MR ASAP: Multicentre Randomised trial of Acute Stroke treatment in the Ambulance with a nitroglycerin Patch - Statistical Analysis Plan

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Table of contents

Introduction

The aim of Multicentre Randomised trial of Acute Stroke treatment in the Ambulance with a nitroglycerin Patch (MR ASAP) is to assess the effect of transdermal glyceryl trinitrate (GTN), started within 3 hours of symptom onset in the prehospital setting, on functional outcome at 90 days in patients with acute ischaemic stroke or intracerebral haemorrhage.(1)

In this statistical analysis plan (SAP) we describe the statistical procedures to estimate the effects of treatment with GTN on primary and secondary outcomes. Additionally, we predefine the subgroup analyses, and the analysis populations.

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 MR ASAP trial. Those subgroup analyses will be made available in subsequent publications or online. Where there is a difference between the protocol (website and published versions) and the following statistical analysis plan (SAP), this document takes precedence.

Outcomes

The primary outcome measure is the score on the modified Rankin Scale (mRS) at 90 days (± 14 days).

Secondary and safety outcomes

On admission to the hospital:

- Vital signs: first measured systolic and diastolic blood pressure, pulse, and body temperature;
- Neurological deficit, as assessed with the National Institutes of Health Stroke Scale (NIHSS);
- Collateral circulation, as assessed with computed tomography (CT) angiography (only in patients in whom CT angiography is performed as part of routine clinical care);
- Lesion size: volume of the perfusion deficit (only in patients in whom CT perfusion is performed as part of routine clinical care) or ICH on the initial CT;

At 24 hours (± 4 hours):

- Vital signs: systolic and diastolic blood pressure, pulse, and body temperature;
- Neurological deficit, as assessed with the National Institutes of Health Stroke Scale (NIHSS);

At 7 days (± 1 day) or at discharge, if earlier:

- Serious adverse events (SAEs) in the first 7 days or until discharge, if earlier;
- Death at 7 days;

At 90 days (± 14 days):

- Death;
- Dichotomized mRS of 0-1 vs. 2-6;
- Dichotomized mRS of 0-2 vs. 3-6;
- Dichotomized mRS of 0-3 vs. 4-6;
- Disability assessed with the score on the Barthel Index (BI);
- Quality of life assessed with the EuroQol 5D-5L (EQ-5D-5L);

- Home time: the number of nights among the first 90 days since stroke onset that are spent in the patient's own home or a relative's home. Where final follow-up occurs earlier, the last known placement will be extrapolated to 90 days;
- Patient location over first 90 days (± 14 days): hospital; rehabilitation service; chronic nursing facility; home.

Missing data and death

We will report proportions of missing values for all reported variables. For descriptive analyses, non-imputed data will be presented.

For the regression analyses, missing baseline data will be imputed using multiple imputation (n=5). If all variables used in the analysis have <1% missing, simple imputation with the median or mode will be used. Outcome data will not be imputed.

For patients who died before the outcome assessment, we will assign the worst score for all unassessed clinical outcome measures and use those for analyses:

- Barthel Index score = 0;
- EQ-5D-5L score = 0;
- NIHSS score = 42.

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.

To maximise time for analysis and interpretation of the results, it may be necessary to perform a soft-lock and preliminary analysis and interpretation once the last patient has had their primary outcome recorded, this involving SB, SUV, HL, DN, PJ, HB; they will not be involved in resolving any final queries to maintain the integrity and blinding of the final database. A hard-lock will be performed once final data cleaning has completed. The approach of soft-lock then hard-lock is a standard approach in large trials and does allow more time to be spent on considering the results of a trial and their interpretation, and preparing for conference presentation and publication.(2,3)

Upon completion, the database will be locked. Data analyses will be performed by SB and SUV under supervision of the independent trial statistician. The final results will then be shared for consideration with the steering committee of the trial. Within 3 months after closure of the database, a manuscript describing the main results of the trial will be submitted for publication.

Statistical analyses

Primary outcome

The primary effect estimate will be a shift on the mRS score at 90 days (± 14 days) assessed by means of ordinal logistic regression, and will be expressed as a common odds ratio with a 95% confidence interval.

The distribution of the mRS scores per trial allocation will be shown as a figure (Figure 2).

The statistical analyses will be adjusted for the following baseline characteristics: age, sex, stroke type, score on the FAST at study inclusion, systolic blood pressure at randomisation, Eye and Motor score of the Glasgow Coma Scale at randomisation, time of symptom onset to randomisation, pre-stroke score on the mRS and ambulance region.

Secondary and safety outcomes

Binary logistic regression will be used for binary outcomes. Ordinal logistic regression will be used for ordered categorical data. For continuous outcomes, regression beta coefficients are reported as estimated with multiple linear regression. Outcomes that are not normally distributed will be transformed prior to the analysis. All analyses will be adjusted for the baseline characteristics mentioned above. To express statistical uncertainty, 95% confidence intervals will be reported for all analyses.

Confidence intervals and P values

Analyses will be two-sided $P < 0.05$ with 95% confidence intervals presented. The trial is testing the effect of the intervention on mRS as primary endpoint. Analyses in subgroups and on other outcomes are considered hypothesis-generating. No adjustment will be made for multiplicity of testing.

Analysis populations

The primary efficacy analyses will be performed on the intention-to-treat population. A target population analysis will be performed for patients with final diagnosis stroke or TIA, excluding stroke mimics.
A sensitivity analysis for safety will be performed on all randomized patients, including those for whom no deferred consent was obtained. For non-consenting patients, a limited set of variables was collected, including death at 7 days, serious adverse events in the first 7 days, and death at 90 days for patients with intracerebral haemorrhage. The robustness of the primary and key secondary analyses will be assessed in the per-protocol population.

The following population definitions will be used:

▪ Intention-to-treat in primary efficacy analysis: all randomised participants with deferred consent and with a valid mRS score recorded at 90 days.

▪ Intention-to-treat in primary safety analysis: all randomised participants with deferred consent with a vital status recorded at 7 days or at 90 days.

▪ Target population analysis: all randomised participants with deferred consent and with a valid mRS score recorded at 90 days, with final diagnosis stroke (ischaemic stroke or intracerebral haemorrhage) or TIA.

▪ Sensitivity safety analysis: all randomised patients (including patients who did not provide deferred consent) for whom a vital status at 7 days is available; all randomised patients with intracerebral haemorrhage for whom a vital status at 90 days was available.

▪ Per-protocol: participants from the target population who are deemed to have no major protocol violations that could interfere with the objectives of the study. This population consists of:

- Patients with stroke (ischaemic stroke or intracerebral haemorrhage) or TIA (built on the assumption that GTN will only alter outcome in patients with stroke);
- Treatment group: Patients allocated to GTN who received GTN, without documented removal of the patch within the 24 +/- 2 hours window;
- Control group: Patients allocated to the control group who did not receive GTN;
- Randomisation within 3 hours of symptom onset;
- Systolic blood pressure ≥ 140 mmHg;
- FAST score > 1.

Compliance

Compliance with allocated treatment will be tabulated. If GTN was not given for 24 +/- 2 hours, the reason will be given (withdrawn informed consent, death, human error, other reason, unknown).

Subgroup analyses

Comparison of the primary outcome in the intervention group vs. control will be performed in the following pre-specified subgroups:

- Age (≤70, >70 years);
- Sex (male, female);
- Stroke type (ischaemic stroke, intracerebral haemorrhage); TIA; stroke mimic;
- In patients with ischaemic stroke: visible occlusion of the proximal intracranial arteries (internal carotid artery (ICA); basilar artery (BA); first or second segment middle cerebral artery (MCA), anterior cerebral artery (ACA), or posterior cerebral artery (PCA)) versus no proximal visible occlusion;
- Intravenous thrombolysis with alteplase (yes; no), irrespective of dose received;
- Endovascular treatment (yes; no);
- Blood pressure (prehospital), dichotomised at the median;
- Time to randomisation (<1 hour; 1-2 hours; ≥2 hours).

Ordinal regression models adjusted for the same variables as the primary analysis, with and without a multiplicative interaction term of the abovementioned variables and the treatment allocation, will be compared to determine whether the added interaction term significantly improves model fit. In the interest of statistical power, for the subgroups that are based on a continuous variable, the continuous variable will be used in the statistical analysis of interaction with treatment (e.g. the whole range of age instead of a trichotomised variable). Statistical significance is defined by $p<0.05$. Results will be shown in a forest plot (figure 3).

In addition, we will perform hypothesis-generating subgroup analyses for other outcomes.

SAE cause definitions

Ischaemic stroke progression (primary diagnosis ischaemic stroke)

Neurological deterioration of 4 points or more from admission NIHSS, or 2 points or more on one item, not explainable by intracranial haemorrhage on imaging.

- It is mandatory to have imaging performed at the time of deterioration; if no neuroimaging was performed, the SAE will be classified as "Neurological deterioration (including death) from ischaemic stroke."
- It is not mandatory to find an explanation for neurological deterioration on imaging (e.g. oedema formation, infarct growth), so a normal CT scan can be compatible with "stroke progression".

Neurological deterioration (including death) from ischaemic stroke (primary diagnosis ischaemic stroke)

- Neurological deterioration of 4 points or more from admission NIHSS, or 2 points or more on one item, including death, as a direct or indirect consequence of ischaemic stroke, when no follow-up imaging was performed

Haemorrhagic stroke progression (primary diagnosis ICH)

Neurological deterioration of 4 points or more from admission NIHSS or 2 points or more on one item, or death, explained by progression of ICH on repeated imaging

- May be due to haemorrhage expansion, oedema formation, new or increased intraventricular haemorrhage, hydrocephalus.
- If no neuroimaging was performed, the SAE will be classified as "Neurological deterioration (including death) from ICH", see definition below.

Neurological deterioration (including death) from ICH (primary diagnosis ICH)

Neurological deterioration of 4 points or more from admission NIHSS or 2 points or more on one item, including death, as direct or indirect consequence of the ICH, when no follow-up imaging was performed.

- For example: patients with severe ICH who died hours or days after onset, or where palliative treatment was started soon after admission because of poor prognosis.

If a patient suffers from, for instance, pneumonia and palliative treatment is started because of poor prognosis due to the combination of pneumonia and the ICH, two separate SAE's should be registered (Pneumonia and Death from ICH).

New ischaemic stroke

New stroke symptoms or recurrence of symptoms after initial complete recovery, compatible with new acute ischaemic stroke in the same or a different vascular territory.

Intracranial haemorrhage

This applies only to symptomatic intracranial haemorrhage after infarction and not to primary intracerebral haemorrhage (SAE due to progression of intracerebral haemorrhage should be categorised as “Stroke progression” or “Neurological deterioration from ICH” or “death from ICH”).

- Symptomatic intracranial haemorrhage is defined according to Heidelberg criteria, with the addition that sICH led to death or was identified as the predominant cause of the neurological deterioration (4 or more points from admission NIHSS, or 2 or more on one item).(4)
- Brain imaging can be unscheduled triggered by symptoms suggestive of ICH, or scheduled e.g. in case of concomitant inclusion in MR CLEAN-NO IV or MR CLEAN-MED trial.

Pneumonia

All pneumonias are considered serious adverse events, whether or not treated with oral or intravenous antibiotics.

Other infections

Other infections which are treated with oral antibiotics (such as an uncomplicated urinary tract infection or flebitis) are generally considered not SAEs, unless they fulfil the criteria for SAE as described in study protocol paragraph 9.2.2..

Hypotension requiring clinical intervention

To be categorised as a serious AE, clinical intervention implies acute treatment with IV fluids or medication such as norepinephrine.

- Does not apply when treatment (only) exists of bed in Trendelenburg position or stopping oral antihypertensive medication or stopping other medication which causes hypotension.

Hypertension requiring clinical intervention

To be categorised as a serious AE, clinical intervention implies treatment with intravenous antihypertensive treatment, such as intravenous labetalol, and/or hypertension treatment is the indication to be admitted to the intensive (or medium) care unit.

- Does not apply when pre-morbid hypertension is treated as usual or when oral antihypertensive treatment is started.
- Does not apply for one or several intravenous labetalol shots on the emergency ward in order to lower blood pressure before treatment with intravenous thrombolysis.

Non-populated tables and figures

Tables

Table 1. Characteristics of the XXX Patients at Baseline.*		
Characteristic	GTN group (N=xxx)	Control group (N=xxx)
Mean age (SD) – years		
Male sex – no. (%)		
Prehospital FAST score of 3 – no. (%) †		
Mean prehospital systolic blood pressure (SD) – mm Hg		
Mean prehospital diastolic blood pressure (SD) – mm Hg		
Mean prehospital heart rate (SD) – beats per min		
Median duration from symptom onset to randomisation (IQR) - min		
Median prehospital EM score of the Glasgow Coma Scale (IQR) ‡		
Premorbid modified Rankin Scale score > 2 – no. (%)§		
Medical history – no. (%)		
Atrial fibrillation		
Diabetes mellitus		
Hypertension		
Use of blood pressure lowering drugs in the past 3 days – no. (%)		
Qualifying event – no. (%)		
Ischaemic stroke		
Intracerebral haemorrhage		
Transient ischaemic attack		
Stroke mimicking condition		
Treatment with intravenous alteplase for ischaemic stroke – no. (%)¶		
Endovascular treatment for ischaemic stroke – no. (%)¶		

Missing data: xxx

* GTN denotes glyceryl trinitrate, IQR interquartile range, SD standard deviation

† The Face-Arm-Speech test is a 3-item prehospital stroke scale, a score of 3 indicates the combination of facial drooping, arm weakness and speech difficulty

‡ EM denotes Eye and Motor combined sum score

§ The modified Rankin Scale score ranges from 0 (no functional limitations) to 6 (death), with a higher score indicating more severe functional disability. A score of 2 or less indicates functional independence.

¶ Not a baseline characteristic

Table 2. Efficacy Outcomes.*						
Outcome	No. of patients with data	GTN (N=xxx)	Control (N=xxx)	Effect Measure	Unadjusted Value (95% CI)	Adjusted Value† (95% CI)
Primary outcome: 90-day modified Rankin Scale score ‡						
Median score (IQR)				Common odds ratio		
Secondary outcomes for all patients						
Dichotomized 90-day modified Rankin Scale score – no. (%)						
0-1				Odds ratio		
0-2				Odds ratio		
0-3				Odds ratio		
Median NIHSS score (IQR) §						
At hospital admission				Beta coefficient		
At 24 hours				Beta coefficient		
Mean systolic blood pressure (SD) at hospital admission				Beta coefficient		
Mean diastolic blood pressure (SD) at hospital admission				Beta coefficient		
Mean heart rate (SD) at hospital admission				Beta coefficient		
Collateral score – no. (%)¶				Common odds ratio		
0						
1						
2						
3						
Median volume of infarct core (IQR) - ml				Beta coefficient		
Median volume of penumbra (IQR) - ml				Beta coefficient		
Median volume of intracerebral hemorrhage (IQR) - ml ||				Beta coefficient		
Median 90-day EQ-5D-5L (IQR)**				Beta coefficient		
Median Barthel Index after 90 days – (IQR) ††				Beta coefficient		
Home time – no. (%)‡‡				Beta coefficient		
Patient location – no. (%)						
Hospital						
Rehabilitation service						
Chronic nursing facility						
Home						

Missing data: xxx

* GTN denotes glyceryl trinitrate, ICH intracerebral haemorrhage, IQR interquartile range, SD standard deviation

† Adjustments were made for age, sex, stroke type, score on the FAST at study inclusion, systolic blood pressure at randomisation, EM-score of the Glasgow Coma Scale at randomisation, time of symptom onset to randomisation, pre-stroke score on the mRS and ambulance region.

‡ The modified Rankin Scale score ranges from 0 (no functional limitations) to 6 (death), with a higher score indicating more severe functional disability. A score of 2 or less indicates functional independence.

§ The National Institutes of Health Stroke Scale (NIHSS) is a 15-item scale ranging from 0 to 42. Higher scores indicate a more severe neurological deficit.

¶ The collateral score quantifies the extent of collateral flow visible on CT-angiography, if assessed, in ischaemic stroke patients. The score ranges from 0 indicating no collaterals, to 3 indicating collateral flow to 100% of the affected territory.
|| Volume of intracerebral haemorrhage, in patients with final diagnosis intracerebral haemorrhage.
** The EuroQoL Group 5-Dimension 5-Level Self-Report Questionnaire (EQ-5D-5L) is an ordinal scale ranging from XX to XX, to measure quality of life. A higher score indicates a better health status.
†† The Barthel index quantifies performance of self-care activities in daily living, ranging from 0 (severe disability) to 100 (no disability).
‡‡ Home time: the number of nights among the first 90 days since stroke onset that are spent in the patient's own home or a relative's home

Table 3. Safety Outcomes. *						
Outcome	No. of patients with data	GTN (N=xxx)	Control (N=xxx)	Effect Measure	Unadjusted Value (95% CI)	Adjusted Value† (95% CI)
Death within 90 days – no. (%)				Odds ratio		
Death within 7 days – no. (%)				Odds ratio		
Any SAE within 7 days – no. (%)				Odds ratio		
Hypotension – no. (%)‡				Odds ratio		
Hypertension – no. (%)‡				Odds ratio		
Symptomatic intracranial haemorrhage – no. (%)§				Odds ratio		
Progression or neurological deterioration from ischaemic stroke ¶				Odds ratio		
Progression or neurological deterioration from intracerebral haemorrhage ||				Odds ratio		

GTN denotes glyceryl trinitrate, ICH intracerebral hemorrhage, SAE serious adverse event

* SAEs were registered in the first 7 days after randomisation, or until discharge, if earlier

† Adjustments were made for age, sex, stroke type, score on the FAST at study inclusion, systolic blood pressure at randomisation, EM-score of the Glasgow Coma Scale at randomisation, time of symptom onset to randomisation, pre-stroke score on the mRS and ambulance region.

‡ Requiring clinical intervention with continuous IV fluid or medication.

§ Symptomatic intracranial haemorrhage in ischaemic stroke patients was scored by a serious adverse event committee using the Heidelberg criteria.

¶ Neurological deterioration of 4 points or more from admission NIHSS, or 2 points or more on one item, not explainable by intracranial haemorrhage on repeated imaging, or as consequence of ischaemic stroke when no follow-up imaging was performed

|| Neurological deterioration of 4 points or more from admission NIHSS, or 2 points or more on one item, explained by progression of intracerebral haemorrhage on repeated imaging, or as consequence of intracerebral haemorrhage when no follow-up imaging was performed

Figures

Figure 1. CONSORT-style flowchart of randomisation, inclusion, and treatment of patients.
In a 1:1 ratio, patients were randomly allocated to the control arm or the intervention arm (transdermal glyceryl trinitrate). Due to deferral of consent, XXX patients were randomised but not included (XX in the intervention arm, XX in the control arm).

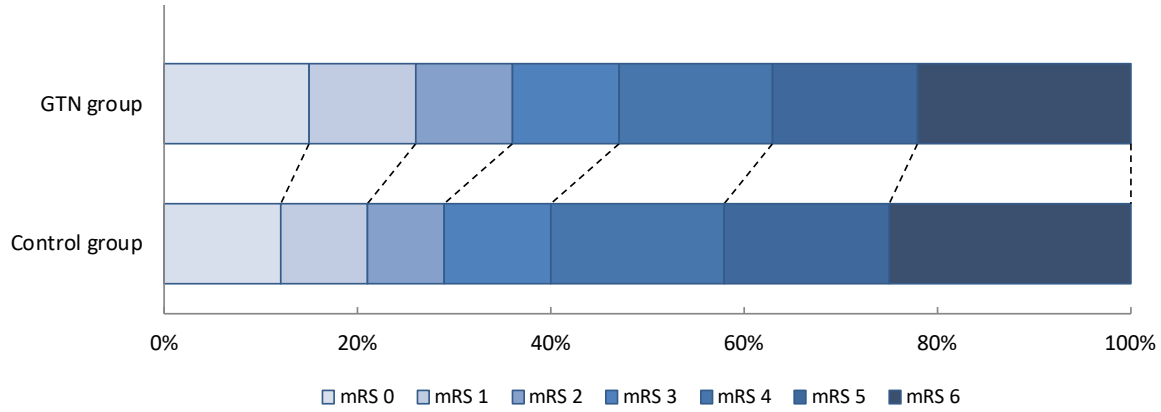

Figure 2. Distribution of the 90-day modified Rankin Scale score in the Intention-to-Treat population.
GTN denotes glyceryl trinitrate, mRS modified Rankin Scale.
Shown are the modified Rankin Scale Scores of all included patients with 90-day follow-up data available. Scores are as follows: 0 (no symptoms or disability after stroke), 1 (no clinically relevant disability), 2 (slight disability), 3 (moderate disability), 4 (moderate to severe disability, 5 (severe disability, complete dependence of daily care), and 6 (death).
Glyceryl trinitrate was XX (adjusted common odds ratio XX; 95% CI, XX-XX).
Note: this figure is an example, with dummy scores.

Variable	GTN	control	acOR (95%CI)	p for interaction
Age				
≤70				
>70				
Sex				
Male				
Female				
Stroke type				
Ischaemic stroke				
Intracerebral haemorrhage				
TIA				
Stroke mimic				
Large vessel occlusion				
Yes				
No				
Intravenous thrombolysis				
Yes				
No				
Endovascular treatment				
Yes				
No				
Blood pressure at randomisation				
Min-median				
Median-max				
Time to randomisation				
≤1 hour				
1-2 hours				
≥2 hours				

Figure 3. Effect of glyceryl trinitrate versus control on 90-day modified Rankin Scale score in prespecified subgroups in the Intention-to-Treat population.

GTN denotes glyceryl trinitrate, TIA transient ischaemic attack, NIHSS National Institutes of Health Stroke Scale.
Data are odds ratio (95% confidence intervals) and interaction test. Comparison by ordinal logistic regression adjusted for age, sex, stroke type, score on the FAST at study inclusion, systolic blood pressure at randomisation, EM-score of the Glasgow Coma Scale at randomisation, time of symptom onset to randomisation, pre-stroke score on the mRS and ambulance region.
Note: will be shown as forest plot.

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