NOVIS trial – Statistical Analysis Plan

1 Administrative information

Title	NOn-invasive Vagus nerve stimulation in acute Ischemic Stroke (NOVIS)
Trial registration	NCT04050501, clinicaltrials.gov
Protocol ID	NL64702.058.18
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Project leader	M.J.H. Wermer, MD, PhD Department of Neurology Leiden University Medical Center (LUMC), Leiden, the Netherlands Head of Department of Neurology University Medical Center Groningen (UMCG), Groningen, the Netherlands
Prinicpal investigators	M.J.H. Wermer, MD, PhD Department of Neurology LUMC Head of Department of Neurology UMCG m.j.h.wermer@umcg.nl A. van der Meij, MD Department of Neurology LUMC Leiden a.van_der_meij@lumc.nl
Co-investigators (in alphabetical order)	M.D. Ferrari, MD, PhD Department of Neurology LUMC N.D. Kruyt, MD, PhD Department of Neurology LUMC M.A.A. van Walderveen, MD, PhD Department of Radiology LUMC E.W. van Zwet, PhD Department of Medical Statistics LUMC
Sponsor	LUMC, Albinusdreef 2, 2333ZA Leiden, the Netherlands

2 Introduction

The study protocol of the NOn-invasive Vagus nerve stimulation in acute Ischemic Stroke (NOVIS) trial has been previously published.[1] Here we provide a summary and updated statistical analysis plan (SAP) with additional detail regarding the scoring of neuro-images, the handling of missing data, and the planned primary and secondary analyses. This SAP was finalized prior to database lock and commencement of the final analysis. Please note that not all pre-specified analyses outlined in this SAP may be included in the main manuscript, due to word count restrictions.

2.1 Research questions

The primary objective is to investigate whether treatment with non-invasive vagus nerve stimulation (nVNS) in addition to standard treatment results in smaller infarct volumes in acute ischemic stroke patients compared with those receiving standard treatment alone. Secondary objectives include assessing the feasibility and tolerability of nVNS, its effect on penumbral recovery, clinical outcomes, and the occurrence of seizures, depression and headache after ischemic stroke. Furthermore, overall survival was added as a secondary objective following the publication of the original protocol.

3 Study methods

3.1 Trial design

NOVIS is a prospective randomized clinical trial with blinded outcome assessment (PROBE design). Patients are recruited from the stroke unit of the Leiden University Medical Center (LUMC).

3.2 Randomization

150 patients are randomized on a 1:1 basis to nVNS on top of best medical practice or best medical practice alone (including intravenous thrombolysis (IVT) and/or endovascular treatment (EVT)). Randomization is performed with Castor (castoredc.com) and is without stratification.

3.3 Blinding

Patients and investigators are not blinded to treatment allocation due to the nature of the intervention. The primary endpoint will be assessed by two experienced neuroradiologists who are blinded to treatment allocation. The 90-day modified Rankin Scale (mRS) will be assessed by a trained research nurse blinded to treatment allocation.

4 Trial population

4.1 Inclusion criteria

- Ischemic stroke comprising the supratentorial region
- Age 18 years or over
- NIHSS 1 or higher
- Perfusion deficit on the admission CT perfusion scan; the penumbra must comprise at least 1/3 of the total ischemic area (ischemic core and penumbra)
- The infarct has to comprise the supratentorial region
- Treatment has to start within 12 hours after stroke onset
- Patients or their legal representatives need to sign informed consent

4.2 Exclusion criteria

- Life expectancy less than three months
- Pre-stroke mRS 3 or higher
- Contraindication for contrast CT agents
- Contraindications for nVNS:
    - An active implantable medical device such as a pacemaker, deep brain stimulator, or any implanted electronic device
    - Symptomatic stenosis or dissection of the carotid artery (in these patients the other side will be stimulated unless a significant stenosis or dissection on the other side is present as well)
    - Structural abnormality e.g. lymphadenopathy, previous surgery or abnormal anatomy (in these patients the other side will be stimulated)
    - Metal cervical spine hardware or metallic implant near the stimulation site
    - Cervical vagotomy (in these patients the other side will be stimulated)
    - Pregnancy

4.3 Patient selection – flow chart

All consecutive patients of 18 years or older presenting on the emergency department of the LUMC and subsequently admitted to the stroke unit with an ischemic stroke and time of onset or time last seen well less than 12 hours ago will be screened for eligibility. A flow chart will be made showing all in- and excluded patients and the reasons for exclusion.

5 Trial endpoints

5.1 Primary endpoint

Infarct volume on day 5 on MRI among those who are alive.

5.2 Secondary endpoints

- Feasibility; defined as more than 75% of the nVNS treated patients completing treatment for five days or until discharge
- Tolerability; defined as less than 10% of the patients treated with nVNS having to abort treatment due to side effects
- Proportion of patients in whom <50% of the penumbra turned into ischemic core on non-contrast CT on day 3
- Clinical outcome (modified Rankin Scale, mRS) on day 90
- Occurrence of seizures or headache in the first 90 days
- Symptoms of depression, quality of life and cognitive status on day 90
- Overall survival in the first 90 days – *added after protocol publication*

Two of the secondary endpoints mentioned in the publication of the study protocol are removed:

- Degree of blood-brain barrier leakage on day three measured with CT perfusion – *removed due to the inability to reliably quantify this parameter.*
- NIHSS on day 5 or on day of discharge if earlier – *removed because it was considered not to have added value in addition to the radiological outcome and the 90-day clinical outcome.*

5.3 Assessment of endpoints

Infarct volumes on MRI will be calculated by manually tracing hyperintense areas on the Diffusion-Weighted Imaging (DWI) sequence using MeVisLab version 3.4.4. Assessments will be done independently by two trained neuro-radiologists blinded to all patient information except for the side of the ischemic stroke. The average infarct volume of the two ratings will be used as the final infarct volume. A Bland-Altman plot will be made to describe interrater agreement. In case of substantial discrepancies between the two assessments the two raters will review their ratings and will trace the final infarct volume together in a consensus meeting.

Perfusion deficits (penumbra and core volumes) will be quantified using automated software (RAPID, iSchemaView).

The 90-day modified Rankin Scale (mRS) will be assessed with a telephone interview by a trained research nurse blinded to treatment allocation.

Symptoms of depression on day 90 will be assessed using the hospital anxiety and depression scale (HADS) questionnaire, quality of life with the EQ-5D and EQ VAS questionnaires, and cognitive status using the Telephone Interview for Cognitive Status (TICS) questionnaire. All questionnaires will be conducted by trained research personnel not blinded to treatment allocation.

6 Statistical analysis

6.1 Baseline Characteristics

For continuous variables, the mean and standard deviation (SD), or median and interquartile range (IQR) will be reported in both groups, whichever is appropriate. For discrete variables, the number of subjects in each category and the percentage based on the amount of subjects without missing values will be reported in each treatment group.

6.2 Primary endpoint analysis

Linear regression analysis will be performed to assess the mean difference (MD) in the final infarct volumes between the two groups with corresponding 95% confidence intervals (CIs). The analysis is based on the intention-to-treat principle. Given the randomized design of the study no adjustments for potential outcome variation will be done with multivariable regression analysis. This decision was made after protocol publication. Instead, a sensitivity analysis will be performed with age, NIHSS at randomization and baseline infarct core on CTP as pre-specified covariates.

6.3 Secondary endpoint analyses

Secondary endpoints will be compared using t-tests for continuous variables and chi-square tests for dichotomous variables. Feasibility and tolerability of nVNS will be assessed binary as “reached” or “not reached”. Overall survival in the first 90 days will be assessed using a Kaplan-Meier estimator. See table for the method of analysis of the other secondary endpoints. For all secondary analyses, no adjustments will be made unless age, NIHSS at randomization and/or baseline infarct core on CTP appear to be unbalanced between the two treatment groups. Effect estimates are expressed as MD or (common) odds ratios (OR) with 95% confidence intervals (CI).

7 Missing data and death

For all variables the proportion of missing values will be reported. For the primary endpoint, missing infarct volumes among those patients who are alive on day 5 will be imputed using a model containing the following information: NIHSS at randomization, baseline infarct core volume on CTP, infarct volume on NCCT on day 3 (if available), infarct volume on NCCT on day 5 (if available instead of MRI), treatment with IVT and/or EVT, TICI score post EVT, discharge before day 5, and death after day 5. We will use multiple imputation to generate 10 imputed datasets.

In case of missing questionnaires, these patients will be excluded from analyses on secondary endpoints regarding 90 day follow up. Furthermore, a subanalysis using the lowest and the highest values on the various scales will be conducted.

8 Locking of the database

After 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 AM and EZ; they will not be involved in resolving any final queries to maintain the integrity and blinding of the final database.[2-4] 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.[2-4] Data analyses will be performed by AM and EZ.

9 References

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Table

Secondary endpoint	Method of analysis	Effect variable
Proportion of patients in whom <50% of the penumbra turned into ischemic core on non-contrast CT on day 3	Binary logistic regression model	OR (95% CI)
Shift on the 90 day mRS	Ordinal logistic regression model	cOR (95% CI)
Occurrence of seizures or headache in the first 90 days	Binary logistic regression model	OR (95% CI)
Symptoms of depression on day 90 (HADS)	Linear regression model	MD (95% CI)
Quality of life on day 90 (EQ-5D and EQ VAS)	Linear regression model	MD (95% CI)
Cognitive status on day 90 (TICS)	Linear regression model	MD (95% CI)

Secondary endpoints, methods of analysis and effect variable.
NIHSS = National Institutes of Health Stroke Scale, mRS = modified Rankin Scale, HADS = Hospital Anxiety and Depression Scale, TICS = Telephone Interview for Cognitive Status, OR = Odds Ratio, CI = Confidence Interval, MD = Mean Difference, cOR = common Odds Ratio