular risk factors	Hypertension, atrial fibrillation, diabetes mellitus, smoking, hypercholesterolemia
Laboratory parameters	INR, kreatinine, GFR (Cockroft-Gault), serum glucose, CRP. SMARTIS substudy: 5x9 cc venous blood sample (before randomization)
Neuro imaging[5]	CT: location, ASPECTS score, hemorrhagic transformation (NINDS/ECASS classification), hyperdense artery sign. CTA: location, clot burden score/collateral score If possible CTP: location, infarct core size, penumbra size, penumbra/infarct index. If possible DWI: location and infarct core size.
Treatment	Intended mode of endovascular treatment

[5] Neuro-imaging parameters will be assessed by a central subcommittee.

Intervention	
Modalities	Actual treatment: i.a. thrombolysis (urokinase, alteplase, other), mechanical treatment (device /type).
Timing	Time of: onset (last seen well), admission, plain CT, CT angiography, (CTP), DWI start of i.v. alteplase, end of i.v. alteplase, start of endovascular procedure (needle in groin), first i.a. bolus, end of revascularization, end of procedure.
Dosages of thrombolytics	Dose of urokinase, alteplase, other agent.
Effect of intervention	mTICI score at start of procedure, mTICI score at end of procedure
Complications	Procedure-related complications Neurological deterioration

Follow-up	
Clinical assessment at 24 hours	NIH Stroke Scale, NIHS supplemental motor scale, Complications
Laboratory parameters	SMARTIS substudy: 5x9 cc venous blood sample
Neuro-imaging at 24 hours	Plain CT: location, ASPECTS score, hemorrhagic transformation (NINDS/ECASS classification), hyperdense artery sign. CT angiography: location, Clot Burden Score/Collateral score CT Perfusion: location, infarct core size, penumbra size, penumbra/infarct index
Neuro imaging at 5-7 days	Plain CT: location, ASPECTS score, hemorrhagic transformation (NINDS/ECASS classification), hyperdense artery sign.
Clinical assessment at 1 wk or discharge	NIH stroke scale; Barthel Index; Global assessment of improvement or deterioration; clinical complications: hemorrhages; Laboratory: GFR.
Clinical assessment at 90 days.	Modified Rankin score, Barthel index, EQ5D.
Laboratory parameters	SMARTIS substudy: 5x9cc venous blood

APPENDICES

APPENDIX 1. LIST OF COLLABORATING INVESTIGATORS

COORDINATING INVESTIGATORS

Puck Fransen, Dept of Neurology, Erasmus MC Rotterdam.
Debbie Beumer, Dept of Neurology, UMC Maastricht.
Olvert Berkhemer, Dept of Radiology, AMC Amsterdam.
Lucie van den berg, Dept of Neurology, AMC Amsterdam.

PRINCIPAL INVESTIGATORS

Diederik Dippel, neurologist, Erasmus MC Rotterdam, Charles B Majoie, neuroradiologist, AMC Amsterdam, Yvo Roos, neurologist, AMC Amsterdam, Robert van Oostenbrugge, neurologist, UMC Maastricht, Aad van der Lugt, neuroradiologist, Erasmus MC Rotterdam.

LOCAL PRINCIPAL INVESTIGATORS:

No	Center	Neurologist	Interventionist
1	Erasmus MC Rotterdam	D. Dippel	P.A. Brouwer
2	Amsterdam Medical Center	Y. Roos	C. Majoie
3	Maastricht Medical center	R. v Oostenbrugge	W. van Zwam
4	UMC Utrecht	J. Kappelle	R. Lo
5	LUMC te Leiden	M. Wermer	M. van Walderveen
6	UMC Nijmegen	E. van Dijk	J. de Vries
7	Haaglanden Ziekenhuis Den-Haag	J. Boiten	G. Lycklama
8	HAGA Ziekenhuis Den-Haag	S. de Bruyn	L. van Dijk
9	UMC Groningen	P. Vroomen	O. Eshgi
10	St. Elisabeth Zkh Tilburg	P. de Kort	W. van Rooy
11	Isala klinieken Zwolle	P. van den Bergh	B. van Hasselt
12	Catharina Ziekenhuis Eindhoven	K. Keizer	X. Tielbeek
13	St. Antonius Nieuwegein	W. Schonewille	J. de Vos
14	Rijnstate Ziekenhuis Arnhem	J. Hofmeijer	J. van Oostayen
15	VU Medisch Centrum Amsterdam	M. Visser	Randomisation only
16	Reinier de Graaf Delft	L. Aerden	Randomisation only*
17	Atrium Heerlen	T. Schreuder	R. Heijboer
18	Medisch Spectrum Twente	H. den Hertog	D. Gerrits
19	Sint Lucas Andreas Ziekenhuis	R. van den Berg-Vos	Randomisation only

* patients may be treated locally by the team from HAGA ziekenhuis.

APPENDIX 2. STUDY COMMITTEES

DATA MONITORING COMMITTEE

Chair: Professor Martin Brown, National Hospital for Neurology & Neurosurgery, London, UK.

Member: Thomas Liebig

Independent Statistician: Theo Stijnen

EXECUTIVE AND WRITING COMMITTEE

Diederik Dippel, neurologist, Erasmus MC Rotterdam, Charles B Majoie, neuroradiologist, AMC Amsterdam, Yvo Roos, neurologist, AMC Amsterdam, Wim van Zwam, neuroradiologist, UMC Maastricht, Robert van Oostenbrugge, neurologist, UMC Maastricht, Aad van der Lugt, neuroradiologist, Erasmus MC Rotterdam,

Puck Fransen, research coordinator, Erasmus MC Rotterdam.

Debbie Beumer, research coordinator, Maastricht UMC.

Olvert Berkhemer, research coordinator, AMC Amsterdam.

Lucie van den Berg, research coordinator on the economic evaluation and long-term follow-up, AMC Amsterdam.

Hester Lingsma, methodologist, Erasmus MC Rotterdam.

IMAGING ASSESSMENT COMMITTEE

Charles B Majoie, neuroradiologist, AMC Amsterdam, Aad van der Lugt, neuroradiologist, Erasmus MC Rotterdam, , Wim van Zwam, neuroradiologist, UMC Maastricht, Rene van den Berg, neuroradiologist, AMC Amsterdam, Geert Lyclama a Nijeholt, neuroradiologist, MC Haaglanden, Den Haag, Sjoerd Jenniskens, neuroradiologist, UMC Nijmegen, Henk Marquering, medical physicist, AMC Amsterdam, Ludo Beenen, ED radiologist, AMC Amsterdam, Marianne van Walderveen, radiologist LUMC, Leiden, Olvert Berkhemer, PhD student, AMC Amsterdam.

OUTCOME ASSESSMENT COMMITTEE

Yvo Roos neurologist, AMC Amsterdam (chair) Peter J Koudstaal, neurologist, Erasmus MC Rotterdam, Ewoud van Dijk, neurologist UMC St. Radboud, Nijmegen , Jelis Boiten, neurologist MC Haaglanden, Den Haag.

ADVERSE EVENT ADJUDICATION COMMITTEE

Robert van Oostenbrugge, neurologist, UMC Maastricht (chair) .
Zwenneke Flach, Radiologist, Isala Kliniek Zwolle, Marieke Wermer, Neurologist, LUMC Leiden.

TRIAL STATISTICIANS

Hester Lingsma, methodologist, Erasmus MC Rotterdam, Ewout Steyerberg, methodologist, Erasmus MC Rotterdam.

INDEPENDENT (ANGIOGRAPHIC) CORE LAB

Albert Yoo (chair), Massachusetts General Hospital Boston, USA

ADVISORY BOARD

Peter Koudstaal, neurologist, Erasmus MC Rotterdam, Tommy Andersson, neuro interventionist, Karolinska Hospital, Stockholm, Sweden, Heinrich Mattle, neurologist, University hospital, Bern, Switzerland, Nils Wahlgren, neurologist, Karolinska Hospital, Stockholm, Sweden.

APPENDIX 3 RECOMMENDATIONS OF THE STEERING COMMITTEE WITH REGARD TO ENDOVASCULAR TREATMENT PROCEDURES, THROMBOLYTIC AGENTS, AND TYPE OF MECHANICAL THROMBECTOMY.

GENERAL

Randomization, inclusion in the trial and subsequent endovascular treatment should be started as soon as possible after presentation in all eligible patients. The time-path below gives an indication about how soon the following steps need to take place.

TABLE A1. OPTIMAL TIME-PATH FOR TREATMENT AND INCLUSION IN MR CLEAN OF PATIENTS WITH ACUTE ISCHEMIC STROKE AND RELEVANT ANTERIOR CIRCULATION ARTERIAL INCLUSION

Procedure	Tx with i.v. alteplase	No tx with i.v. alteplase
Arrival at ER	0	0
Start neuroimaging	20 min	20
Start iv-alteplase	30 min	-
Randomization	60 min	30
Start endovascular treatment	90 min (since ER)	60

The selection process is shown in Figure 3 of the protocol. Three clinical situations can be distinguished:

1 “No response to i.v. alteplase”: in these patients, there is no favorable response to treatment with intravenous alteplase. Vascular neuroimaging (CTA, MRA, TCD) may follow treatment with i.v. alteplase, but the steering committee recommends that vascular neuroimaging is done at the start of i.v. treatment. The exact definition of favorable response is left to the discretion of the local investigator, but the steering committee suggests the following: neurological recovery to a level that would obviate the indication for i.v. alteplase would the patient present with these symptoms. No minimum time period between assessment of the response to i.v. alteplase and end of treatment is required, but the steering committee recommends that randomization and inclusion into the trial, andsubsequent endovascular treatment should be started as soon as possible.

2 “Outside therapeutic window for i.v. alteplase”: these patients present in the 4.5 to 6 hour window. They can be included in the study, and randomized for endovascular treatment or no endovascular treatment.

3 “ Contra-indications for i.v. alteplase”: these patients have contraindications for i.v. alteplase that do not apply to mechanical thrombectomy, for example a recent cerebral infarction in a different vascular area, previous cerebral hemorrhage, treatment with coumarines leading with INR 1.7 to 3.0). They can be included in the study, and randomized for endovascular treatment or no endovascular treatment. Intra-arterial thrombolysis is not recommended for these patients, but mechanical thrombectomy ís.

NEUROIMAGING

Neuroimaging studies to assess vessel patency should preferably be done before or simultaneously with treatment with i.v. alteplase, in order not to lose time and brain. Especially, the delay between end of i.v. infusion and start of endovascular treatment should be minimized to less than 1 hour.

THROMBOLYTIC AGENTS, DOSE AND TYPE

For intra-arterial thrombolysis urokinase or alteplase may be used. A dose of 1 mg alteplase is considered to be equivalent to 12.500 U (10.000-15.000 U) urokinase. [44]

Patients who have been pre-treated with i.v. alteplase should not receive more than 30 mg alteplase during intra-arterial treatment, or an equivalent dose of 400,000 U urokinase. The steering committee recommends that the thrombolytic agent is delivered in shots of 5 mg alteplase or 50.000 – 100.000 U urokinase , in 5-10 minutes time intervals. Vessel patency should be checked after each shot. Before the last i.a. shot is given, the interventionist has to decide to go for mechanical thrombectomy. After mechanical thrombectomy the shot can be given, in order to prevent distal re-occlusion.

Patients should be treated with intra-arterial thrombolyis until recanalization is reached or the maximum cumulative dose is reached. When intra-arterial treatment will be delivered directly (i.e. within 30 minutes) after intravenous alteplase, the clinical investigator may consider giving only 2/3 of the total i.v. alteplase dose. The safety of these dosing schedules is discussed in section 1.2 of the protocol.

TABLE A2-A: DOSING SCHEME FOR INTRAVENOUS AND INTRA ARTERIAL THROMBOLYSIS WITH ALTEPLASE OR UROKINASE

Weight (kg)	Alteplase Bolus i.v (mg)	Alteplase 2/3 dose (mg)	Alteplase 1/3 dose (mg)	Urokinase equivalent 1/3 Dose (U)	Alteplase Max total dose (mg)*	Urokinase equivalent max total dose (kU)
50	5	25	15	200	45	600
55	5	28	17	200	50	600
60	5	31	18	225	54	675
65	6	34	19	250	59	750
70	6	36	21	250	63	750
75	7	38	23	275	68	825
80	7	41	24	300	72	900
85	8	43	26	325	77	975
90	8	46	27	350	81	1,050
95	9	48	29	375	86	1,125
100	9	51	30	400	90	1,200
>100	9	51	30	400	90	1,200

*urokinase dose is rounded 25,000 U.

MECHANICAL THROMBECTOMY

Randomized clinical trials of devices for mechanical thrombectomy have not been done.

General criteria for the use of devices in MR CLEAN are publication of a case series at least 20 patients treated with the device, with an acceptable rate of complications and a TIMI 2/3 recanalization rate of more than 50%.

Currently, two devices for mechanical thrombectomy (MERCI and PENUMBRA), specifically designed for treatment of acute intracranial arterial occlusions have been evaluated in case series, and have been FDA approved for this purpose. One catheter system, EKossonics SV, designed for delivery of ultrasound-waves to the occluding thrombus, has been evaluated in IMS2 and will be evaluated together with the MERCI system in IMS3. These devices have been shown to be capable of recanalization, with an acceptable rate of complications.[39] Therefore, Currently the MERCI, Penumbra, Solitaire and Ekossonics SV devices, fulfill the requirements as formulated by the steering committee (Table A3).

Two stent devices have been approved for intracranial treatment, but not for treatment of patients with acute ischemic stroke, moreover published experience with treatment is limited. The steering committee of MR CLEAN will be actively seeking evidence that will make specific stenting devices acceptable for use in the trial.

TABLE A3. LIST OF MECHANICAL THROMBECTOMY DEVICES THAT ARE AVAILABLE IN THE NETHERLANDS, THEIR MODE OF ACTION AND CURRENT STATUS.

Device	Mode of action[6]	Manufacturer	NL Dealer	Approval[7]	Evaluation studies[8]
Allowed					
Merci retriever	R,A	Concentric Medical	Top Medical	FDA	CS
Penumbra System	A,F	Penumbra Incm US	Penumbra	FDA	CS
Solitaire Stent (ev3)	S	EV3	Ev3	CE	CS
EKossonic SV	U	EKOS Bothell Wash	Angiocare	CE	CR
Pending					
Wingspan stent	A	Boston Scientific	Boston sci	FDA[9]	CS
CATCH device	R,A	Balt Extrusion	Angiocare	CE	CR
Revasc	S	Micrus	Angiocare	CE	?
Moses	S	Micrus	Angiocare	CE	?
Fast	A	Micrus	Angiocare	CE	?
Vasco+35ASPI	A	Balt	Angiocare	CE	?

[6] R=retraction, A=aspiration, S=Stenting, F=Fragmentation, U= ultrasound enhanced lysis

[7] FDA = Federal Drugs Agency, CE = *Conformité Européenne*, which means in concordance with European legislation.

[8] U=unpublished studies only, CR=case reports, CS=case series

[9] Approved only for elective treatment of intracranial stenosis.

APPENDIX 4 INTERVENTION-RELATED ANGIOGRAPHIC IMAGING

WHEN

1) The pre-intervention angiogram should be performed via the guiding catheter to evaluate the site of vessel occlusion, extent of thrombus, territories involved, concomittant pathologies and to assess collateral flow. [45]

2) After passing the occlusion site a microcatheter injection should be performed to assess the distal vascular bed.

3) After each bolus of thrombolytic agent a control angiogram via a microcatheter injection and/or guiding catheter injection should be performed to assess vessel patency.

4) After each passage of a mechanical device, a control angiogram should be performed via the guiding catheter to assess vessel patency.

5) At the end of the procedure the angiogram should be repeated via the guiding catheter to assess the final angiographic outcome

HOW

1. Pre-intervention and end-of-procedure angiogram:

The angiogram should include the internal carotid artery (or common carotid in case of occlusion or severe stenosis of internal carotid) feeding the target vessel as demonstrated on CTA.

To completely assess the collateral circulation, injections of the contralateral internal carotid artery (or common carotid in case of occlusion or severe stenosis of internal) and the dominant vertebral artery are preferred. However, they are not necessary for inclusion in the study.

AP views and lateral views of the intracranial arteries are obtained. It is essential that the angiograms include both the arterial and venous phases of the injection to evaluate the collateral pathways and perfusion of the distal vascular bed.

The angiograms should be performed via the guiding catheter with the same catheter position, contrast injection volume and rate (6-8ml with 4ml/s for internal carotid, 8-10ml with 4-6ml/s for common carotid and 6-8ml with 4-5ml/s for vertebral artery), and angiographic views before, after the procedures to adequately assess the results of therapy.

2. After passing the occlusion site, after each bolus of thrombolytic agent and after each pass of a mechanical device:

At least one view, at the discretion of the interventionalist.

The complete series of the angiograms and microcatheter injections should be saved according to the DICOM standard and a copy should be sent to the imaging assessment committee.

APPENDIX 5: PROTOCOL AMENDMENT MR CLEAN TRIAL; SUBSTANTIAL PROTOCOL 3.1.

Amendment 1:
Add under Add under Specific exclusion criteria for intended intra-arterial thrombolysis:

" Current treatment with oral thrombin antagonists, such as argatroban and dabigatran or treatment with rivaroxaban, a selective Factor Xa inhibitor."
Rationale: the new oral factor Xa inhibitors and thrombin antagonsists provide a level of anticoagulation that is comparable with cumarines or heparin. It is very likely that concomittant treatment with alteplase of urokinase leads to an increased risk of (local) hemorrhage. The risk of treatment with mechanical devices is likely to be unchanged.

Amendment 2:
Add under substudies:
''SMARTIS: study of haemostatic markers in intra-arterial treatment for acute ischemic stroke''.
Rationale: the study was planned to be carried out on the role of heamostatic factors as effect modifiers in endovascular treatment

Amendment 3:
Replace vacancy under after AMC Amsterdam under appendix 1, coordinating investigators by: Olvert Berkhemer

Amendment 4:
Replace vacancy under after UMC Maastricht under appendix 1, coordinating investigators by: Debbie Beumer

Amendment 5:
Replace Trude Leertouwer by Sjoerd Jenniskens, neuroradiologist, UMC Nijmegen
under appendix 2,Imaging assessment committee

Amendment 6:
Replace vacancy under appendix 2, outcome assessment committee by Ewoud van Dijk, neurologist UMC St. Radboud, Nijmegen , Jelis Boiten, neurologist MC Haaglanden, Den Haag.

Amendment 7:
Replace vacancy under appendix 2, adverse event committee by Zwenneke Flach, Radiologist, Isala Kliniek Zwolle, Marieke Wermer, Neurologist, LUMC Leiden

APPENDIX 6 : PROTCOL AMENDMENT MR CLEAN TRIAL SUBSTANTIAL PROTOCOL 3.2

Amendment 1: Add on page 2/3 under participating centers and independent physicians three new centers (listed above). Added corrected one independent physicians. The newly added centers will only randomize patients since there is no interventional radiologist present. For further explanation see Amendment 3.

Amendment 2: Added and replaced under "List of authors under the main title": JR2 was changed into Debbie Beumer. JR3 was changed into Olvert A Berkhemer.

Amendment 3: Add under 4.3 Participating centers and center eligibility. Three new centers are added where patients can be randomized. Prior to randomization the neurologist of that center will contact the closet center MEC approved MR CLEAN center. If there is a possibility for intra arterial treatment in that particular center the so called 'randomization center' will continue to randomize the patients. If the patient is randomized in the intra arterial treatment arm, the patient will be transferred to the MEC approved MR CLEAN center. If the patient is randomized in the control arm the patient will be monitored in this randomization center.

Amendment 4: Add under substudies: "DIANE: Value of Diffusion-Weighted MRI for Selection of Patients for IA Treatment for Acute Ischemic Stroke in the NEtherlands." For further explanation we refer to the separate protocol.

Amendment 5: Add under Tables, “Table 1 Abbreviations”: DWI “Diffusion Weighted Imaging”

Amendment 6: Added M2 branche 2 and adjusted M2 branche 1 under “Table 5c CLOT BURDEN SCORE’. Sum score is now 10.

Amendment 7: Add under Tables, Table 6b. List of study data, Baseline: “If possible DWI: location and infarct core size. And under intervention: DWI

Amendment 8: Deleted under Tables, Table 6b: List of study data, Follow-up: Deleted NIHSS stroke scale at 90 days.

Amendment 9: Appendix 1, coordinating investigators: Formatting and hierarchy of names was changed. Added Dept of Radiology to Olvert Berkhemer

Amendment 10: Added three local principal investigators changed two others, to the table in APPENDIX 1 LIST OF COLLABORATIONG INVESTIGATORS. Table “Local principal investigators”.

Amendment 11: Added 2 members to the imagine committee in Appendix 2 “Imaging Assessment Committee”

APPENDIX 7: PROTCOL AMENDMENT MR CLEAN TRIAL; SUBSTANTIAL PROTOCOL 3.3

Substantial changes:

Amendment 1: Added under “List of authors under the main title”: junior researcher 4 : Lucie A. van den Berg was added,
Rationale of change: this junior researcher is added to investigate the economic evaluation and long-term follow up of the MR CLEAN trial.

Amendment 2: On page 18 under paragraph 6. Methods, under Functional Outcome, removed from the original text: *Score on the Academic Medical Centre Linear Disability Scale at 90 Days and Score of Telephone Interview on Cognitive Status (TICS) at 90 days*.
Rationale of change: two measurements were removed due to practical problems of implementing the Academic Medical Centre Linear Disability Scale and time management problems during the telephone interview at three months.

Amendment 3: On page 18 under paragraph 6. Methods, under Data for cost-effectiveness analysis, added to the original text: *Therefore a separate substudy on cost-effectiveness will be carried out (see 10.2 substudies and the separate substudy protocol for more detailed information*).
Rationale of change: sentence was added to announce a substudy on economic evaluation.

Amendment 4: On page 20 under paragraph 6.4 Study procedures, removed from the original text*: Academic Medical Centre Linear Disability Scale and TICS.*
Rationale of change: see Amendment 2.

Amendment 5: On page 23 under paragraph 10 Administrative aspects and publication, under 10.2 substudies, added to the original text: Furthermore we will carry out a third substudy on long term follow-up and cost-effectiveness of endovascular treatment (Roos) . For this study the follow-up will be extended to two years. We refer to a separate substudy protocol for a detailed description (‘CLOT-MR CLEAN: Cost-effectiveness analyses and LOng Term follow-up in patients randomised in a multicenter randomized clinical trial of endovascular treatment for acute ischemic stroke in The Netherlands’).

Rationale of change: The MR CLEAN trial is an ideal setting for measuring the societal costs related to endovascular treatment after stroke , thereby enabling a full economic evaluation of cerebral endovascular treatment against standard care. The time horizon will be two years. Within this time horizon the MR CLEAN trial itself will answer the question whether higher recanalization rates improve functional outcome and quality of life (see separate substudy protocol CLOT-MR CLEAN).

Amendment 6: On page 36, under TABLE 6A. Schedule of study activities, removed from table*: Academic Medical Centre Linear Disability Scale and TICS.*
Rationale of change: see Amendment 2.

Amendment 7: On page 38, under TABLE 6B. List of study data, removed from table: *Academic Medical Centre Linear Disability Scale and TICS*. Rationale of change: see Amendment 2.

Non substantial administrative changes:

Non substantial amendment 1: On page 23 under paragraph 10 Administrative aspects and publication, under 10.2 substudies, the original text *:* replaced *Another* for *A second*

Non substantial amendment 2: On page 23 under paragraph 10 Administrative aspects and publication, under 10.2 substudies, The original text *A substudy on costs and cost-effectiveness of endovascular treatment (Roos), and on clinical and radiological predictors of recanalization (Majoie) and functional outcome after treatment (Dippel). Interobserver and validation studies of rapid reperfusion scores will be carried out,* was replaced by: *A substudy on clinical and radiological predictors of recanalization (Majoie) and functional outcome after treatment (Dippel) as well as interobserver and validation studies of rapid reperfusion scores will be carried out.*

Non substantial amendment 3: On page 39, under Appendix 1. List of collaborating investigators, under Coordinating investigators, one new junior researcher was added: *Lucie van den berg, Dept of Neurology, AMC Amsterdam, see* .

Non substantial amendment 4 : On page 39, under Appendix 1. List of collaborating investigators, under local principal investigators *J. de Vries* was replaced by *J. van Dijk* as interventionist at the HAGA Ziekenhuis Den Haag.

Non substantial amendment 5: On page 40, under Appendix 2. Study committees, under Executive and Writing committee, one new junior researcher was added : Lucie van den Berg, research coordinator on the economic evaluation and long-term follow-up, AMC Amsterdam and one methodologist: Hester Lingsma, methodologist, Erasmus MC Rotterdam,was added.

APPENDIX 8: PROTOCOL AMENDMENT MR CLEAN TRIAL; SUBSTANTIAL PROTOCOL 3.4

Substantial changes:

Amendment 1: Added two new centers. One center, Medisch Spectrum Twente, will be a MR CLEAN intervention center and will start patient treatment after complying with the protocol guidelines. The other center, Sint Lucas Andreas, will only randomize patients; intra-arterial treatment will be provided by the Academic Medical Center Amsterdam. (Adjustments can be found on page 2, 3 and 39)

Amendment 2: Moved the exclusion criterion: “Cerebral infarction in the distribution of the relevant occluded artery in the previous 6 weeks” from “specific exclusion criteria for intended intra-aterial thrombolysis” to “general exclusion criteria”. Rationale; this criterion was accidently placed under specific instead of general.

Amendment 3: Changed on page 39, the status of the Atrium MC to active MR CLEAN treatment center. The Atrium MC may start treatment after complying with the protocol guidelines. Treatment of patients is done in close collaboration with Maastricht University Medical Center.

Non substantial changes administrative changes:

Non substantial amendment 1: Correction of table 5C “Clot Burden score for CTA and MRA’ on page 35. Added an extra M2 branch in the table, this was a typo in the original table.

Non substantial amendment 2: Added in Appendix 2 “Study Committees”. Added O.A. Berkhemer to the Imaging Assessment committee.

APPENDIX 9: PROTOCOL AMENDMENT MR CLEAN TRIAL; PROTOCOL 3.5

(Non) substantial changes / administrative changes:

Non substantial amendment 1: Correction of the word amendment on title page and correction and added one amendment date on page 5.

Non substantial amendment 2: Corrected neurological decline on the NIHSS scale to 4 or more points (page 18).

Substantial amendment 3: We removed the option for use of transcranial Doppler ultrasound to confirm the intracranial arterial occlusion from the inclusion criteria. Rationale: this option was not operationalized, and transcranial Doppler was not used in the trial (page 15).

Non substantial amendment 4: We updated the TICI scale to the modified TICI scale and are following the definitions as formulated in “Recommendations on Angiographic Revascularization Grading Standards for Acute Ischemic Stroke: A Consensus Statement” published in Stroke. 2013;44:2650-2663 to grade reperfusion on the DSA's acquired during the intervention. (page 34). We replaced the term TICI with m(odified) TICI throughout the manuscript.

Non substantial amendment 5: Added APPENDIX 10: (predefined) Statiscal Analysis Plan (SAP). Rationale: SAP was added as an appendix to the manuscript we submitted to Trials, which now has been provisionally accepted. The SAP defines our prespecified subgroup analyses before closure of the database and deblinding (page 50).

APPENDIX 10 STATISTICAL ANALYSIS PROTOCOL (SAP)

MR CLEAN SAP (STATISTICAL ANALYSIS PLAN).

Diederik Dippel, Hester Lingsma, Olvert Berkhemer.

for the MR CLEAN steering committee.

V 3.0 May 14, 2014.

INTRODUCTION

The purpose of the Multi Center Randomized Clinical trial of Endovascular treatment in The Netherlands (MR CLEAN) is to assess the safety and effect on functional outcome of intra-arterial treatment in patients with acute ischemic stroke caused by intracranial arterial, anterior circulation occlusion.

Here we will summarize the statistical analysis of the data. We will describe how missing data will be handled and subgroup analyses will be performed. Moreover, we will describe the time path of the analyses and the process of deblinding, as well as reporting to the Data Monitoring and Safety Committee (DMSC).

Although we list an extensive number of analyses, we do not imply that all these will be described in the main paper, because of space restrictions.

STATUS OF THE TRIAL

The trial is running well. Inclusion has ended on March 16, 2014. In total, 502 patients have been randomized, but 2 patients have withdrawn consent, leaving 500 patients who have been registered in the database.

RESEARCH QUESTIONS

PRIMARY RESEARCH QUESTIONS

The primary objective of this study is to estimate the effect of endovascular treatment on functional outcome after acute ischemic anterior circulation stroke of less than six hours duration, in patients with a symptomatic intracranial occlusion.

SECONDARY RESEARCH QUESTIONS

The secondary objectives are to assess the safety of endovascular treatment with regard to the occurrence of hemorrhagic and ischemic complications, the efficacy with regard to obtaining recanalization, and to evaluate predictors of recanalization, including imaging aspects and hemostatic parameters. Moreover, we want to assess the safety and efficacy of different types of endovascular treatment (i.e. mechanical treatment, intra-arterial thrombolysis) different combinations of treatment (i.e. with intravenous alteplase) and different timings of treatment.

TRIAL DESIGN

This is a multicenter clinical trial with randomized treatment allocation, open label treatment and blinded endpoint evaluation (PROBE design).

The intervention contrast is intra-arterial treatment (alteplase or urokinase, and/or mechanical treatment) versus no intra-arterial treatment. The treatment is provided in addition to best medical management, which may include intravenous alteplase.

Randomization was stratified for center, dichotomized score on the National Institutes of Health Stroke Scale (NIHSS),[1] treatment with iv alteplase and intended mechanical treatment.

INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria are:

- A clinical diagnosis of acute stroke, with a deficit on the NIH stroke scale of 2 points or more.
- CT or MRI scan ruling out intracranial hemorrhage.
- Intracranial arterial occlusion of the distal intracranial carotid artery or middle (M1/M2) or anterior (A1/A2) cerebral artery, demonstrated with CTA, MRA, DSA.
- The possibility to start treatment within 6 hours from onset.
- Informed consent given.
- Age 18 or over.

General exclusion criteria are:

- Arterial blood pressure > 185/110 mmHg.
- Blood glucose < 2.7 or > 22.2 mmol/L.
- Intravenous treatment with thrombolytic therapy in a dose exceeding 0.9 mg/kg alteplase or 90 mg.
- Intravenous treatment with thrombolytic therapy despite contra-indications, i.e. major surgery, gastrointestinal bleeding or urinary tract bleeding within the previous 2 weeks, or arterial puncture at a non-compressible site within the previous 7 days.
- Cerebral infarction in the distribution of the relevant occluded artery in the previous 6 weeks.

Specific exclusion Criteria for intended mechanical thrombectomy are:

- Laboratory evidence of coagulation abnormalities, i.e. platelet count <40 x 10^9/L, APTT>50 sec or INR >3.0.

Specific exclusion criteria for intended intra-arterial thrombolysis are:

- History of intracerebral hemorrhage.
- Severe head injury (contusion) in the previous 4 weeks.
- Clinical or laboratory evidence of coagulation abnormalities, i.e. platelet count <90 x 10^9/L, APTT>50 sec or INR >1.7.

PRIMARY AND SECONDARY OUTCOMES

The primary outcome is the score on the modified Rankin Scale at 90 days. Secondary outcomes concern imaging parameters, clinical parameters and safety parameters.

Imaging parameters are:

- Vessel recanalization at 24 hours after treatment, assessed by CTA or MRA. The criteria for recanalization on CTA or MRA are based on the Arterial Occlusive Lesion (AOL) scale,[2] and the Clot Burden Score.[3]
- Infarct size assessed by CT on day 5-7, using standard methods, including manual tracing of the infarct perimeter and semiautomated pixel thresholding.[4, 5]

Clinical parameters are:

- Score on the National Institutes of Health Stroke Scale (NIHSS) at 24 hours.
- Score on the NIHSS at 1 week or at discharge.
- Score on the EQ5D at 90 days,[6]
- Barthel index at 90 days.[7]

The primary safety parameter was neurologic deterioration within 24 hours from inclusion in the study. Neurological deterioration was defined as any decline in NIHSS of more than 4 points. In these patients, urgent brain CT is mandatory. This serious adverse event was further classified as due to intracranial hemorrhage, ischemia or other (undetermined) cause.

BLINDING

It was not possible to view the treatment allocation before the patient was registered in the study database, nor was it possible to remove the patient from the study base after treatment assignment has become known. Both patient and treating physician were aware of the treatment assignment. Information on outcome at three months was assessed through standardized forms and procedures by a dedicated research nurse. Assessment of outcome on the modified Rankin scale was based on this information, by assessors who were blind to the treatment allocation. Results of neuroimaging were also assessed in a blinded manner. Information on treatment allocation and 90-day outcome was kept separate from the main study database. The steering committee members were kept unaware of the results of interim analyses of efficacy and safety. The trial statistician combined data on treatment allocation with the clinical data in order to report to the data monitoring and safety committee (DMSC) in a closed session.

STATISTICAL ANALYSIS PROPER

PRIMARY EFFECT ANALYSIS

The main analysis of this trial consists of a single comparison between the trial treatment groups of the primary outcome after 90 days. The analysis is based on the intention-to-treat principle. The primary effect parameter takes the whole range of the modified Rankin scale (mRS) into account and is defined as the relative risk for improvement on the mRS estimated as an odds ratio with ordinal logistic regression.[8] Multivariable regression analysis will be used to adjust for chance imbalances in main prognostic variables between intervention and control group in the primary effect analysis, but also in all secondary analyses and subgroup analyses.[9] These key variables are:

- age,
- stroke severity (NIHSS) at baseline
- time to randomization
- previous stroke,
- atrial fibrillation,
- diabetes mellitus and
- carotid top occlusion versus no carotid top occlusion

PRIMARY EFFECT ANALYSIS IN SUBGROUPS

The effect of intervention on the modified Rankin scale will be analyzed in the following subgroups:

- Age 80 or over versus age less than 80
- NIHSS in tertiles (2-15, 16-19 and 20 or higher)
- Carotid top occlusion present versus no carotid top occlusion
- Time since onset to randomization 120 minutes or less versus more than 120 minutes.
- Extracranial >50% carotid stenosis or occlusion versus no >50% carotid stenosis or occlusion
- ASPECTS 0-4 / 5-7 / 8-10
- Thrombus length >7mm versus 7 mm or less.

Secondary effect parameters will be the improvement according to the classical dichotomizations of the modified Rankin scale, neurological deficit at 24 hours and 1 week measured with the NIHSS, quality of life at 90 days measured with EQ5D and vessel patency at 24 hours as well as infarct size at 5-7 days on CT or MRI.

1. mRS 0-1 versus 2-6 at 90 days
2. mRS 0-2 versus 3-6 at 90 days
3. mRS at 0-3 versus 4-6 at 90 days
4. Barthel index 19-20 versus <19 at 90 days
5. Score on the EQ5D at 90 days
6. Score on the NIHSS at 24 hours
7. Score on the NIHSS at 7 days or discharge
8. Vessel patency (clot burden score) on CTA at 24 hours
9. Infarct size (automated volume measurement) on CT at 5-7 days.

Effect parameter in these first four analyses will be the odds ratio, estimated with multiple logistic regression. The effect parameter on the fourth to sixth outcome (EQ5D and NIHSS) will be a regression parameter beta, estimated with multiple linear regression models. Outcome data may have to be log-transformed. The clot

burden score will be dichotomized into 10 or less than 10 and the effect parameter will be the odds ratio, estimated with multiple logistic regression. The effect on infarct size will be estimated with a multiple linear regression model.

In all analyses, statistical uncertainty will be expressed by means of 95% confidence intervals. Although the size of this study does not allow for precise estimates of treatment effect in subgroups, we assess heterogeneity of effects, and analyze consistency of effects on secondary outcomes.

MISSING DATA AND DEATH

Patients who die within the study period will be assigned the worst score on all outcome measures and taken into the analysis. Proportions of missing values for all variables will be reported. Variables that will be used to adjust the primary and secondary effect analyses (age, NIHSS at baseline, time to randomization, previous stroke, atrial fibrillation, diabetes mellitus and site of intracranial occlusion are designated as key variables. Missing values for these variables (if any) will be analysed for randomness and imputed with standard methods.

TIME PATH OF THE ANALYSES AND LOCKING OF THE DATABASE.

To maximize time for analysis and interpretation of the results and allow presentation of the final results at the World Stroke Conference by the end of October 2014, a soft-lock and preliminary analysis will be performed once the last patients have had their final outcome recorded and the data has been reviewed by the DMSC by mid July 2014. Following final data cleaning on the last patients to be recruited, a hard-lock will be performed by mid September. The results of this analysis will be considered by the Trial Steering Committee by the end of September, 2014. The preliminary interpretation will be performed after soft-lock by DD, CM and HL; they will not be involved in resolving any final queries to maintain the integrity and blinding of the final database. The approach of soft-lock then hard-lock is a standard approach in large trials and allows more time to be spent on considering the results of a trial, their interpretation and presentation for publication.

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FIGURES

FIGURE 1: TRIAL LOGO.

FIGURE 2

Assumed distribution of the primary outcome, according to the modified Rankin scale in group receiving no intra-arterial treatment (mRS-C) (control) and the group receiving intra-arterial treatment (mRS-I). THe cumulative proportion of patients in mRS 0-3 has increased with 10%.

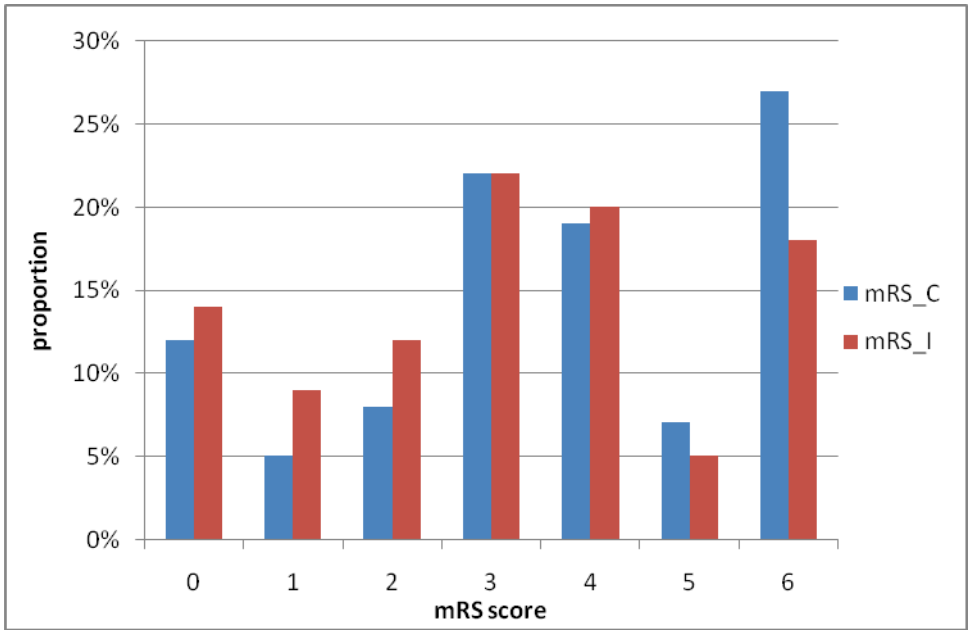
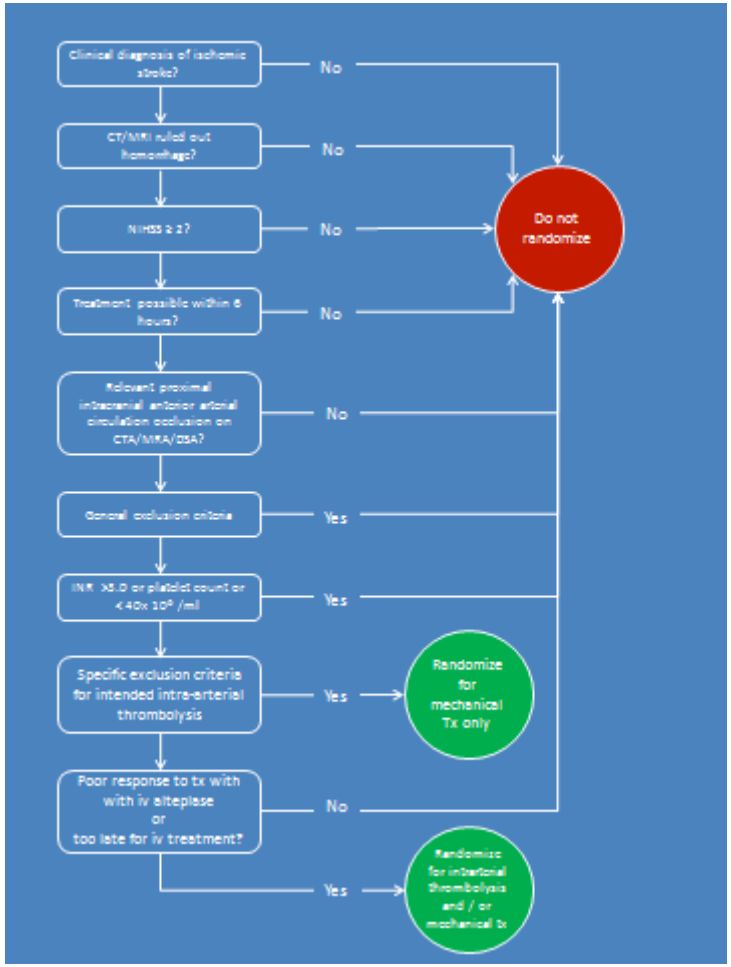


FIGURE 3. RANDOMIZATION AND INCLUSION OF PATIENTS IN THE TRIAL



Inclusion criteria
- Clinical diagnosis of ischemic stroke
- CT/MRI ruled out hemorrhage
- NIHSS ≥ 2
- Treatment possible within 6 hours
- Relevant proximal intracranial anterior circulation occlusion on CTA/MRA/DSA
- Informed consent given.
- Age 18 or over.

General exclusion criteria
- Arterial blood pressure > 185/110 mmHg.
- Blood glucose < 2.7 or > 22.2 mmol/L.
- Intravenous treatment with thrombolytic therapy in a dose exceeding 0.9 mg/kg rtPA or 90 mg.
- Intravenous treatment with thrombolytic therapy despite contra-indications, i.e.
  - major surgery, gastrointestinal bleeding or
  - urinary tract bleeding within the previous 2 weeks,
  - arterial puncture at a non-compressible site within the previous 7 days.`

Exclusion criteria for intended mechanical thrombectomy
- Laboratory evidence of coagulation abnormalities, i.e. platelet count <40 x 10*9/L, APTT>50 sec or INR >3.0.

Exclusion criteria for intended intra-arterial thrombolysis
- Cerebral infarction in the distribution of the relevant occluded artery in the previous 6 weeks.
- History of intracerebral hemorrhage.
- Severe head injury (contusion) in the previous 4 weeks.
- Clinical or laboratory evidence of coagulation abnormalities, i.e. platelet count <90 x 10*9/L, APTT>50 sec or INR >1.7.

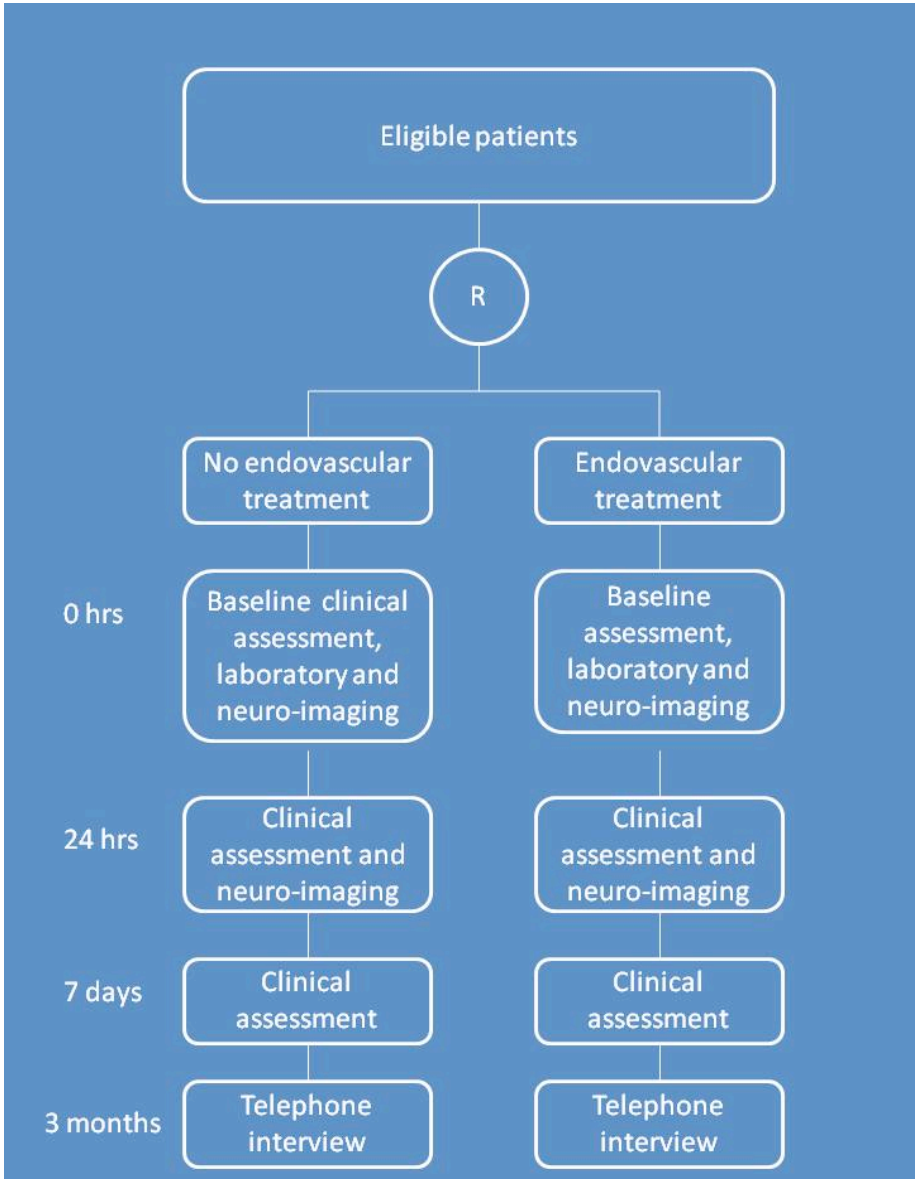


FIGURE 4. PATIENT FLOW IN THE TRIAL

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