MR CLEAN-MED, Statistical Analysis Plan

Wouter van der Steen, Rob van de Graaf, Bob Roozenbeek, Hester Lingsma, Aad van der Lugt, Diederik Dippel, on behalf of the MR CLEAN-MED investigators.

V2.0 20-08-2021

Version number	Change	Date
1.0	Initial version	01-07-2021
2.0	- Corrected typo of secondary outcome 'reperfusion grade' to 'recanalization grade (modified arterial occlusive lesion [mAOL] score)' - Changed statistical analysis procedure for adjusting for the early termination of moderate-dose unfractionated heparin arms (After consultation with independent statistician)	20-08-2021

Introduction

The aim of *MR CLEAN-MED (Multicenter Randomized CLinical trial of Endovascular treatment for Acute ischemic stroke in the Netherlands. The effect of periprocedural MEDication: acetylsalicylic acid, unfractionated heparin, both, or neither)* is to assess the effect of acetylsalicylic acid (ASA) and unfractionated heparin (UFH), alone, or in combination, in patients with acute ischemic stroke (AIS), who undergo endovascular thrombectomy (EVT) for a confirmed intracranial large vessel occlusion of the anterior circulation. In this statistical analysis plan we described the rationale behind the trial, the design of the trial, the methodology to assure adequate blinding, and the statistical procedures to estimate the primary effect. Additionally, we predefined the most important subgroup analyses. Last, we specified the time-path after follow-up of the final patient to publication. Please note that, due to word count restrictions, it is possible that not all pre-specified analyses listed in this statistical analysis plan will be included in the publication on the primary outcomes of the trial. Those analyses will be made available in subsequent publications or online. This paper should be read as an adjunct to the study protocol, published earlier. (1)

Rationale

EVT by means of retrievable stents, in patients with AIS with confirmed intracranial large vessel occlusion of the anterior circulation in whom the procedure can be started within 6 hours from

onset, has been proven safe and effective. This was first reported in the MR CLEAN trial – a landmark trial performed by members of our collaboration – and later confirmed in 6 other trials. (2-8) Still, a considerable proportion of patients do not recover despite fast and complete recanalization. (9) This is for a major part attributable to incomplete microvascular reperfusion (IMR). (10) IMR has been linked to distal microvascular damage or dysfunction as a result of tissue necrosis and cell death, intervention simply being late, but also to distal microvascular occlusion. This may be due to occlusion of distal vessels by pericyte contraction, distal embolization of microthrombi from the original occluding the proximal artery, in situ formation of microthrombi and cellular plugs caused by platelet activation and increased hemostasis, activated by formation of neutrophil extracellular traps (NETs). (11) It is important to target treatment on the reduction of IMR. We consider it likely that in patients with AIS treated with EVT, periprocedural antiplatelet or anticoagulant treatment improves distal reperfusion, but whether it improves clinical outcome is unknown.

The use of antiplatelet agents in AIS in general has a small beneficial effect, (12) but its use in patients treated with IV alteplase is associated with increased risk of intracranial hemorrhage. (13, 14) However, there are no trial data on acute treatment with antiplatelet agents in patients treated with EVT. (15) Previous trials on the effect of UFH in AIS have failed to demonstrate a beneficial effect. (12) However, the use of UFH in EVT has not been tested in randomized controlled trials. The risk of hemorrhage due to treatment with low or moderate dosages of heparin are low in patients with moderate or severe ischemic stroke. (12) A recent review confirms that there are no data on periprocedural antithrombotic treatment, and consequently, there is large practice variation. (15)

In conclusion, what we need is an answer to the question whether treatment with antithrombotic or antiplatelet agents directly before the start of mechanical thrombectomy is beneficial and leads to better outcomes, despite the potential extra risk of symptomatic intracranial hemorrhage.

Research Questions

The primary objective of this trial is to assess the effect of ASA and UFH, alone, or in combination on functional outcome at 3 months in patients with AIS caused by a confirmed intracranial large vessel occlusion of the anterior circulation, who undergo intra-arterial treatment with or without prior intravenous thrombolysis according to standard care.

The secondary objective is to assess the safety and effect of ASA and UFH, alone, or in combination, on neurological outcome, revascularization and infarct size in patients who undergo intra-arterial treatment for AIS caused by a confirmed intracranial large vessel occlusion of the anterior circulation.

The tertiary objectives are 1) to collect (waste) biomaterials (thrombo-emboli, aspirate blood) and to analyze biomarkers in blood samples with respect to their potential for treatment effect modification, 2) to collect and analyze data regarding the deferred consent procedure and its association with patient recall and satisfaction at three months from randomization, and 3) to study the efficiency of national EVT implementation, given the availability of EVT hospitals and capacity, and travel times of ambulance services. To this end, we aim to collect data (time delays and diagnostics) from each step in the acute stroke pathway as input parameters for a simulation model. This way we can study the regional set-up of the EVT organizational model.

Trial Design

MR CLEAN-MED (ISRCTN76741621) is a national multicenter phase III clinical trial with randomized treatment allocation, open label treatment, and blinded endpoint evaluation (PROBE design) with a 2x3 factorial design. (1) Patients were randomized for periprocedural intravenous treatment with ASA (300mg bolus) or no ASA, and for low-dose UFH (5000IU bolus, followed by 500IU/hour for 6 hours), moderate-dose UFH (5000IU, followed by 1250IU/hour for 6 hours) or no UFH. In April 2019, after discontinuing inclusion in the moderate-dose UFH arms because of a safety issue, the trial continued in a 2x2 factorial design. Only stent retrievers and aspiration devices, which are CE marked approved for EVT, and approved for use in the study by the steering committee are allowed in the trial as a first line of defense.

Randomization is stratified for center and for inclusion in the active treatment arm of the Multicenter Randomized trial of Acute Stroke treatment in the Ambulance with a nitroglycerin Patch (MR ASAP). In MR ASAP, the effect on functional outcome of prehospital transdermal nitroglycerin treatment within 3 hours of ischemic or hemorrhagic stroke onset is determined (http://www.mrasap.nl, ISRCTN99503308). Participation in the ARTEMIS project was not considered an exclusion criterium. In ARTEMIS, patients were randomized into a group with real-time feedback to the physicians on the times from admission to administration of alteplase

and time to groin puncture, or into a group without direct feedback (https://clinicaltrials.gov/ct2/show/NCT02808806).

Inclusion criteria

- a clinical diagnosis of acute ischemic stroke;
- caused by intracranial large vessel occlusion of the anterior circulation (distal intracranial carotid artery or middle (M1/proximal M2) cerebral artery) confirmed by neuro-imaging (CTA or MRA);
- CT or MRI ruling out intracranial hemorrhage;
- treatment possible (groin puncture) within 6 hours from symptom onset or last seen well;
- a score of at least 2 on the NIH Stroke Scale;
- age of 18 years or older;
- informed consent in writing (deferred).

Exclusion criteria

- Pre-stroke disability which interferes with the assessment of functional outcome at 90 days, i.e. mRS >2;
- Treatment with IV alteplase despite the following contra-indications for IV alteplase:
  o cerebral infarction in the previous 6 weeks with residual neurological deficit or signs of recent infarction on neuroimaging,
  o intracerebral hemorrhage within the previous 3 months,
  o INR exceeding 1.7, or prior use of direct oral anticoagulant (DOAC),
  o IV alteplase infusion >4.5 hours after symptom onset;
- Contra-indications for ASA/UFH, for instance: allergy, recent surgery, heparin induced thrombocytopenia;
- Therapeutic heparin use;
- INR exceeding 3.0;
- Known hemorrhagic diathesis or known thrombopenia ($<90^9$/L);
- Participation in medical or surgical intervention trials other than current (or MR ASAP (http://www.mrasap.nl, ISRCTN99503308) / ARTEMIS (https://clinicaltrials.gov/ct2/show/NCT02808806)).

Outcomes

The primary outcome is the score on the modified Rankin Scale at 90 days +/- 14 days after randomization

Secondary outcomes are:

- Reperfusion grade (Extended treatment in cerebral ischaemia [eTICI] score) on final angiography of EVT
- Recanalization grade (modified arterial occlusive lesion [mAOL] score) at 24 +/- 12 hours, assessed with CTA or MRI 3D TOF
- Score on the NIHSS at 24 hours and 5-7 days, or at discharge
- Final infarct volume, assessed with NCCT at 5-7 days or with DWI-MRI at 24 +/- 12 hours after EVT. Final infarct volume will be assessed with the use of automated, validated algorithms (16)
- All possible dichotomizations of the mRS at 90 days (± 14 days)
- Score on the EQ-5D-5L and Barthel index at 90 days (± 14 days)

Safety endpoints are the following:

- Intracerebral hemorrhage according to the Heidelberg Bleeding Classification (17)
- Symptomatic intracerebral hemorrhage (sICH) scored according to the Heidelberg Bleeding Classification, with the addition of sICH that led to death and that was identified as the predominant cause of the neurologic deterioration (17)
- Extracranial hemorrhages requiring transfusion or resulting in death
- Embolization in new territory on angiography during EVT
- Infarction in new territory assessed with NCCT at 5-7 days or DWI-MRI at 24 +/- 12 hours after EVT.
- Death from all causes within 90 days (+14 days)

Blinding

The trial features a PROBE design. Both patient and treating physician will be aware of the treatment assignment. Information on outcome at three months will be assessed in a telephone interview through standardized forms and procedures, by a trained investigator unaware of treatment allocation. Interviews will be recorded. Final assessment of the mRS score at 90 days

will be performed by the outcome committee, consisting of trained investigators blinded to the treatment allocation, based on masked reports of the telephone interview.

Results of neuroimaging will also be 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 independent trial statistician will combine data on treatment allocation with the clinical data in order to report to the data safety monitoring board (DSMB).

Status of the trial

As of this writing, a total of 15 centers have been initiated and included patients in the Netherlands. On the 16th of April 2019, after enrollment of 132 patients, and after receipt of the 4th safety report, the Trial Steering Committee (TSC) followed the unanimous advice of the DSMB to stop inclusions in the moderate-dose UFH arm of the trial. This decision was made because of a safety issue. Inclusion in the other treatment arms was considered safe and continued. Then, on the 27th of January 2021, after receipt of the combined 2nd interim analysis and 11th safety analysis, the DSMB advised that the TSC should be unblinded to the outcome data (all remaining treatment groups) in order to make a decision about stopping or continuing one or both of the remaining arms of the trial. The TSC again followed the advice of the DSMB. After analyses of the DSMB report the TSC decided to stop inclusion of patients into the trial. Since then, follow-up and validation and completion of the database continued with masked data, or else, blinded colleagues. The database will be locked in July 2021. In total, 663 patients were randomized (safety cohort), of whom 628 patients gave deferred consent for main analyses, and 6 patients gave deferred consent for the use of already collected data.

Missing data and death

We will report proportions of missing values for all collected variables. For descriptive analyses, only the crude, non-imputed data will be presented. For the regression analyses, missing data will be imputed using multiple imputation methods. For patients who died within the study period we will assign the worst score for all unassessed clinical outcome measures and use those for analyses.

Time path of the analysis and locking of the database

After the follow-up of the final patient, the last records of the database will be cleaned and checked for completeness within two months. Upon completion, the database will be locked. The final analysis will be performed by the study coordinators and principal investigators of the trial and will be reported to the independent trial statistician. The final results will then be shared for consideration with the Trial Steering Committee. Within 3 months after obtaining the final results, a manuscript describing the main results of the trial will be submitted for publication. The syntax and output will be made available upon request.

Statistical Analysis

The analysis will be in accordance with the CONSORT guidelines. Baseline data by treatment allocation will be reported with standard statistical procedures. All analyses will be performed according to the intention-to-treat principle. To express statistical uncertainty, 95% confidence intervals will be reported for all analyses.

We will analyze the treatment effect estimates for ASA versus no ASA, and for UFH versus no UFH. Additionally, we will evaluate the treatment effect for the different dosages (i.e. moderate-dose or low-dose) of UFH versus no UFH. To correct for the earlier termination of the moderate-dose UFH arms, we will adjust for an extra variable – inclusion before or after stopping the moderate-dose UFH arms. For the moderate-dose UFH versus no UFH analysis we will only evaluate patients who were included prior to stopping the allocation of moderate-dose UFH.

Primary effect analysis

The primary effect parameter will be the common odds ratio, estimated with ordinal logistic regression, which represents the shift on the 7-category modified Rankin scale, measured at 3 months from randomization. The treatment effect estimates for both ASA versus no ASA and heparin versus no heparin will be adjusted for prognostic variables: age, pre-stroke mRS, time from onset to door of EVT center, time from door EVT center to groin puncture, stroke severity (baseline NIHSS) and collateral score. Adjusted and unadjusted estimates with corresponding 95% confidence intervals will be reported.

Primary effect analysis in subgroups

Pre-specified subgroups will be performed by testing for interaction between the specific baseline characteristic and treatment. The effect of intervention on the modified Rankin scale will be analyzed in subgroups determined by the following variables:

- Tertiles of age
- Sex
- Tertiles of (systolic) blood pressure at baseline
- Tertiles of NIHSS at baseline
- Tertiles of time from onset of symptoms to randomization, groin puncture and revascularization
- Diabetes mellitus
- Atrial fibrillation
- Extracranial carotid obstruction
- Occlusion location
- ASPECTS
- Collateral score
- Type of device
- IVT versus no IVT
- Prior use of antiplatelet agents, vitamin K antagonists or direct oral anticoagulants
- Inclusion in active treatment arm of MR ASAP

Secondary, tertiary and safety analyses

Secondary, tertiary and safety effect parameters will be determined using linear, logistic or ordinal regression analyses as appropriate, with the same adjustment variables as the primary outcome.

Last, a cost utility analysis will be performed to assess the cost-effectiveness of the intervention under study. The outcome parameters of the cost-effectiveness analysis will be the cost per patient with good functional outcome and the cost per QALY.

References

1. Chalos V, van de Graaf RA, Roozenbeek B, van Es ACGM, den Hertog HM, Staals J, et al. Multicenter randomized clinical trial of endovascular treatment for acute ischemic stroke. The effect of periprocedural medication: acetylsalicylic acid, unfractionated heparin, both, or neither (MR CLEAN-MED). Rationale and study design. Trials. 2020;21(1):644.
2. Fransen PS, Beumer D, Berkhemer OA, van den Berg LA, Lingsma H, van der Lugt A, et al. MR CLEAN, a multicenter randomized clinical trial of endovascular treatment for acute ischemic stroke in the Netherlands: study protocol for a randomized controlled trial. Trials. 2014;15:343.
3. Bracard S, Ducrocq X, Mas JL, Soudant M, Oppenheim C, Moulin T, et al. Mechanical thrombectomy after intravenous alteplase versus alteplase alone after stroke (THRACE): a randomised controlled trial. Lancet Neurol. 2016;15(11):1138-47.
4. Campbell BCV, Donnan GA, Lees KR, Hacke W, Khatri P, Hill MD, et al. Endovascular stent thrombectomy: the new standard of care for large vessel ischaemic stroke. Lancet Neurol. 2015;14(8):846-54.
5. Goyal M, Demchuk AM, Menon BK, Eesa M, Rempel JL, Thornton J, et al. Randomized assessment of rapid endovascular treatment of ischemic stroke. N Engl J Med. 2015;372(11):1019-30.
6. Jovin TG, Chamorro A, Cobo E, de Miquel MA, Molina CA, Rovira A, et al. Thrombectomy within 8 hours after symptom onset in ischemic stroke. N Engl J Med. 2015;372(24):2296-306.
7. Muir KW, Ford GA, Messow CM, Ford I, Murray A, Clifton A, et al. Endovascular therapy for acute ischaemic stroke: the Pragmatic Ischaemic Stroke Thrombectomy Evaluation (PISTE) randomised, controlled trial. J Neurol Neurosurg Psychiatry. 2017;88(1):38-44.
8. Saver JL, Goyal M, Bonafe A, Diener HC, Levy EI, Pereira VM, et al. Stent-retriever thrombectomy after intravenous t-PA vs. t-PA alone in stroke. N Engl J Med. 2015;372(24):2285-95.
9. Fransen PS, Berkhemer OA, Lingsma HF, Beumer D, van den Berg LA, Yoo AJ, et al. Time to Reperfusion and Treatment Effect for Acute Ischemic Stroke: A Randomized Clinical Trial. JAMA Neurol. 2016;73(2):190-6.
10. Dalkara T, Arsava EM. Can restoring incomplete microcirculatory reperfusion improve stroke outcome after thrombolysis? J Cereb Blood Flow Metab. 2012;32(12):2091-9.
11. Yemisci M, Gursoy-Ozdemir Y, Vural A, Can A, Topalkara K, Dalkara T. Pericyte contraction induced by oxidative-nitrative stress impairs capillary reflow despite successful opening of an occluded cerebral artery. Nat Med. 2009;15(9):1031-7.
12. Sandercock P CR, Counsell C, et al. The International Stroke Trial (IST): a randomised trial of aspirin, subcutaneous heparin, both, or neither among 19435

patients with acute ischaemic stroke. International Stroke Trial Collaborative Group. Lancet. 1997;349(9065):1569-81.
13. Anderson CS, Robinson T, Lindley RI, Arima H, Lavados PM, Lee TH, et al. Low-Dose versus Standard-Dose Intravenous Alteplase in Acute Ischemic Stroke. N Engl J Med. 2016;374(24):2313-23.
14. Zinkstok SM, Roos YB, investigators A. Early administration of aspirin in patients treated with alteplase for acute ischaemic stroke: a randomised controlled trial. Lancet. 2012;380(9843):731-7.
15. Widimsky P, Kocka V, Rohac F, Osmancik P. Periprocedural antithrombotic therapy during various types of percutaneous cardiovascular interventions. Eur Heart J Cardiovasc Pharmacother. 2016;2(2):131-40.
16. Boers AM, Marquering HA, Jochem JJ, Besselink NJ, Berkhemer OA, van der Lugt A, et al. Automated cerebral infarct volume measurement in follow-up noncontrast CT scans of patients with acute ischemic stroke. AJNR Am J Neuroradiol. 2013;34(8):1522-7.
17. von Kummer R, Broderick JP, Campbell BC, Demchuk A, Goyal M, Hill MD, et al. The Heidelberg Bleeding Classification: Classification of Bleeding Events After Ischemic Stroke and Reperfusion Therapy. Stroke. 2015;46(10):2981-6.