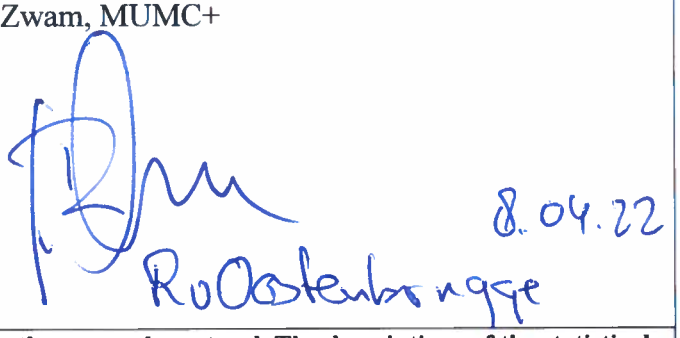
STATISTICAL ANALYSIS PLAN - MR CLEAN LATE TRIAL

Section 1: Administrative Information

Title	Multicenter Randomized Clinical Trial of Endovascular Treatment of Acute Ischemic Stroke in The Netherlands for Late arrivals: MR CLEAN-LATE
Trial registration	NL58246.078.17, ISRCTN19922220, Registered on 11 December 2017
Protocol ID	MEC-2017-367
SAP version & date	1.0, April 2022
Protocol version	1.8, June 2021
SAP revision	None
Roles & responsibility	**Coordinating PhD student:** Susanne G. H. Olthuis, MUMC+ **Statistical advisor:** Prof. dr. Hester F. Lingsma, EMC **Independent trial statistician:** Daan Nieboer, EMC **Chief Investigators/Principal Investigators (PI):** Prof. dr. Robert J. van Oostenbrugge, MUMC+ Prof. dr. Wim H. van Zwam, MUMC+ Signature PI & date:  8.04.22
This document should be read as an adjunct to the research protocol. The descriptions of the statistical analyses in this document are, however, leading.	

Section 2: Introduction

Background and rationale:

For the background and rationale of the MR CLEAN LATE trial see the research protocol(1).

Research questions / Objectives:

The primary objective of this trial is to assess the effect of EVT with best medical treatment compared with best medical treatment alone on functional outcome in patients with AIS, caused by an intracranial large vessel occlusion of the anterior circulation and at least some collateral flow (poor to good collaterals), who can be treated between 6 and 24 hours (LATE-EVT) after symptom onset or last seen well.

The secondary objectives are to assess whether LATE-EVT compared with best medical treatment alone has a beneficial effect on neurological recovery (NIHSS), recanalization at 24h, infarct size the occurrence of (symptomatic) intracerebral hemorrhage (ICH), and 90-day mortality.

Status of the trial:

As of this writing, 18 centers in the Netherlands have included 502 patients for the MR CLEAN LATE trial. The final inclusion (500th patient with registered consent) was on 27-01-2022, the last follow-up will be around the beginning of May 2022, and we aim to close the database in June 2022.

Timing analysis and locking of the database:

After the 90-day follow-up of our final inclusion we aim to complete data collection and cleaning within one month, after which the database will be locked. After locking the database the treatment allocation will be revealed and the primary and secondary analyses will be performed by the study-coordinator and principal investigator of the trial, and will be reported to the independent trial statistician. The results confirmed by the independent trial statistician will then be shared for consideration with the executive committee, the DSMB and then with the steering committee of the trial. The results will be processed into a manuscript describing the main trial results which we aim to submit for publication within 3 months after obtaining the final results.

Section 3: Study Methods

Trial design:

The MR CLEAN LATE (ISRCTN19922220) is a multicenter phase III clinical trial with randomized treatment allocation, open-label treatment, and blinded endpoint evaluation (PROBE design). The intervention contrast is EVT versus no EVT. The treatment is provided in addition to best medical management.

Randomization & blinding:

The randomization procedure will be computer- and web-based, using permuted blocks (of varying block size, unknown until after closure of the database).
Back-up by telephone will be provided. Randomization is allowed when the occlusion and degree of collaterals has been established by CTA. Randomization will be stratified for center and for inclusion in the active treatment arm of MR ASAP (for details see the research protocol). Inclusion and randomization of patients with poor collaterals will be restricted. After 100 patients with poor collaterals have been included and randomized, we will only allow inclusion of patients with moderate to good collaterals.

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 database 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, by a trained investigator blinded for treatment allocation. Interviews will be recorded. Assessors who are blinded to the treatment allocation will base assessment of outcome on the modified Rankin scale on this information. Results of neuroimaging will also be assessed in a blinded manner. Information concerning treatment allocation is kept separate from the 90-day follow-up outcome 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 (DSMB).

Sample size calculation:

We estimated that a sample size of 500 patients would provide a power of 85% with two sided alpha 0.05, using simulation with 5000 runs per assumed sample in R.
For the power calculations we assume the distribution over the 7-point modified Rankin Scale (mRS) in the control group which is similar to the distribution of mRS in the control group of the MR CLEAN trial: mRS 0: 0%; mRS 1: 6%; mRS 2 13%; mRS 3 16%; mRS 4: 30%; mRS 5: 12%; mRS 6: 22%. We assume a favorable effect of treatment with a common odds ratio of 1.52, which would lead to an 7% absolute increase in the proportion of patients with mRS 0-2. The aim is to include 500 patients. In the analysis covariate adjustment will be used, which reduces the required sample size by approximately 25%. In a simulation with 5000 runs we computed the proportion of positive trials, for a given sample size. The corresponding effective sample size was 670, providing 84% power to detect a true treatment effect (two-sided alpa=0.05).

Framework:

Superiority trial.

Interim analysis:

In order to increase the safety of the intervention, the trial will be monitored by an independent data safety monitoring board (DSMB). The DSMB will be chaired by a neurologist, and include a neuro-interventionist and an independent methodologist/statistician. The DSMB will meet frequently, at least annually or after inclusion of the next 100 patients (whichever comes first), 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 DSMB, along with any other analyses that the Board may request. In the light of these analyses, DSMB will advise the chairman of the Steering Committee if, in their view, the randomized comparisons in the trial 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.
No futility criterion will be applied.

Section 4: Trial population

Inclusion criteria:
In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Clinical diagnosis of Acute Ischemic Stroke, caused by proximal intracranial anterior circulation occlusion (distal intracranial carotid artery or middle (M1/M2)) cerebral artery confirmed by neuro-imaging (CTA or MRA), and presence of poor*, moderate or good collateral flow as shown by neuroimaging (CTA)
- CT or MRI ruling out intracranial hemorrhage,
- Start of IA treatment (groin puncture) possible between 6 and 24 hours after symptom onset or last seen well < 24 hours including wake-up strokes,
- A score of at least 2 on the NIH Stroke Scale
- Age of 18 years or older
- Written informed consent (deferred).

* Inclusion and randomization will be restricted to patients with moderate or good collaterals when 100 patients with poor collaterals have been included in the study.

Exclusion criteria:
A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Distal intracranial carotid artery occlusion/M1 occlusion, NIHSS ≥10, infarct core ≤25ml and a mismatch ratio total ischemic volume/ infarct core ≥2 (patient is eligible for direct EVT treatment, based on DAWN/DEFUSE 3 patient profile)
- Pre-stroke disability which interferes with the assessment of functional

outcome at 90 days, i.e. mRS >2

- Cerebral infarction in the previous 6 weeks with residual neurological deficit or signs of large recent infarction on neuroimaging in the territory of the middle cerebral artery
- Clinical evidence of hemorrhagic diathesis, confirmed by an INR > 3 and/or a platelet count < 40 x 109/L and/or an APTT > 50 sec. (In case there is no clinical indication to test for hemorrhagic diathesis, it may be assumed that the INR, APTT and platelet count are within these limits).
- Clearly demarcated hypodensity in >1/3 of the middle cerebral artery territory, consistent with current symptoms
- Participation in medical or surgical intervention trials other than current (or MR ASAP and ARTEMIS)

Outcomes:

Primary outcome:

- Score on the modified Rankin Scale at 90 days (+/- 14 days)

Secondary outcomes:

- Recanalization rate at 24 hours after randomization, assessed with NCCT/CTA or MRI
- Score on the NIHSS at 24 hours and 5-7 days after randomization, or at discharge
- Final infarct volume on NCCT at 5-7 days or 24-48h MRI. Final infarct volume will be assessed with the use of an automated, validated algorithm. Infarct size at day 5-7 will be compared with plain CT and perfusion CT or MRI results (if available) at baseline
- Dichotomized mRS of 0-1 vs. 2-6 at 90 days (± 14 days)
- Dichotomized mRS of 0-2 vs. 3-6 at 90 days (± 14 days)
- Dichotomized mRS of 0-3 vs. 4-6 at 90 days (± 14 days)
- Death at 90 days (± 14 days)
- Score on the Barthel index and EQ5D-5L at 90 days (± 14 days)

Safety endpoints:

- Hemorrhages according to the ECASS radiological classification
- sICH scored according to the Heidelberg criteria
- Embolization in new territory on angiography during EVT

- Occurrence of aneurysm spurium
- Occurrence of groin hematoma
- Infarction in new territory at 1 week
- All-cause mortality at 90 days

Section 4: Statistical Principles & Analysis

For all descriptive analyses, we will use crude (non-imputed) data. Imputed data will be used for all regression analyses. 95% confidence intervals will be reported to express statistical uncertainty. All analysis will be performed according to the intention-to-treat principle.

For continuous outcome measures, log transformation will be used if necessary, to correct for non-normally distributed data. In case this does not resolve the normality issues we will resort to categorization of the variable. In our analyses, we will handle the NIHSS score, ASPECTS and collateral grades as continuous variables.

Missing data and death:

Proportions of missing values will be made insightful and all missing data will be imputed using multiple imputation. The data set will include calculated/sum scores for certain variables, as well as their sub-items.

For subjects with unassessed NIHSS scores due to death at the time of assessment, we will assign the worst score (i.e. 42)(2, 3), for comatose patients the maximum score is 39(4). For patients that have a score above 39, but who are not deceased at the time of assessment we will set their scores to the maximum of 39. We will assign the value "0" to unassessed EQ-5D-5L and/or Barthel scores that are missing due to death at the time of assessment. These scores will be used in all analyses.

Primary effect analysis:

A direct comparison between the two trial arms will be made concerning the score on the mRS at 90 days after randomization. The primary effect parameter will be the common odds ratio, estimated with ordinal logistic regression, which represents the shift towards better outcome on the full distribution of modified Rankin scale at 3 months. The estimate will be adjusted for the prognostic variables:

- age, pre-stroke mRS, time from onset/last seen well to randomization, baseline stroke severity (NIHSS), collaterals, and unwitnessed stroke.

Adjusted estimates with corresponding 95% confidence intervals will be reported.

Primary effect analysis in subgroups:

The effect of intervention on the modified Rankin Scale will be analyzed in subgroups determined by the following variables:

- Tertiles of age
- Sex (m/f)
- Tertiles of (systolic) blood pressure at baseline
- Tertiles of NIHSS at baseline
- Tertiles of time from onset of symptoms/last seen well to randomization
- Tertiles of time from time first noticed symptoms to randomization
- Diabetes mellitus (y/n)
- Atrial fibrillation (y/n)
- Extracranial carotid obstruction (y/n)
- Occlusion location (ICA, ICA-T, M1, M2/3)ASPECTS (0-4, 5-7, 8-10)
- Collaterals (none, poor, moderate, good)
- Unwitnessed stroke (y/n)
- Intravenous alteplase received (y/n)

The abovementioned pre-defined subgroup analyses will be performed by testing for interaction between these characteristics and treatment allocation. In the interest of statistical power, for subgroups that are based on a continuous variable, the continuous variable will be used in the statistical analysis of the interaction with treatment (i.e. the whole range of age instead of the categorized variable). Statistical significance is defined as $p<0.05$.

Secondary outcome analysis:

Secondary outcomes will be analyzed using linear, logistic, or ordinal regression analyses as appropriate, and we will report beta coefficients, odds ratios, and common odds ratios with their 95% confidence intervals respectively. Adjusted results will be reported. We will use the same adjustment variables to analyze the secondary outcomes as we will use for the primary outcome

Safety cohort analysis:

Safety outcomes are in-hospital mortality and occurrence of in-hospital symptomatic ICH (sICH). These outcomes will be analyzed with unadjusted logistic regression according to the intention-to-treat principle, in the safety cohort.

Additional analysis:

<u>As-treated analyses:</u>

In addition to the intention-to-treat- analyses we will also perform an as-treated analysis for our primary, secondary, and safety outcomes.

<u>The as-treated population:</u>

- Intervention group – as treated analysis:
    - All subjects allocated to the intervention group, who actually underwent groin puncture in order to receive EVT
    - All subjects allocated to the control group, who did eventually undergo groin puncture in order to receive EVT (i.e. cross-overs).
- Control group – as treated analysis:
    - All subjects allocated to the control group, who did not undergo groin puncture in order to receive EVT
    - All subjects allocated to the intervention group, who did eventually not undergo groin puncture to receive EVT (i.e. cross-overs).

References:

1. Pirson F, Hinsenveld WH, Goldhoorn RB, Staals J, de Ridder IR, van Zwam WH, et al. MR CLEAN-LATE, a multicenter randomized clinical trial of endovascular treatment of acute ischemic stroke in The Netherlands for late arrivals: study protocol for a randomized controlled trial. Trials. 2021;22(1):160.
2. Chalos V, van der Ende NAM, Lingsma HF, Mulder M, Venema E, Dijkland SA, et al. National Institutes of Health Stroke Scale: An Alternative Primary Outcome Measure for Trials of Acute Treatment for Ischemic Stroke. Stroke. 2020;51(1):282-90.
3. van der Steen W, van de Graaf RA, Chalos V, Lingsma HF, van Doormaal PJ, Coutinho JM, et al. Safety and efficacy of aspirin, unfractionated heparin, both, or neither during endovascular stroke treatment (MR CLEAN-MED): an open-label, multicentre, randomised controlled trial. Lancet. 2022;399(10329):1059-69.
4. Lyden P. Using the National Institutes of Health Stroke Scale: A Cautionary Tale. Stroke. 2017;48(2):513-9.