THE ASSESSMENT OF CEREBRAL HAEMODYNAMICS IN PATIENTS WITH TRANSIENT ISCHAEMIC ATTACK: A TRANSCRANIAL DOPPLER STUDY

Date and Version No: **Version 1.0 20th February 2019**

Sponsor Reference No: **UNOLE 0694**

Ethics Reference No: **19/LO/0501**

Chief Investigator: **Professor Thompson Robinson**

Professor of Stroke Medicine

Department of Cardiovascular Sciences, University of Leicester

Room 228, Robert Kilpatrick Clinical Sciences Building

Leicester Royal Infirmary, Leicester, LE2 7LX

Email: tgr2@le.ac.uk Tel: 0116 252 3187

Investigators: **Mr Angus Batterham**

Intercalated MSc student

Department of Cardiovascular Sciences, University of Leicester

Room 237, Robert Kilpatrick Clinical Sciences Building

Leicester Royal Infirmary, Leicester, LE2 7LX

Email: ab786@student.le.ac.uk Tel: 0116 252 5802

Dr Victoria Haunton

Honorary Senior Lecturer

Department of Cardiovascular Sciences, University of Leicester

Room 210, Robert Kilpatrick Clinical Sciences Building

Leicester Royal Infirmary, Leicester, LE2 7LX

Email: vjh12@le.ac.uk Tel: 0116 252 5802

Professor Ronney Panerai

Emeritus Professor

Department of Cardiovascular Sciences, University of Leicester

Room 225, Robert Kilpatrick Clinical Sciences Building

Leicester Royal Infirmary, Leicester, LE2 7LX

Email: tgr2@le.ac.uk Tel: 0116 252 3183

Sponsor: University of Leicester

Research Governance Office

uolsponsor@le.ac.uk

0116 258 4099 / 4867 / 8305

Author

Dr Victoria Haunton BM MD DGM MRCP(Geriatric Medicine)

Honorary Senior Lecturer

Department of Cardiovascular Sciences, University of Leicester

Room 210, Robert Kilpatrick Clinical Sciences Building

Leicester Royal Infirmary, Leicester, LE2 7LX

Email: vjh12@le.ac.uk

Tel: 0116 252 5802

Confidentiality Statement

Signatures:

The approved protocol should be signed the author and/or person(s) authorised to sign the protocol

Signature Page

Chief Investigator Name: _____

Chief Investigator Signature: _____

Date: _____

Sponsor Representative Name: _____

Sponsor Representative Signature: _____

Date: _____

TABLE OF CONTENTS

1. AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made

2. SYNOPSIS

Study Title	THE ASSESSMENT OF CEREBRAL HAEMODYNAMICS IN PATIENTS WITH TRANSIENT ISCHAEMIC ATTACK: A TRANSCRANIAL DOPPLER STUDY
Internal ref. no.	UNOLE 0694
Trial Design	Single-centre, prospective, case control study
Trial Participants	Participants with Transient Ischaemic Attack (TIA) and healthy adults (HC)
Planned Sample Size	40 participants in total; 20 participants with TIA and 20 HC
Follow-up duration	N/A
Planned Trial Period	6 months
Primary Objective	To determine the beat-to beat cerebral blood flow velocity (CBFv) of both TIA and HC subjects during performance of a squat-stand manoeuvre
Secondary Objective	To determine other beat-to-beat cerebral haemodynamic parameters, specifically autoregulation index (ARI), of both TIA and HC subjects during performance of a squat-stand manoeuvre
Outcome Measures	This is not an intervention study, and therefore it would not be appropriate to assess classical primary and secondary outcome measures such as death and disability for this study. However, we intend to evaluate the following relevant outcomes: 1. The number of participants successfully recruited 2. The number of recruited participants able to comply with the full measurement protocol. 3. The number of measurements rejected because of aspects related to data quality during the analysis protocol, with recorded reasons. 4. Overall, the number of recruited subjects in whom values for the following parameters can be derived: • % change of cerebral blood flow velocity (CBFv) at baseline in response to performance of a squat-stand manoeuvre • Autoregulation index

3. ABBREVIATIONS

AE	Adverse event
AR	Adverse reaction
ARI	Autoregulation Index
BP	Arterial Blood Pressure
CA	Cerebral Autoregulation
CBF	Cerebral Blood Flow
CBFv	Cerebral Blood Flow Velocity
CHIASM	Cerebral Haemodynamics in Ageing and Stroke Medicine
CI	Chief Investigator
CPP	Cerebral Perfusion Pressure
CrCP	Critical Closing Pressure
CRF	Case Report Form
CT	Computerised Tomography
dCA	Dynamic Cerebral Autoregulation
ECG	Electrocardiogram
$EtCO_2$	End-Tidal Partial Pressure of Carbon Dioxide
fMRI	Functional Magnetic Resonance Imaging
GCP	Good Clinical Practice
HC	Healthy Control
HR	Heart Rate
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ISF	Investigator Site File
LRI	Leicester Royal Infirmary
MCA	Middle Cerebral Artery
PET	Positron Emission Tomography
PIL/S	Participant Information Leaflet/Sheet
RAP	Resistance Area Product
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SCA	Static Cerebral Autoregulation
SSM	Squat stand manoeuvre
SOP	Standard Operating Procedure
SPECT	Single photon emission computed tomography
SUSAR	Suspected Unexpected Serious Adverse Reactions
TCD	Transcranial Doppler Ultrasound
TFA	Transfer Function Analysis
TIA	Transient Ischaemic Attack
TMF	Trial Master File
UK	United Kingdom

4. BACKGROUND AND RATIONALE

Adequate cerebral blood flow (CBF) is essential for brain survival and function. Brain metabolism is almost entirely dependent on oxidative mechanisms, particularly the metabolism of glucose, and the brain has a very limited capacity for anaerobic function. Adequacy and constancy of cerebral blood flow are therefore vital in ensuring both a steady supply of oxygen, glucose and other nutrients, and the removal of waste products of metabolism.

Cerebral autoregulation (CA) refers to the ability of the brain to maintain a relatively constant CBF in response to significant changes in cerebral perfusion pressure (CPP). Thus, in response to an increase or decrease in CPP, there is a vasodilatation or vasoconstriction of cerebral vessels. This variable resistance to flow occurs mainly in the cerebral arteriolar bed; the major cerebral arteries are essentially conductive rather than compliant [Itoh et al 2012, Kuga et al 2009, Kontos et al 1978]. The classic concept of CA whereby, under normal physiological conditions, CBF is around 50ml/100g/min, and this is maintained across a wide range of blood pressures (MAP of 60-160mmHg), was first described by Lassen in 1959. CA is usually described as being static (sCA), reflecting the integrity of such mechanisms over time, or dynamic (dCA), occurring in response to sudden fluctuations in perfusion pressure. CA is accomplished through the complex interplay of a variety of myogenic, metabolic and neurogenic mechanisms.

In recent years, there has been growing evidence that CA is altered in various disease states including ischaemic stroke [Eames et al 2002], head injury [Czosynka et al 1996], carotid stenosis [Reinhard et al 2004], pre-eclampsia [van Veen et al 2013], chronic diabetes mellitus [Chiu et al 2005], Alzheimer's dementia [Claassen et al 2011], and idiopathic Parkinson's disease [Haunton 2013].

In ischaemic stroke, impaired CA is related to neurological deterioration, the necessity for decompressive surgery, and poor prognosis [Aries et al 2010]. CA has also been shown to correlate with stroke severity and functional outcomes [Llwyd et al 2018].

Transient ischaemic attack (TIA) is defined as "a transient episode of neurologic dysfunction caused by focal brain, spinal cord or retinal ischemia without acute infarction" [Easton et al 2009]. Approximately 46,000 people in the UK each year have a first TIA [Rothwell et al 2005] and one in 12 people will have a full stroke within a week of having a TIA [Coull et al 2004]. Urgent assessment in a specialist TIA clinic ensures timely and appropriate investigations and rapid interventions (such as antiplatelet therapy, blood pressure-lowering medication, statins, anticoagulation and carotid

endarterectomy or stenting where appropriate) [Rothwell et al 2007]. TIA and acute ischaemic stroke share the same pathophysiological mechanisms and, whilst there have been a significant number of studies investigating cerebral haemodynamics and CA in acute stroke, there have been very few studies exploring CA and TIA. A study by Atkins et al reported no impairment of cerebrovascular haemodynamic control following TIA [Atkins et al 2010]. However, recent abstract level data suggest altered CA is predictive of patients who go on to have further TIAs and strokes [Musthak et al 2017].

Cerebral haemodynamics can be studied using techniques such as functional magnetic resonance imaging (fMRI), positron emission tomography (PET) or single-photon emission computed tomography (SPECT). However, these techniques are expensive, in the case of SPECT involve radiation, and there are feasibility issues including the need to lie still for prolonged periods and to have no metal implants. Transcranial Doppler Ultrasonography (TCD) is a simple, non-invasive imaging modality with high temporal resolution which allows for continuous and bilateral recording of cerebral blood flow velocity (CBFv) through the major cerebral arteries [Aaslid et al, 1982]. TCD has the advantage that CBFv measurements can be made in standing, seated, tilted and supine positions. Continuous measurements of CBFv using TCD have shown that CBFv is affected by pertubations in arterial blood pressure (BP) [Aaslid et al 1989].

The most widely used, validated, modelling technique in the analysis of the relationship between CBFv and BP is transfer function analysis (TFA) [Claassen et al 2016]. TFA makes it possible to examine the effect of changes in (BP) on CBFv. It quantifies the extent to which the input signal, BP, is reflected in the output signal, CBFv, and was first proposed by Giller.
Cerebral haemodyamic data which can be obtained using TFA include values of coherence, phase, gain, autoregulatory index (ARI), critical closing pressure (CrCP) and resistance area product (RAP). The ARI was derived in 1995 by Tiecks et al using of equations and curves based on the CBFv response to a sudden fall in induced by thigh cuff deflation. These equations and curves allow for the calculation of an ARI value from 0 to 9, where 0 represents absence of autoregulation i.e. CBF dependent on CPP, (a 'pressure-passive relationship') and 9 represents best measurable autoregulation. 'Normal' autoregulation is represented by an ARI of 5±1.

Whilst CA can be evaluated using spontaneous fluctuations in BP [Brodie et al 2009], coherence between oscillations at these low frequencies (<0.07Hz) is generally low (0.5), which often leads to the exclusion of such data from analysis in studies of CA [Claassen et al 2016]. Furthermore, larger and induced perturbations of BP allow us to study the CBF response to BP with increased certainty that there is a causal relationship. Larger magnitude BP changes can be induced using challenges

such as bilateral thigh cuff inflation to suprasystolic blood pressure and their simultaneous release, lower body negative pressure, and the cold pressor stimulus (placing a hand in ice water). These can be uncomfortable for participants. More recently, squat-stand manoeuvres (SSMs) have also been shown to produce large changes in BP and thus CBFv [Claassen et al 2009a, Smirl et al 2015, Barnes et al 2017]. Squatting induces a rapid but transient rise in BP with its peak after approximately 2-3 seconds (s). This increase in BP has been attributed to a sudden increase in cardiac output from increased venous return from the lower limbs. The subsequent reduction in BP on standing up has been attributed to a combination of reduction in peripheral vascular resistance and a reduction in cardiac output due to rapid return of central blood volume to the lower limbs [Smirl et al 2015]. SSMs can be more comfortable for participants and are arguably more representative of physiological BP challenges in daily life, e.g. bending down to pick something up from the floor and standing up after tying a shoelace, than those other BP challenges detailed above. When SSMs are performed repeatedly, large and periodic changes in BP and CBFv are created. SSMs have been shown to be safe and well tolerated in various populations including heart transplant recipients [Smirl et al 2014], young and old healthy controls [Oudegeest-Sander et al 2014, Barnes et al 2017], and patients with Alzheimer's dementia [Claassen JA et al 2009b]. It has recently been shown that reliable CA data can be obtained from as few as three SSMs [Barnes et al 2018].

The proposed study will investigate and compare the beat-to beat CBFv changes observed during performance of a SSM protocol in 20 participants with acute (within 7 days) TIA and 20 HC.
The proposed study will also investigate whether performance of a SSM is associated with differences in other beat-to-beat cerebral haemodynamic parameters, specifically autoregulation index (ARI), between participants with acute (within 7 days) TIA and 20 HC. In doing so, it is hoped that we will be able to establish if CA is impaired in patients with TIA.

5. OBJECTIVES

5.1 Primary Objective

To determine the beat-to beat cerebral blood flow velocity (CBFv) of both TIA and HC subjects during performance of a SSM.

5.2 Secondary Objective

To determine other beat-to-beat cerebral haemodynamic parameters, specifically autoregulation index (ARI), of both TIA and HC subjects during performance of a SSM.

6. STUDY DESIGN

6.1 Summary of Trial Design

This is a single-centre, prospective, case control study which will run for six months. 20 patients with acute TIA (within 7 days), and 20 healthy controls will be recruited from the specialist TIA clinic at University Hospitals of Leicester NHS Trust. Participants will be eligible if they are aged over 18 and can consent to participate. They won't be able to participate if they have severe heart failure, an irregular heartbeat, blocked neck blood vessels, severe breathing problems, if they do not understand written or verbal English, if they have inadequate ultrasound windows, or if they are pregnant.

TIA participants will be given a minimum of 1 hour, and a maximum of 48 hours to decide whether or not to take part in the study, but must undertake the study assessment within 7 days of their TIA. Healthy control participants will be given a minimum of 1 hour, and a maximum of 7 days to decide whether or not to take part in the study. There is no time restriction on when they undertake the study assessment.

All participants will undergo an assessment of brain blood flow using TCD, during which their heart rate, breathing and blood pressure will also be monitored. During the assessment participants will sit quietly before being asked to stand and then complete a squat-stand manoeuvre in time with a computer sequence. The research visit will take approximately 90 minutes, the TCD assessment itself will take approximately 1 hour and participants only need to attend once.

6.2 Primary and Secondary Endpoints/Outcome Measures

This is not an intervention study, and therefore it would not be appropriate to assess classical primary and secondary outcome measures such as death and disability for this study. However, we intend to evaluate the following relevant outcomes:

a) The number of participants recruited
b) The number of participants able to comply with the full measurement protocol
c) The number of measurements rejected because of aspects related to data quality during the analysis protocol, with recorded reasons
d) Overall, the number of recruited subjects in whom values for the following parameters can be derived:

    % change of cerebral blood flow velocity (CBFv) at baseline in response to performance of a squat-stand manoeuvre

    Autoregulation index

7. TRIAL PARTICIPANTS

7.1 Overall Description of Trial Participants

20 patients with acute TIA (within 7 days), and 20 healthy controls will be recruited from the specialist TIA clinic at University Hospitals of Leicester NHS Trust.

TIA participants will be given a minimum of 1 hour, and a maximum of 48 hours to decide whether or not to take part in the study, but must undertake the study assessment within 7 days of their TIA.

Healthy control participants will be given a minimum of 1 hour, and a maximum of 7 days to decide whether or not to take part in the study. There is no time restriction on when they undertake the study assessment.

7.2 Inclusion Criteria

HEALTHY CONTROLS:

- Willing to participate
- Capacity to consent to the study
- Aged over 18 years
- Able (in the Investigator's opinion) and willing to comply with all study requirements
- Good understanding of written and verbal English

TIA PATIENTS:

- Willing to participate
- Capacity to consent to the study
- Aged over 18 years
- Able (in the Investigator's opinion) and willing to comply with all study requirements
- A diagnosis of acute (≤7 days) TIA, made by a specialist in the TIA clinic at University Hospitals of Leicester NHS Trust that fulfils the 2009 American Heart Association definition of TIA
- Good understanding of written and verbal English

7.3 Exclusion Criteria

ALL PARTICIPANTS (HEALTHY CONTROLS AND TIA PATIENTS)

- Unwilling to take part
- Unable to consent
- Aged under 18 years
- Unable (in the Investigator's opinion) or unwilling to comply with any study requirements
- Female participants who are pregnant, lactating or planning pregnancy during the course of the study
- A diagnosis of atrial fibrillation
- A diagnosis of severe heart failure (Ejection Fraction <30%)
- Severe respiratory disease
- Inadequate bilateral transcranial Doppler windows (see section 8.3)
- Carotid stenosis ≥70% (unilateral or bilateral)
- Participant enrolled in an interventional research study.
- Poor understanding of written and verbal English

8. STUDY PROCEDURES

8.1 Screening and Eligibility Assessment

Patients attending the specialist TIA clinic at University Hospitals of Leicester NHS Trust will be eligible for recruitment into the study.

Medical records will be reviewed by the direct care team to ensure participants meet the inclusion and exclusion criteria. Eligibility screening will only be undertaken by the direct clinical care team for potential participants.

Participants will be approached at their clinical consultation in the specialist TIA clinic by consultants, registrars or specialist nurses to determine study interest prior to referral to the researcher and provision of study specific information. A poster advertising the study will be displayed in the TIA clinic waiting room. Both participants with TIA, and healthy controls (those without a diagnosis of TIA) will be recruited from the clinic. Healthy spouses, friends or partners of patients with TIA will be asked if they would like to participate in the study as a healthy control subject.

TIA participants will be given a minimum of 1 hour, and a maximum of 48 hours to decide whether or not to take part in the study, but must undertake the study assessment within 7 days of their TIA.

Healthy control participants will be given a minimum of 1 hour, and a maximum of 7 days to decide whether or not to take part study. There is no time restriction on when they undertake the study assessment.

8.2 Informed Consent

We will only invite participants who have capacity to give us consent in this research study. The researcher will have primary responsibility for assessing the capacity of the individual to participate in research, according to the Mental Capacity Act 2005 and under the direct supervision of the Chief Investigator of the project.

Informed consent will be undertaken by Mr Angus Batterham in the Cerebral Haemodynamics in Ageing and Stroke Medicine (CHIASM) laboratory located in the Windsor building, Leicester Royal Infirmary.

The participant must personally sign and date the latest approved version of the informed consent form before any study specific procedures are performed. Written and verbal versions of the

current approved Participant Information Leaflet (PIS) and Participant Consent Form will be presented detailing no less than: the exact nature of the study; the implication and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care and with no obligation to give the reason for withdrawal. However, should this situation occur the research team would like to keep all the information and data that has been collected so far for the final analysis. This will be clearly stated in both the PIS and Participant Consent Form.

Participants with TIA will be allowed up to 48 hours (minimum of one hour) to consider the information, providing they will still be able to participate in the study within 7 days of their TIA.

Healthy control participants will be given a minimum of 1 hour, and a maximum of 7 days to decide whether or not to take part in the study. There is no time restriction on when they undertake the study assessment.

Participants will be given the opportunity to question the Investigator, their GP or other independent parties to decide whether they will participate in the study. Written Informed Consent will then be obtained by means of participant dated signature and dated signature of the person who presented and obtained the informed consent.

Volunteers will also be allowed up to 48 hours (minimum of one hour) to consider the information, and the opportunity to question the Investigator or other independent parties to decide whether they wish to participate in the study. Written informed consent will then be obtained by means of volunteer dated signature and dated signature of the person who presented and obtained the informed consent.

The person who obtains the consent will be suitably qualified and experienced, and have been authorised to do so by the Chief/Principal Investigator as detailed on the Delegation of Authority and Signature log for the study. The original signed form will be retained at the study site within the Trial Master File (TMF) or Investigator Site File (ISF). A copy of the signed Informed Consent will be given to participants and a copy retained in the participant medical notes.

8.3 TCD window insonation check

Once a consent form has been signed, and before collecting baseline information on demographics and handedness, or undertaking the SSM, participants will undergo a brief Transcranial Doppler

Ultrasound (TCD) check to ensure they have adequate acoustic temporal windows (inadequacy rates of 5-37% well documented in the literature) [Itoh et al 1993, Marinoni et al 1997].

If the participant is found to have inadequate acoustic windows, then they will be unable to participate in the study any further, and will be recorded as a screen failure.

8.4 Collection of demographic data

To avoid repetition and undue burden to participants, consent will be obtained from participants to record the following data from their TIA clinic letter as detailed below:

- Date of birth
- Gender
- Height
- Weight and BMI
- Past Medical History
- Current Medications
- GP name and address
- Smoking status
- Capillary blood glucose
- Carotid ultrasound result
- Type and result of brain imaging

Healthy controls will be asked the above information directly, although will be unable to provide capillary blood glucose, carotid ultrasound or brain imaging results.

- Participants will be asked to detail their ethnicity according to the 18 standardised ethnic categories outlined in the 2011 Office of National Statistics census (Appendix A)
- Handedness will be determined for all participants using the Edinburgh Handedness Inventory [Oldfield 1971] (Appendix B)

8.5 TCD Assessment

For all subjects, all assessments will be undertaken in a dedicated cardiovascular research laboratory at Leicester Royal Infirmary (LRI), which is at a controlled temperature (20-24°C) and is free from distraction. Participants will be asked to refrain from heavy meals, strenuous exercise, alcohol, smoking and caffeine if possible for four hours prior to attending the research laboratory. Baseline brachial casual BP will be measured using a validated UA767 BP monitor. Beat-to-beat non-invasive BP will be recorded continuously using the Finometer cuff device (Finapres Medical Systems; Amsterdam, The Netherlands) attached to the middle finger of the right hand which will be supported in an arm sling. R-R interval will be recorded using a 3-lead electrocardiogram (ECG). Respiratory rate will be recorded, and end-tidal partial pressure of carbon dioxide ($EtCO_2$) will be monitored using small nasal cannulae placed at the base of the nose (Salter Labs, ref 4000) attached to a capnograph (Capnocheck Plus) to monitor the breathing. A tilt sensor will be attached to the thigh of the participant's right leg by means of a Velcro strap to measure the efficiency and angle of the squatting motion. Simultaneous bilateral insonation of the middle cerebral arteries (MCAs) will be performed using TCD with 2MHz probe using a DWL Doppler box, with the subject either sitting or lying supine on a couch (in the case of difficult to insonate windows). The vessels will be located via the temporal bone window, and identified as the MCAs by their waveform, depth, velocities, and direction of flow. All parameters will be simultaneously recorded onto a computer software system (PHYSIDAS), providing data for subsequent off-line analysis. A head frame will be used to secure the ultrasound probes in position and to minimise their movement. Once satisfactory signals have been obtained, four recordings will be made:

1) A 5 minute baseline recording during which the participant sits quietly with their eyes open
2) A 6 minute recording during which the participant sits for 1 minute before standing and remaining standing quietly with their eyes open for 5 minutes
3) A recording during which the participant is asked to stand for 10 seconds before squatting down and remaining in a squat position for 10 seconds before standing back up and then repeating this process for 5 minutes. A computer program will provide visual cues to the participant to guide the timings of the squat-stand sequence. As each squat manoeuvre (consisting of a squat down and stand back up) will last for 20 seconds, the participant will be required to perform a total of 15 squats in the 5 minute period. This recording will begin and end with a period of 90 seconds baseline recording where the participant stands quietly with their eyes open.

A period of instruction will precede recording 3 during which the SSM will be demonstrated. During squats, participants will be asked to breathe through their nose and to avoid straining if possible. Participants will be instructed to squat down as low as they feel able. They will be informed that they will need to perform a maximum 15 squats and to take this into account when choosing their depth. Participants will be informed that they should try to complete all 15 squats if possible. However, if they wish to stop the SSM before this, they should make the investigator aware during a standing period and ideally remaining standing for a further 90 seconds before being disconnected from the equipment.

During the recordings the internal plethysmography servo-adjust of the Finometer will be switched off, but this will be switched back on in between each recording to allow for accurate calibration of BP. A brachial BP will also be taken in between each recording using the same validated UA767 BP monitor as at the beginning of the baseline assessment.

Participants will be allowed to rest for as long as needed before leaving the laboratory at the end of the study measurement. Refreshments will be provided, and travel expenses will be refunded.

There is a very small chance that the 3 lead ECG could detect a heart abnormality (arrhythmia) in either the healthy controls or the patient participants. Should this situation occur, we would explain our findings to the participant and notify their GP. Formal written consent will be obtained to cover this circumstance.

8.6 Definition of End of Trial

The end of trial is the date of the last visit of the last participant.

8.7 Discontinuation/Withdrawal of Participants from Study Treatment

As detailed in section 8.3, if the participant is found to have inadequate acoustic windows, then they will be unable to participate in the study any further. They will therefore be recorded as a screen failure and withdrawn from the study.

Furthermore, each participant has the right to withdraw from the study at any time and without giving a reason.

In addition, the investigator may discontinue a participant from the study at any time if the investigator considers it necessary for any of the following reason:

- An inability to comply with study procedures

- Significant protocol deviation
- Consent withdrawn

All of the above will be recorded in the case report form (CRF).

8.8 Source Data

Source documents are original documents, data, and records from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence. Medical records will be used as source documents for the TIA participants.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data). The CRF will therefore be considered the source document for the healthy control participants.

All documents will be stored within a locked filing cabinet, within a locked office in an access restricted University of Leicester site. On all study-specific documents, other than the signed consent, the participant will be referred by the study participant number/code, not by name.

9. TREATMENT OF TRIAL PARTICIPANTS

9.1 Storage of Study Equipment of Related Apparatus

All study specific equipment is stored securely in the CHIASM laboratory and maintained annually by the Medical Physics department at University Hospitals of Leicester NHS Trust.

9.2 Clinical Care

Throughout the study, TIA patients will continue to receive standard clinical care, which includes (but is not limited to): specialist multi-disciplinary assessment, further radiological investigations, and appropriate investigation and secondary prevention interventions (including antithrombotic/ anticoagulant, BP-lowering and cholesterol-lowering therapies).

10. SAFETY REPORTING

10.1 Definitions

Adverse Event (AE)

An AE or adverse experience is:

Any untoward medical occurrence in a patient or clinical investigation participant, which does not necessarily have to have a causal relationship with his/her treatment.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the study, whether or not considered related to the study.

Adverse Reaction (AR)

All untoward and unintended responses related to the study.

All cases judged by either the reporting medically qualified professional or the sponsor as having a reasonable suspected causal relationship to the study qualify as adverse reactions.

Severe Adverse Events (SAE)

To ensure no confusion or misunderstanding of the difference between the terms “serious” and “severe”, which are not synonymous, the following note of clarification is provided:

The term “severe” is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as “serious”, which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a participant’s life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

Serious Adverse Event or Serious Adverse Reaction (SAR)

A serious adverse event or reaction is any untoward medical occurrence that:

- Results in death
- Is life-threatening

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

- Requires inpatient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect
- Other important medical events*

*Other events that may not result in death, are not life threatening, or do not require hospitalisation, may be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

Expected Serious Adverse Events/Reactions

See Appendix D for a complete list of expected adverse events not subject to immediate reporting.

Suspected Unexpected Serious Adverse Reactions (SUSAR)

A serious adverse reaction, the nature or severity of which is not consistent with the applicable product information.

10.2 Reporting procedures for All Adverse Events

All AEs occurring during the study observed by the investigator or reported by the participants, whether or not attributed to study, will be recorded on the CRF.

The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to study, other suspect device and action taken. Follow-up information should be provided as necessary.

AEs considered related to the study as judged by a medically qualified investigator or the sponsor will be followed until resolution or the event is considered stable. All related AEs that result in a participant's withdrawal from the study or are present at the end of the study, should be followed up until a satisfactory resolution occurs.

It will be left to the investigator's clinical judgment whether or not an AE is of sufficient severity to require the participant's removal from the study. A participant may also voluntarily withdraw from the study due to what he or she perceives as an intolerable AE.

The severity of events will be assessed on the following scale: 1 = mild, 2 = moderate, 3 = severe.

The relationship of AEs to the study will be assessed by a medically qualified investigator.

10.3 Reporting Procedures for Serious Adverse Events

All SAEs, except those expected ones defined in Appendix D that do not require immediate reporting (see Appendix D), must be reported to the Sponsor within 24 hours of discovery or notification of the event. The Sponsor will perform an initial check of the information and ensure that it is reviewed at the next R&D Management meeting. All SAE information must be recorded on an SAE form and sent to the Sponsor using the appropriate reporting form and the contact details on there. Additional information received for a case (follow-up or corrections to the original case) needs to be detailed on a new SAE form which must be sent to the Sponsor using the appropriate reporting form and the contact details on there.

The Sponsor will report all SUSARs to the Research Ethics Committee concerned. Fatal or life-threatening SUSARs must be reported within 7 days and all other SUSARs within 15 days. The CI will inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants.

In addition to the expedited reporting above, the CI shall submit once a year throughout the study or on request an Annual Report to the Ethics Committee which lists all SAEs / SUSARs that have occurred during the preceding 12 months.

11. STATISTICS

11. 1 Statistical analysis

The null hypotheses being tested are:

- cerebral haemodynamic parameters, including CBFv and ARI, do not differ between baseline recordings and during performance of squat-stand manoeuvres
- cerebral haemodynamic parameters, including CBFv and ARI, do not differ between TIA patients and HC

All data will be tested for normality prior to statistical testing using Shapiro-Wilks. Categorical data will be reported as absolute number and percentages. Continuous data will be plotted and assessed for normality; normally distributed continuous variables will be described as mean (SD) and continuous variables with skewness as median (IQR). Statistical testing for differences between HC and TIA groups will be by two-way ANOVA for continuous, parametric data, Friedman 2 way analysis of variance for continuous, non-parametric data, and Chi-Square for nominal data.

Data will be recorded in Microsoft Excel. Statistical analyses will be performed using the latest versions of SPSS for Windows or Statistica, and graphs will be produced using the latest version of Statistica.

11.2 Sample size calculation

A formal sample size calculation was not possible for this study therefore a realistic number of participants has been selected based on previous experience of similar research studies by Dr Haunton, Professor Robinson and Professor Panerai.

Brodie FG et al reported in 2009 that a sample of 11 patients per group will allow the detection of a difference in ARI of 2 units between groups, with 80% power at the 5% significance level.

11.3 The Level of Statistical Significance

Statistical significance will be set at $p<0.05$

11.4 Procedure for Accounting for Missing and Unused Data

Unused data will be reported, with reasons in the final report after study completion, and in any publication. Spurious and missing data will be reported, but not included in final analyses.

12. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

13. QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with the current approved protocol, ICH GCP, relevant regulations and standard operating procedures.

The University of Leicester operate a risk based monitoring and audit programme to which this study will be subject.

14. CODES OF PRACTICE AND REGULATIONS

14.1 Ethics

All informed consent taken from TIA patients and HC subjects will be taken following GCP guidelines.

14.2 Sponsor Standard Operating Procedures

All relevant Sponsor SOPs will be followed to ensure that this study complies with all relevant legislation and guidelines.

14.3 Declaration of Helsinki

The Investigator will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki (last amended October 2000, with additional footnotes added 2002 and 2004).

14.4 ICH Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical Practice (CPMP/ICH/135/95) July 1996.

14.5 Approvals

Once Sponsor authorisation has been confirmed, the protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), Health Research Authority (HRA), and host institution(s) for written approval.

Once Sponsor authorisation has been confirmed, the Investigator will submit and, where necessary, obtain approval from the above parties for all substantial and non-substantial amendments to the original approved documents.

14.6 Participant Confidentiality

The trial staff will ensure that the participants' anonymity is maintained. The participants will be identified only by initials and a participants ID number on the CRF and any electronic database. All documents will be stored securely and only accessible by trial staff and authorised personnel. The

study will comply with the Data Protection Act which requires data to be anonymised as soon as it is practical to do so.

15. DATA HANDLING AND RECORD KEEPING

15.1 Data Extraction

As supportive evidence of the diagnosis of TIA in TIA participants, results and significant findings from Computerised Tomography (CT) head/Brain Magnetic Resonance Imaging (MRI) and Carotid Ultrasound, if undertaken, will be recorded on the CRF.

Other information which will be extracted from the medical notes of TIA participants and recorded on the CRF include smoking status, capillary blood glucose, medications, past medical history, height, weight and BMI.

The medical notes of healthy control participants will not be reviewed, and participants will instead be asked their smoking status, medications, past medical history, height and weight directly. Answers to these questions will be recorded on the CRF.

Data regarding participants' age, date of birth, ethnicity and handedness will also be recorded onto the CRF. All other parameters, including CBFv, squat angle, heart rate (HR), BP, and $ETCO_2$, will be recorded onto a computer system (PHYSIDAS), providing data for subsequent analyses.

15.2 Data Analysis

Off-line analyses will be undertaken using software designed by the University of Leicester's Medical Physics Group.

15.3 Data Management

Baseline and demographic data will be recorded on the CRF, which will be retained in the study site file. This will be stored within a locked filing cabinet, within a locked office in a building which requires swipe card or security review for access at the University of Leicester. CRF data will be anonymised for storage on a secure drive on encrypted, password protected, university desktop computers.

Personal data that cannot be anonymised (e.g. contact details and enrolment logs) will be stored within a locked filing cabinet, within a locked office in a building which requires swipe card or security review for access at the University of Leicester and/or within a password protected file, on an encrypted, password protected, desktop computer which is kept within a locked office. No personal data will be accessed or stored on personal laptops.

All physiological parameters (signals) that are collected during the measurement will be saved using a coded filename. The participant name and other identifying detail will NOT be included in any study data electronic file. All study data will be entered into Microsoft Excel for Windows on an encrypted, password protected, desktop computer which is kept within a locked office. .

Anonymised outcome data will be held on an encrypted, password protected desktop computer which is kept within a locked office. Anonymised outcome data may also be held on an encrypted, password protected personal laptop belonging to researchers named on the authorisation log.

Participants will be identified by a study specific participants number and/or code in any database. Names and any other identifying detail will NOT be included in any study data electronic file.

Anonymised research data may be shared with researchers from other Departments or Universities conducting similar research. Information regarding this will be detailed in the PIS, and explicit written consent for the sharing of such data will be obtained from participants.

16. STUDY GOVERNANCE

The trial management group, which consists of the CI, co-investigators, and other research staff, will meet monthly to monitor study progress, and recruitment targets. As this is a prospective case control study, and not a therapeutic study, a data safety monitoring committee is not required.

17. FINANCING AND INSURANCE

In this study Dr Haunton and Professor Robinson will supervise the researcher who is responsible for participant recruitment, data collection and analysis. Dr Haunton will take a lead role in preparing the experimental procedures and then guiding participant recruitment. Professor Panerai will supervise data analysis and will also have a key role in the interpretation of results.

In this study, the assessments on participants will be carried out by the researcher, and all the equipment to be used is provided, serviced and maintained by the Department of Medical Physics, University of Leicester.

There are no NHS treatment or support costs.

18. PUBLICATION POLICY

On all publications, the host institution will be acknowledged, and each author will be required to disclose details of their own involvement/contribution in the study (specific publication).

19. REFERENCES

Aaslid R, Markwalder TM, Nornes H. Noninvasive transcranial Doppler ultrasound recording of flow velocity in basal cerebral arteries. *J.Neurosurg.* 1982;57:769-74

Aaslid R, Lindegaard KF, Sorteberg W et al. Cerebral autoregulation dynamics in humans. *Stroke* 1989; 20: 45-52

Aries MJ, Elting JW, De Keyser J, et al. Cerebral autoregulation in stroke: a review of transcranial Doppler studies. *Stroke* 2010; 41(11):2697-704.

Atkins ER, Brodie FG, Rafelt SE et al. Dynamic Cerebral Autoregulation Is Compromised Acutely following Mild Ischaemic Stroke but Not Transient Ischaemic Attack *Cerebrovasc Dis* 2010; 29: 228–235

Barnes SC, Ball N, Panerai RB et al. Random squat/stand maneuvers: a novel approach for assessment of dynamic cerebral autoregulation? *J Appl Physiol* 2017; 123(3): 558-566.

Barnes SC, Ball N, Haunton VJ et al. How many squat-stand manoeuvres to assess dynamic cerebral autoregulation? *Eur J Appl Physiol.* 2018 [Epub ahead of print] doi: 10.1007/s00421-018-3964-2.

Brodie FG, Atkins ER, Robinson TG et al. Reliability of dynamic cerebral autoregulation measurement using spontaneous fluctuations in blood pressure. *Clin Sci (Lond).* 2009; 116(6): 513-20

Chiu C, Yeh S, Liau B. Assessment of CA dynamics in diabetics using time-domain cross-correlation analysis. *J Med Biol Eng* 2005; 25: 53–59

Claassen JAHR, Levin BD, Zhang R. Dynamic cerebral autoregulation during repeated squat-stand maneuvers. *J Appl Physiol* 2009a; 106: 153-160

Claassen JA, Diaz-Arrastria R, Martin-Cook K et al. Altered cerebral hemodynamics in early Alzheimer disease: a pilot study using transcranial Doppler. *J Alzheimer's Dis* 2009b; 17(3): 621–629

Claassen JAHR, Zhang R. Cerebral autoregulation in Alzheimer's disease. *J Cereb Blood Flow Metab* 2011; 31(7): 1572-1577

Claassen JA, Meel-van den Abeelen AS, Simpson DM et al. Transfer function analysis of dynamic cerebral autoregulation: A white paper from the International Cerebral Autoregulation Research Network. *J Cereb Blood Flow Metab* 2016; 36(4): 665-80

Coull AJ, Lovett JK, Rothwell PM. Population based study of early risk of stroke after transient ischaemic attack or minor stroke: implications for public education and organisation of services. *BMJ* 2004; 328: 326

Czosnyka M, Smielewski P, Kirkpatrick P et al. Monitoring of cerebral autoregulation in head-injured patients. *Stroke* 1996; 27: 1829-1834

Eames PJ, Blake MJ, Dawson SL et al. Dynamic cerebral autoregulation and beat to beat blood pressure control are impaired in acute ischaemic stroke. *J Neurol Neurosurg Psych* 2002; 72: 467-472

Easton JD, Saver JL, Albers GW et al.Definition and evaluation of transient ischemic attack: a scientific statement for healthcare professionals from the American Heart Association/American Stroke Association Stroke Council; Council on Cardiovascular Surgery and Anesthesia; Council on Cardiovascular Radiology and Intervention; Council on Cardiovascular Nursing; and the Interdisciplinary Council on Peripheral Vascular Disease. The American Academy of Neurology affirms the value of this statement as an educational tool for neurologists. *Stroke* 2009; 40(6): 2276-93.

Giller CA. The frequency-dependent behavior of cerebral autoregulation. *Neurosurgery* 1990; 27: 362-368

Haunton, VJ. (2013). Cerebral autoregulation in idiopathic Parkinson's disease. MD Thesis. University of Leicester, UK. Available at http://hdl.handle.net/2381/28752

Itoh T, Matsumoto M, Handa N et al. Rate of successful recording of blood flow signals in the middle cerebral artery using transcranial Doppler sonography. *Stroke* 1993; 24: 1192–1195

Itoh Y, Suzuki N. Control of brain capillary blood flow. *J Cereb Blood Flow Metab* 2012; 32: 1167 – 76

Kontos HA, Wei EP, Navari RM et al. Responses of cerebral arteries and arterioles to acute hypotension and hypertension. *Am J Physiol* 1978; 234: H371-H383.

Kuga N, Hirata T, Sakai I et al. Rapid and local autoregulation of cerebrovascular blood flow: a deep-brain imaging study in the mouse. *J Physiol* 2009; 587(4): 745–752

Lassen NA. Cerebral blood flow and oxygen consumption in man. *Physiol Rev* 1959; 39: 183-238

Llwyd O, Salinet ASM, Panerai RB et al. Cerebral Haemodynamics following Acute Ischaemic Stroke: Effects of Stroke Severity and Stroke Subtype. Cerebrovasc Dis Extra. 2018; 8(2): 80-89.

Marinoni M, Ginanneschi A, Forleo P et al. Technical limits in transcranial Doppler recording: Inadequate acoustic windows. *Ultrasound Med Biol* 1997; 23: 1275–1277

Musthak A, Dimassi D, Akhtar N et al. Cerebral blood flow and auto-regulation in acute TIA patients from a general hospital in Qatar. *Neurology* 2017; 88 (16S)

Oldfield, R. C. The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsycholoigia* 1971; 9: 97-113.

Office of National Statistics Final recommended questions for the 2011 Census in England and Wales https://www.ethnicity-facts-figures.service.gov.uk/ethnicity-in-the-uk/ethnic-groups-and-data-collected

Oudegeest-Sander MH1, van Beek AH, Abbink K et al. Assessment of dynamic cerebral autoregulation and cerebrovascular CO2 reactivity in ageing by measurements of cerebral blood flow and cortical oxygenation. *Exp Physiol* 2014; 99(3): 586-98

Reinhard M, Roth M, Muller T et al. Effect of carotid endarterectomy or stenting on impairment of dynamic cerebral autoregulation. *Stroke* 2004; 35: 1381-1387

Rothwell, P, Coull AJ, Silver LE et al. Population-based study of event-rate, incidence, case fatality, and mortality for all acute vascular events in all arterial territories (Oxford Vascular Study). *Lancet* 2005; 366: 1773-1783

Rothwell PM, Giles MF, Chandratheva A et al. Effect of urgent treatment of transient ischaemic attack and minor stroke on early recurrent stroke (EXPRESS study): a prospective population-based sequential comparison. *Lancet* 2007; 370(9596): 1432-1442.

Smirl JD, Haykowsky MJ, Nelson MD et al. Relationship between cerebral blood flow and blood pressure in long-term heart transplant recipients. *Hypertension* 2014; 64(6): 1314-20

Smirl JD, Hoffman K, Yu-Chieh Tzeng et al. Methodological comparison of active- and passive-driven oscillations in blood pressure; implications for the assessment of cerebral pressure-flow relationships. *J Appl Physiol* 2015; 119: 487–501

Tiecks FP, Lam AM, Aaslid R et al. Comparison of static and dynamic cerebral autoregulation measurements. *Stroke* 1995; 26: 1014-1019

Van Beek A, Claasen J, Rikkert M et al. Cerebral autoregulation: an overview of current concepts and methodology with special focus on the elderly. *J Cereb Blood Flow Metab* 2008; 28: 1071-1085

van Veen TR, Panerai RB, Haeri S et al. Cerebral autoregulation in normal pregnancy and preeclampsia. *Obstet Gynecol* 2013; 122(5): 1064-9

20. APPENDIX A: CATEGORISATION OF ETHNICITY

White

1. English / Welsh / Scottish / Northern Irish / British

2. Irish

3. Gypsy or Irish Traveller

4. Any other White background, please describe

Mixed / Multiple ethnic groups

5. White and Black Caribbean

6. White and Black African

7. White and Asian

8. Any other Mixed / Multiple ethnic background, please describe

Asian / Asian British

9. Indian

10. Pakistani

11. Bangladeshi

12. Chinese

13. Any other Asian background, please describe

Black / African / Caribbean / Black British

14. African

15. Caribbean

16. Any other Black / African / Caribbean background, please describe

Other ethnic group

17. Arab

18. Any other ethnic group, please describe

21. APPENDIX B: EDINBURGH HANDEDNESS INVENTORY

Please indicate with a check (✓) your preference in using your left or right hand in the following tasks.

Where the preference is so strong you would never use the other hand, unless absolutely forced to, put two checks (✓✓).

If you are indifferent, put one check in each column (✓ | ✓).

Some of the activities require both hands. In these cases, the part of the task or object for which hand preference is wanted is indicated in parentheses.

Task / Object	Left Hand	Right Hand
1. Writing		
2. Drawing		
3. Throwing		
4. Scissors		
5. Toothbrush		
6. Knife (without fork)		
7. Spoon		
8. Broom (upper hand)		
9. Striking a Match (match)		
10. Opening a Box (lid)		
Total checks:	LH =	RH =
Cumulative Total	CT = LH + RH =	
Difference	D = RH – LH =	
Result	R = (D / CT) × 100 =	
Interpretation: (Left Handed: R < -40) (Ambidextrous: -40 ≤ R ≤ +40) (Right Handed: R > +40)		

22. APPENDIX C: SCHEDULE OF PROCEDURES

PROCEDURES	VISIT	
	Screening	**Baseline**
Informed consent	0	1
Demographics	1	1
Medical history	1	1
Concomitant medications	1	1
Eligibility assessment	1	0
Ethnicity categorisation	0	1
Handedness	0	1
Cerebral Haemodynamic Assessment (TCD, blood pressure, ECG, $ETCO_2$)	0	1

23. APPENDIX D: EXPECTED EVENTS NOT SUBJECT TO EXPEDITED REPORTING

Acute Coronary Syndromes	Malignancy
Agitation	Myocardial infarction
Angina	Nausea
Anorexia	Personality change
Anxiety	Peripheral Vascular Disease
Cognitive decline	Pressure sores
Constipation	Renal impairment
Delirium	Sedation
Depression	Seizure
Disease progression	Sexual dysfunction
Dysphagia	Sleep disturbance
Electrolyte disturbance	Stroke
Fall	Syncope
Fatigue	Transient ischemic attack
Fracture	Urinary retention/catheterisation
Gastrointestinal disturbance	Urinary tract infection
Hallucinations	Ulceration
Heart failure	Violent behaviour
Incontinence, faecal	Visual loss
Incontinence, urinary	Vomiting
Infections	Wandering
Institutionalisation / Admission to care home	Weakness
Intracerebral Haemorrhage	Weight loss
Loss of ability to function or care for self	