Transauricular nerve stimulation in acute ischaemic stroke requiring mechanical thrombectomy: protocol for a phase 2A, proof-of-concept, sham-controlled randomised trial.

Statistical Analysis Plan

Version 1.0
Date: 26.10.2023

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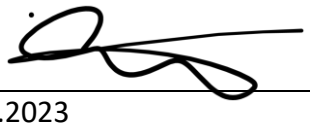
Signature	
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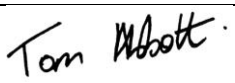
Signature	
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1. Administrative information

Trial Information

REC number:	23/WA/0013
Trial Sponsor:	Queen Mary University of London
Trial Sponsor reference:	011368
Trial Funder:	INNOVATE-UK
Trial registration :	NCT05417009
Protocol version (date):	Version 3.0 (20/04/2023)

Version 1.0 of the SAP for VANS was written before TA had access to unblinded data (i.e. trial dataset with the variables for treatment allocation included).

Remit of the SAP

The purpose of this document is to provide details of the statistical analyses and presentation of results to be reported for the VANS trial. It is important to set these out and to agree them in advance of inspecting the outcome data for the trial, so that data derived decisions in the analysis are avoided. Any exploratory, post hoc or unplanned analysis will be clearly identified as such in the respective study analysis report.

2. Background and study design

Study objectives	**Primary Objective** To determine the effect of auricular nerve stimulation and blood pressure variability, measured by calculating the coefficient of variation of systolic blood pressure. **Secondary Objectives** To determine the effect of auricular nerve stimulation on other measures of systolic and diastolic blood pressure variability in the first 24h after mechanical thrombectomy , heart rate variability And clinical outcomes (NIH Stroke Scale (NIHSS) , organ dysfunction)
Study design	Phase II, single-centre, two-arm, parallel group randomised controlled trial- pre-specified analysis.
Setting	Acute stroke requiring mechanical thrombectomy.
Participants	**Inclusion criteria** • age>18 years; • undergoing mechanical thrombectomy for acute ischaemic stroke; • established hypertension and/or hypertensive on admission (defined as systolic BP >140mmHg; diastolic BP >90mmHg).

	Exclusion criteria • participation in a trial exploring similar biological mechanism; previous enrolment; • anatomical contraindication (local ear abnormalities); • pregnancy.
Interventions	**Active stimulation** bilateral active transauricular nerve stimulation for the duration of the mechanical thrombectomy procedure (AffeX-CT/001 investigational device). The intervention will be repeated for 1h the morning following the mechanical thrombectomy **Sham stimulation** bilateral sham transauricular nerve stimulation for the duration of the mechanical thrombectomy procedure (AffeX-CT/001 investigational device). The intervention will be repeated for 1h the morning following the mechanical thrombectomy
Primary outcome measure	The primary outcome is Blood pressure variability [Time Frame: 0-24h after mechanical thrombectomy], as calculated from the coefficient of variation of systolic blood pressure

3. Outcome measures

Primary outcome measure
The primary outcome is Blood pressure variability [Time Frame: 0-24h after mechanical thrombectomy], as reflected by the coefficient of variation of systolic blood pressure.

Secondary outcome measures

1. Systolic and diastolic blood pressure variability in the first 24h after mechanical thrombectomy [Time Frame: 0-24h after mechanical thrombectomy]: systolic and diastolic blood pressure mean, standard deviation, and coefficient of variation.
2. Heart rate variability [Time Frame: 0-24h after mechanical thrombectomy]: time and frequency domain measures of autonomic cardiac modulation.
3. NIH Stroke Scale (NIHSS) [Time Frame: recorded before, at 24h after mechanical thrombectomy]
4. Organ dysfunction [Time Frame: First seven days after mechanical thrombectomy.]- defined as clinical need for intravenous pharmacological support for blood pressure [pressors or intravenous antihypertensive medication]; arrythmias; myocardial injury [high sensitivity troponin]; hospital-acquired infection.

Exposure variables

The exposure is bilateral active or sham transauricular nerve stimulation for the duration of the mechanical thrombectomy procedure (AffeX-CT/001 investigational device), an intervention that is be repeated for 1h the morning following the mechanical thrombectomy (provided the patient remains in Royal London Hospital).

Sample size and randomisation

Sample size calculation

the sample size is derived from data reported from the secondary analysis of the BEST study, which examined blood pressure variability and neurologic outcome after endovascular thrombectomy.[1] An adjusted sample size of 36 patients is required to have a 90% chance of detecting, as significant at the 5% level, a decrease in the coefficient of variation in systolic blood pressure from 15±4mmHg in the sham group to 10±4mmHg in the VAN group [assuming 5% non-compliance rate in each group]

Randomisation procedure

Randomisation will occur before the surgical procedure is due to start. Block randomisation (blocks of 4) will be undertaken (STATA). To enter a patient into the VANS trial, research staff at the site will log on to a secure web-based randomisation and data entry platform hosted by Queen Mary University of London and complete the patient's details to obtain a unique patient identification number and allocation to a treatment group. A patient's treatment group allocation will only be revealed to the person performing randomisation.

4. Analysis methods for VANS

General analysis principles

Analyses will follow the intention-to-treat principle: all randomised patients with a recorded outcome will be included in the analysis and analysed according to the treatment to which they were randomised [2, 3]. Patients will be included in the analysis, regardless of whether the treatment they received was compliant with the protocol. Missing data for baseline covariates to be included in the analysis model will be accounted for using mean imputation for continuous variables and the missing indicator approach will be used for missing data for categorical variables [4, 5].

For the analysis of the primary outcome, each secondary outcome, and all process measures, we will present the following information:

- The number of patients included in each analysis, by treatment group
- A summary statistic of the outcome (e.g. mean (SD), number (%)), by treatment group
- The estimated treatment effect
- A 95% confidence interval for the estimated treatment effect
- A two-sided p-value

For all analyses, a significance level of 5% will be used.

Representativeness of patients

All participating sites have been asked to keep a log of eligible patients not recruited to the trial. Reasons for non-participation will be categorised and summarised. Participation in the trial, treatment allocation and completeness of follow-up will be illustrated by a CONSORT flow diagram [6].

Baseline characteristics

Baseline characteristics will be summarised for each treatment group by the mean and standard deviation or median and interquartile range for continuous variables, and the number and percent for categorical variables, but not subjected to hypothesis testing. The following baseline characteristics will be summarised by treatment group:

- Demographic: age (years), gender (male/female)
- Co-morbid disease : Chronic obstructive pulmonary disease (COPD), Asthma, Interstitial lung disease or pulmonary fibrosis, Ischaemic heart disease, Congestive heart failure, Diabetes Mellitus, Liver cirrhosis, Active cancer, peripheral vascular disease
- Pre-operative blood test results; Haemoglobin, Creatinine, Neutrophil count, Lymphocyte count, Albumin.
- Cardiovascular medication: Beta-blocker, Calcium channel antagonist (amlodipine/felodipine etc), verapamil/diltiazem, Doxazosin, Diuretic, Statin, Nitrate, Anti-platelet agents (aspirin, clopidogrel), Angiotensin converting enzyme inhibitors (ACE inhibitors), Angiotensin II Receptor Blockers (ARBs), Warfarin, SGLT2 inhibitor, Dabigatran or Apixaban, Any other anticoagulation established pre-operatively (LMWH), Metformin, Insulin.

Analysis software

All analyses will be conducted in Stata Version 14 (StataCorp. 2015. *Stata Statistical Software: Release 14*. College Station, TX: StataCorp LP).

Analysis of primary outcome

Primary analysis

The primary outcome will be the coefficient of variation of systolic blood pressure over the first 24h from start of mechanical thrombectomy. The primary exposures will be analysed by construction of a single categorical variable consisting of active versus sham stimulation.

When participants are randomised according to incorrect baseline information, under the ITT principle they should be analysed in their allocated treatment group, irrespective of the fact that their allocation was based on incorrect information. The incorrect baseline information should be kept in the randomisation record, as this reflects how the randomisation was performed, and the correct information documented for use in an adjusted analysis [7].

Analysis of secondary outcomes

1. Blood pressure variability in the first 24h after mechanical thrombectomy (standard deviation, average real variability, successive variation and residual standard deviation).

BPV formulae are provided in supplementary data. These measures take into account the distribution and linear trends of BP in an effort to reduce the dependency of BPV on mean BP level.

2. Autonomic cardiac modulation in the first 24h after mechanical thrombectomy (Holter monitoring).

3. Neurological recovery will be reported by recording serial NIH Stroke Scale (NIHSS) scores, an FDA-approved primary outcome measure for stroke in early phase II trials before and 24h after mechanical thrombectomy.[8]

Exploratory objectives

Biomarkers for risk of infection (whole blood leukocyte transcriptomic changes after incubation at 37ºC with sterile saline or 100ng/ml lipopolysaccharide for 4h), infections within 7 days of MT and myocardial injury (high-sensitivity troponin) will also be quantified. Cardiac injury will be assessed by measuring high-sensitivity troponin T on admission and 24h after MT.

Sensitivity analysis

For patients discharged before the repeat stimulation intervention or repatriated to referring centres before the repeat intervention, we will undertake a sensitivity analysis. The primary analysis will be repeated once assuming that all patients in the continue group with missing outcomes did not have BPV >20. The analysis will then be repeated again with the opposite assumptions. This will then give the absolute range of how much the results could change if the data were complete. This will also assess the robustness of our analysis of the primary outcome under the assumption that data is missing-not-at-random (MNAR).

5. Other analyses, data summaries and graphs

Clinical management

Clinical management for the treatment allocation will be summarised but not subjected to statistical testing. Numbers (%) and means (SD) or medians (IQR) will be provided separately for each principal exposure group:

- Thrombectomy technique
- General Anaesthesia/ Sedation
- Endotracheal tube inserted?
- Did the patient have any arrhythmias
- Intraoperative hypotension
- Planned level of care.

Process measures

Summary measures will be presented separately for each treatment allocation. All patients with recorded data will be included in the summary. Formal statistical analysis will not be performed. Patient status at follow up (alive/dead; home/hospital) seven days after MT will be presented as number (%).

Safety analyses

Adverse events and serious adverse events will be presented as a number (%) by
treatment allocation. All patients with a recorded outcome will be included in the summary. In addition to this, 'other' adverse events will be reported separately if prevalence is more than 5% across all participants in the trial.

Protocol deviations

Numbers and percentages of protocol deviations will be reported. The following protocol deviations will be reported: (a) Patient who did not receive either or both leads; (b) other deviation. We will report the number of patients in each treatment group with at least one of the above protocol deviations. In addition to this, 'other' protocol deviations will be reported separately of prevalence is more than 5% in the trial.

References

1. Mistry, E.A., et al., *Blood Pressure Variability and Neurologic Outcome After Endovascular Thrombectomy: A Secondary Analysis of the BEST Study.* Stroke, 2020. **51**(2): p. 511-518.
2. White, I.R., et al., *Strategy for intention to treat analysis in randomised trials with missing outcome data.* BMJ, 2011. **342**: p. d40.
3. Montori, V.M. and G.H. Guyatt, *Intention-to-treat principle.* CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne, 2001. **165**(10): p. 1339-1341.
4. White, I.R. and S.G. Thompson, *Adjusting for partially missing baseline measurements in randomized trials.* Statistics in Medicine, 2005. **24**(7): p. 993-1007.
5. Groenwold, R.H.H., et al., *Missing covariate data in clinical research: when and when not to use the missing-indicator method for analysis.* CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne, 2012. **184**(11): p. 1265-1269.
6. Schulz, K.F., et al., *CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials.* BMC Medicine, 2010. **8**(1): p. 18.
7. Yelland, L.N., et al., *Applying the intention-to-treat principle in practice: Guidance on handling randomisation errors.* Clinical Trials, 2015. **12**(4): p. 418-423.
8. Chalos, V., 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): p. 282-290.

Appendix 1: Derived outcomes and variables

Primary outcome

CV systolic blood pressure: $$\mathrm{CV} = \left(\frac{SD}{BP_{mean}} \right) * 100$$

Secondary outcomes

For both systolic BP (SBP) and diastolic BP (DBP), the following indices of blood pressure variability will be calculated:

1. Standard deviation SBP/DBP in the first 24h after mechanical thrombectomy.

$$\mathrm{SD} = \sqrt{(\frac{1}{n-1}) \sum_{(i=1)}^{(n)} \left(BP_i - BP_{mean} \right)^2}$$

2. Average real variability in the first 24h after mechanical thrombectomy.

$$ARV = \frac{1}{\sum w_k} \sum_{k=2}^{n} w_k \times \left| BP_k - BP_{k-1} \right|$$

3. Successive variation in the first 24h after mechanical thrombectomy.

$$\mathrm{SV} = \sqrt{\left(1/\left(n-1\right)\right) \sum_{(i=1)}^{(n-1)} \left(BP_{i+1} - BP_i \right)^2)}$$

4. Residual standard deviation in the first 24h after mechanical thrombectomy.

$$\mathrm{rSD} = \sqrt{\frac{\sum \left(BP - BPest \right)^2}{n-2}}$$

Heart rate variability, time domain measures
RR-interval, SDNN, RMSSD, SD HR, NN, pNN

Frequency domain measures
VLF power, VLF peak, LF power, LF peak, HF power, HF peak, DFA1, DFA2.

Peak level of Troponin-T measured within 24 hours of MT
This is defined as the highest troponin-t value measured within 24 hours of mtwhich is collected on admission and 24h post MT [if applicable]. If only one troponin-T value is available, then this will be used as the peak. If troponin-T data is missing for both timepoints, then the outcome will be set to missing.

Infection up to 7 days after mechanical thrombectomy
This is defined as one or more of the following infections (definitions of each type of infection are the same used in the SPACE protocol):

- Superficial surgical site infection
- Deep surgical site infection
- Organ space surgical site infection
- Urinary tract infection
- Infection, source uncertain
- Laboratory confirmed blood stream infection

Equal to 1 if:
- At least one of the components of infection is listed as occurring Equal to 0 if:
- All of the components of postoperative infection are “None”

Missing if:
- All components are missing
- OR one or more of the components of postoperative infection is missing and all other components are “None”

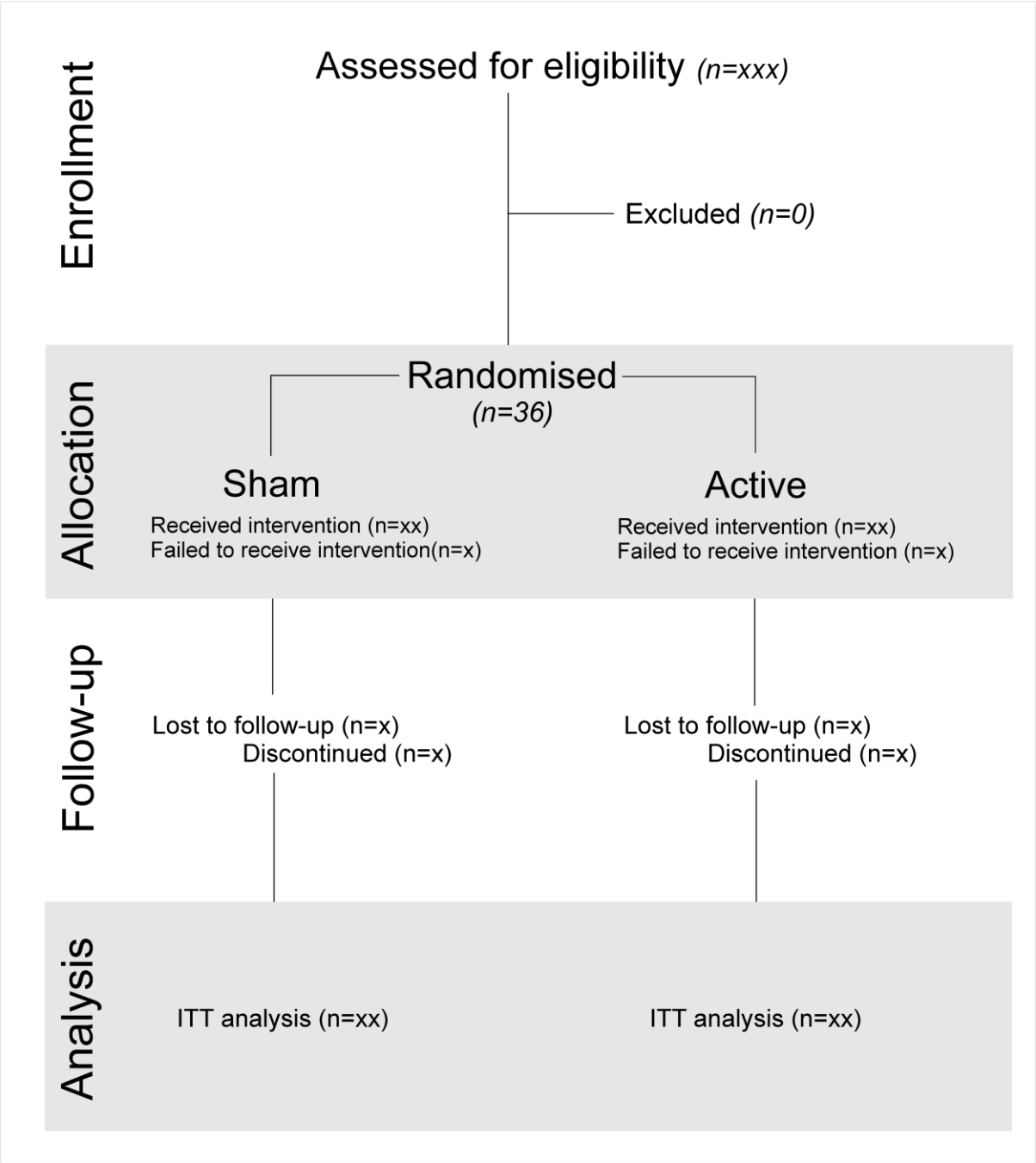
<u>Death and location 7 days after mechanical thrombectomy</u>

Equal to 1 if:
- Patient status at 7-day follow-up is dead/in hospital

Equal to 0 if:
- Patient status at 7-day follow-up is alive / out of hospital

Missing if:
- Patient status at 30-day follow-up is missing
- Patient status at 30-day follow-up is marked as ‘dead’ and missing date of death

Appendix 2: Information for CONSORT flow diagram



Appendix 3: Dummy tables

Table 1: Baseline Characteristics

	Sham	Active
Gender - no. (%)		
Male		
Female		
Age (years)		
Median (IQR)		
Chronic comorbid disease - no. (%)		
COPD		
Asthma		
Interstitial lung disease or pulmonary disease		
Ischaemic heart disease		
Diabetes mellitus		
Heart failure		
Liver cirrhosis		
Active cancer		
Peripheral vascular disease		
Current or previous smoker		
Preoperative blood tests results		
Haemoglobin (d/DL)		
Mean (SD)		
Creatinine (µmol/L)		
Mean (SD)		
Cardiovascular medication - no. (%)		
Beta-blocker		
Calcium channel antagonist (e.g. amlodipine/felodipine)		
verapamil/diltiazem		
Doxazosin		
Diuretic		
Statin		
Nitrate		
Anti-platelet agents (aspirin, clopidogrel)		
ACE inhibitor		
Angiotensin receptor antagonist		
Warfarin		
SGLT2 inhibitor		
NOAC		
Other anticoagulant		
Metformin		
Insulin		

Abbreviations: SD, standard deviation; IQR, Interquartile range; COPD, chronic obstructive pulmonary disease.

Table 2: Clinical management

	Sham	Active
NIHSS score on admission		
Troponin on admission (ng/L)		
Thrombectomy technique - no. (%)		
Aspiration		
Clot breakup		
Thrombolysis		
Anaesthetic technique - no. (%)		
General anaesthesia		
Sedation		
Endotracheal tube inserted		
Systolic blood pressure <90 mmHg - no. (%)		
Arrhythmias - no. (%)		
Planned level of care on the first night after MT - no. (%)		
Critical care unit		
Stroke ward		
Repatriated		

Abbreviations: SD, standard deviation; IQR, Interquartile range

Table 3: Adherence to intervention.

Adherence - no. (%)	Sham	Active
Patients with ≥1 treatment deviation		
Total number of deviations		
Number of treatment deviations per patient		
0		
1		
2		
Other deviation		

Table 4: Primary and secondary outcomes for blood pressure variability.

	Sham	Active	P value
Primary outcome			
Systolic BP coefficient of variation			
Secondary outcomes:			
Systolic blood pressure			
Mean			
Standard deviation			
Coefficient of variation			
Diastolic blood pressure			
Mean			
Standard deviation			
Coefficient of variation			
Heart rate			
Mean			
Standard deviation			
Coefficient of variation			

Table 5: Secondary outcomes for heart rate variability.
Mean (SD)

	Sham	Active	**P value**
Time domain measures			
RR-interval			
SDNN			
RMSSD			
SD HR			
NN			
pNN			
Frequency domain measures			
VLF power			
VLF, peak			
LF, power			
LF, peak			
HF, power			
HF, peak			
DFA1			
DFA2			

Table 6. Process/ exploratory measures.

	ACTIVE	SHAM
NIH Stroke Scale (NIHSS) 24h after mechanical thrombectomy (delta change from admission)		
Health status 7 days after mechanical thrombectomy.		
Alive		
Troponin change from admission (delta change from admission)		

Table 7:Adverse events

Adverse Events - no. (%)	**active**	**sham**
Patients with ≥ 1 adverse event		
Total number of adverse events		
Type of adverse event		
Cardiac arrythmia		
Local skin irritation		
Other		