CEREBRAL HAEMODYNAMIC CHANGES DURING COGNITIVE TESTING: A FUNCTIONAL TRANSCRANIAL DOPPLER (fTCD) STUDY

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1. AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
	0.1	15/10/2015	Dr V Haunton	Pre-sponsor approval version
	0.2	14/02/2017	Dr L Beishon	Revision of recruitment strategy, updated protocol details
	0.3	02/03/2017	Dr L Beishon	Sponsor changes made
	1	06/03/2017	Dr L Beishon	Sponsor approved version
	2	03/04/2017	Dr L Beishon	Revision following REC review
	3	01/06/2017	Dr L Beishon	Major amendment to recruitment strategy

2. SYNOPSIS

Study Title	Cerebral Haemodynamic Changes During Cognitive Testing: A Functional Transcranial Doppler (fTCD) study
Internal ref. no.	
Trial Design	Prospective, observational, case-control study
Trial Participants	Healthy Control Subjects, Patients with Mild Cognitive Impairment, Patients with Vascular Dementia and Patients with Alzheimer's Dementia
Planned Sample Size	11 Healthy Control Patients, 11 Patients with Mild Cognitive Impairment, 11 Patients with Vascular Dementia and 11 Patients with Alzheimer's Dementia
Follow-up duration	None
Planned Trial Period	7 Months
Primary Objective	To compare the beat-to beat cerebral blood flow velocity (CBFv) between patients with Mild Cognitive Impairment (MCI), patients with Vascular Dementia (VascD), patients with Alzheimer's Dementia (AlzD) and age-, gender- and blood pressure (BP)-matched healthy control (HC) subjects during performance of the Addenbrooke's Cognitive III (ACE-III) examination
Secondary Objectives	1) To investigate whether performance of the Addenbrooke's Cognitive III Examination is associated with differences in other beat-to-beat cerebral haemodynamic parameters, specifically autoregulation index (ARI), between MCI patients, VascD patients, AlzD patients, and age-, gender- and BP-matched HC subjects. 2) To assess the acceptability and feasibility of this protocol in patients with cognitive impairment in clinical practice. 3) To assess the reproducibility of measurements in cognitively impaired patient group at 3 months from initial measurement
Outcome Measure	This is not an intervention study, and therefore it would not be appropriate to assess the 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 percentage of recruited subjects (HC, MCI patients, VascD patients and AlzD patients) able to comply with the full measurement protocol. 2. The percentage of measurements rejected because of aspects related to data quality during the analysis protocol, with recorded reasons. 3. Overall, the percentage of recruited subjects (healthy controls, MCI patients, VascD patients and AlzD patients) in whom values for the following parameters can be derived:

	• % change of CBFv at baseline in response to performance of the ACE-III Cognitive Examination • Autoregulation index (using the Tiecks model and from the phase, gain and coherence).

3. ABBREVIATIONS

ACE-III	Addenbrooke's Cognitive Examination
AE	Adverse event
AlzD	Alzheimer's Dementia
AR	Adverse reaction
ARI	Autoregulation Index
BP	Arterial Blood Pressure
CA	Cerebral Autoregulation
CBF	Cerebral Blood Flow
CBFv	Cerebral Blood Flow Velocity
CI	Chief Investigator
CPP	Cerebral Perfusion Pressure
CrCP	Critical Closing Pressure
CRF	Case Report Form
CRO	Contract Research Organisation
CT	Clinical Trials
CVR	Cerebrovascular Resistance
CVRi	Cerebrovascular Resistance Index
dCA	Dynamic Cerebral Autoregulation
EC	Ethics Committee (see REC)
ECG	Electrocardiogram
EHI	Edinburgh Handedness Inventory
$EtCO_2$	End-Tidal Partial Pressure of Carbon Dioxide
FFT	Fast Fourier Transform
GCP	Good Clinical Practice
GP	General Practitioner
GPI	Gosling's Pulsatility Index
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
MCI	Mild Cognitive Impairment
NVC	Neurovascular Coupling
NHS	National Health Service
NICE	National Institute for Health and Care Excellence

NRES	National Research Ethics Service
PET	Positron Emission Tomography
PI	Principal Investigator
PIL/S	Participant/ Patient Information Leaflet/Sheet
RAP	Resistance Area Product
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
TCD	Transcranial Doppler Ultrasound
TFA	Transfer Function Analysis
TMF	Trial Master File
UK	United Kingdom
VMR	Vasomotor Reactivity
VascD	Vascular Dementia

4. BACKGROUND AND RATIONALE

Dementia is a syndrome which comprises an acquired decline in memory and other cognitive functions(s) in an alert (non-delirious) person that is sufficiently severe to affect daily life. It is the most common neurodegenerative disorder in the United Kingdom (UK); 850,000 people currently live with dementia in the UK, and that number is expected to rise to more than 1 million within the next 5 years [Alzheimer's Society 2014]. The annual cost to the UK of dementia is £23.6 billion [Alzheimer's Society 2014]. The most common type of dementia in the population (55%) is Alzheimer's dementia (AlzD), which is characterised pathologically by neuronal loss and the presence of amyloid plaques and tau neurofibrillary tangles. AlzD typically has an insidious onset and a slow progression. There are several diagnostic criteria, and commonly used criteria include; Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) classification of neurocognitive disorders [American Psychiatric Association 2000], ICD-10 classification [World Health Organisation, 1992], and the National Institute on Aging-Alzheimer's Association (NIA/AA) criteria [McKhann, 2011]. The recently published DSM-V criteria have re-classified dementia as major and mild neurocognitive disorders [Simpson, 2014]. Vascular dementia (VascD) is the second commonest form of dementia (15%), and is suggested by the presence of vascular risk factors. The key pathological processes are subcortical white matter ischaemia and/or multiple cerebral infarcts. The onset is often abrupt, and the progression stepwise and irregular; cognitive deficits are often less uniform than those of Alzheimer's dementia. Vascular dementia is diagnosed according to NINDS-AIREN criteria [Roman et al 1993]. Mild cognitive impairment (MCI) affects up to 20% of older adults and describes a set of symptoms rather than a specific medical condition or disease. A person with MCI has subtle problems with one or more of the following: day-to-day memory, planning, language, attention and visuospatial skills [McDade et al 2015]. MCI clinically may comprise a group of heterogeneous conditions and may have differing aetiologies [Petersen 2004]. MCI can be diagnosed using the criteria set out by the International Working Group on Mild Cognitive Impairment [Winblad et al 2004]. Although MCI significantly increases the risk of developing dementia [Busse et al 2006], at present it is not possible to accurately predict which patients with MCI will progress to dementia.

In recent times, our knowledge regarding the pathogenic mechanisms of dementia has changed considerably. In contrast to previous thoughts about the pure neurodegenerative nature of AlzD, it is now well established that vascular dysfunction and haemodynamic disturbances play a role in ALzD, as well as in VascD [Sabayan et al 2012]; with several epidemiological studies reporting an association between vascular risk factors (e.g. hypertension, hyperlipidaemia, type 2 diabetes mellitus) and AlzD [Viswanathan et al 2009]. Furthermore, post-mortem examinations have shown that the brains of AlzD patients contain cerebrovascular pathologies [Cacabelos et al 2003, Gorelick

2004]. Research studies investigating the vascular contributions to cognitive decline have generally reported cerebral hypoperfusion [Keage et al 2012, Sabayan et al 2012], and it is thought that this hypoperfusion affects cellular health which in turn triggers neurodegenerative pathways [Ruitenberg et al 2005, Kalaria 2010].

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 which are particularly problematic for older populations, 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]. Measurements can be taken at rest, or during brain activation tasks such as sensorimotor functions or cognitive tasks. TCD measurement preformed during such brain activation tasks is commonly referred to as functional TCD (fTCD), and is the assessment of blood flow in response to a specific cognitive stimulus or mental operation, a concept known as neurovascular coupling (NVC). Although the exact mechanisms of NVC continue to be explored and evaluated, it is thought that cerebral activation triggers a haemodynamic response involving neurons, astrocytes, vascular cells and local metabolites [Carmignoto et al 2010, Girouard et al 2006]. Cerebral blood flow (CBF) is also strongly influenced by the arterial partial pressure of carbon dioxide ($PaCO_2$) which also modulates the effectiveness of cerebral autoregulation (CA), defined as the ability of the brain to maintain a relatively constant CBF in response to significant changes in cerebral perfusion pressure (CPP). CA is improved by hypocapnia and impaired by hypercapnia [Aaslid et al 1989, Paulson et al 1990]. It has been shown that CA can be reliably assessed by evaluating CBF responses to changes in $PaCO_2$, such as those induced by hypo- and hyperventilation respectively [Dineen et al, 2010]. This concept is known as assessing vasomotor (or CO_2) reactivity (VMR).

In addition to studying CBFv, NVC and VMR, by using transfer function analysis (TFA) further haemodynamic data can be obtained and calculated using TCD and beat-to-beat blood pressure monitoring. These data include coherence, phase, gain, autoregulatory index (ARI), critical closing pressure (CrCP) and resistance area product (RAP). By examining the transfer of BP fluctuations to CBF as a measure of CA, TFA makes it possible to examine the effect of changes in arterial blood pressure (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 in 1990. The parameter which tests the

linearity between the input and output signals at each frequency is called the coherence, with values of coherence ranging between zero and one. Values of coherence approaching one indicate a perfect linear relationship between input and output (i.e. CBFv and BP). Although there is currently no consensus on the threshold at which good coherence is assumed, many studies have adopted a coherence of ≥ 0.5 to accept the relationship as significant [Panerai et al 1998, Panerai et al 2006]. Phase is equivalent to the shift in radians from 0 to 2π (or degrees from 0° to 360°) that would be required to align input (BP) with output (CBFV) at a given frequency, and so gives an indication of the relative timing of the two signals. The magnitude of the phase response may be an indicator of the integrity of the autoregulatory response; phase is positive in intact CA (CBFv recovers faster than changes in BP) and it tends to zero in impaired CA when CBFv tends to follow BP, during steady-state conditions [Panerai et al 1998, Birch et al 1995]. Gain is the ratio of the amplitude of the output signal to the input signal, and so indicates the magnitude of change in CBFv that is due to a change in BP. Although gain alone is not a reliable measure of CA, an increase in gain suggests that CA is impaired, whereas a low gain indicates an efficient CA [Giller 1990, Van Beek et al 2008].

The time domain approach in TFA is used to extract information about the CA mechanism from the analysis of mean BP, CBFv, heart rate (HR) and end-tidal partial pressure of carbon dioxide ($EtCO_2$) with respect to time. In 1995 Tiecks et al derived a set of equations and curves based on the CBF_V response to a sudden fall in induced by thigh cuff deflation. These equations and curves allow for the calculation of an autoregulatory index (ARI) 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. Other parameters obtainable in the time domain may also provide useful information regarding the integrity of dCA, including CrCP and RAP. CrCP is expressed in mmHg and is defined as the arterial pressure below which small vessels collapse and forward blood flow becomes zero, which in the cerebral circulation is equivalent to the sum of ICP and the contributions of vascular smooth muscle tone [Panerai 2003]. RAP is expressed in mmHg.s/cm and is an index of cerebrovascular resistance, which is equal to the total cerebrovascular resistance x cross-sectional area of the vessel [Evans et al 1988].

A meta-analysis of twelve TCD studies in AlzD and VascD [Sabayan et al 2012], reported significantly lower CBFv in patients with ALzD and VascD in comparison to healthy control subjects. CFBv was especially low in patients with VascD. In MCI, although there are limited data available, no significant differences in CBFv have been observed between MCI patients and healthy controls, unless restricting to amnestic MCI [Keage et al 2012].

Four studies which have used fTCD to investigate cerebral haemodynamic responses to brain activation in patients with dementia have reported conflicting findings; two found that individuals with AlzD had attenuated blood flow responses to cognitive demand, and two found no differences between dementia patients and controls. These studies used visual stimulation [Asil et al 2005, Gucuyener et al 2010, Rosengarten et al 2001], and a whispered verbal task, a thumb opposition task and a design discrimination task [Matteis et al 1998].

Cognitive testing with standardised assessment tools such as the Mini Mental State Examination, Montreal Cognitive Assessment and Addenbrooke's-III Cognitive Examination (ACE-III) is a key component of the formal diagnosis of dementia, yet the effects of these tests on cerebral blood flow and haemodynamics is unknown. The ACE-III [Hsieh et al 2013] is a widely used, validated, cognitive screening tool recommended for use by health practitioners and researchers in patients over 50 years old with suspected dementia. The ACE-III is available for free. The copyright is held by Professor John Hodges, ARC Federation Fellow and Professor of Cognitive Neurology at Neuroscience Research Australia, who is happy for the test to be used in clinical practice and research projects.

This protocol has been used successfully by this group to examine changes in CBFv in 40 healthy volunteers from the University of Leicester. The data from this analysis has been presented at an international conference and is currently undergoing peer review for publication. Therefore, this protocol has demonstrated feasibility in a healthy population and warrants further investigation for the utility in a patient population.

Therefore, the proposed study will investigate and compare the beat-to beat cerebral blood flow velocity (CBFv) between patients with Mild Cognitive Impairment (MCI), patients with Vascular Dementia (VascD), patients with Alzheimer's Dementia (AlzD) and age-, gender- and blood pressure (BP)-matched healthy control (HC) subjects during performance of the Addenbrooke's Cognitive III (ACE-III) examination. The proposed study will also investigate whether performance of the Addenbrooke's Cognitive III Examination is associated with differences in other beat-to-beat cerebral haemodynamic parameters, specifically autoregulation index (ARI), between MCI patients, VascD patients, AlzD patients, and age-, gender- and BP-matched HC subjects.

5. OBJECTIVES

5.1 Primary Objective

To compare the beat-to beat cerebral blood flow velocity (CBFv) between patients with Mild Cognitive Impairment (MCI), patients with Vascular Dementia (VascD), patients with Alzheimer's Dementia (AlzD) and age-, gender- and blood pressure (BP)-matched healthy control (HC) subjects during performance of the Addenbrooke's Cognitive III (ACE-III) examination

5.2 Secondary Objectives

1) To investigate whether performance of the Addenbrooke's Cognitive III Examination is associated with differences in other beat-to-beat cerebral haemodynamic parameters, specifically autoregulation index (ARI), between MCI patients, VascD patients, AlzD patients, and age-, gender- and BP-matched HC subjects.
2) To assess the acceptability and feasibility of this protocol in patients with cognitive impairment in clinical practice.
3) To assess the reproducibility of measurements in cognitively impaired patient group at 3 months from initial measurement

6. STUDY DESIGN

6.1 Summary of Trial Design

This is a prospective, observational, case-control study.

Each participant will be required to attend the Cardiovascular Research Laboratory, Level 5, Windsor Building, University Hospitals of Leicester NHS Trust, for one Transcranial Doppler Assessment lasting approximately 90 minutes.

6.2 Primary and Secondary Endpoints/Outcome Measures

This is not an intervention study, and therefore it would not be appropriate to assess the 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 percentage of recruited subjects (HC, MCI patients, VascD patients and AlzD patients) able to comply with the full measurement protocol.
2) The percentage of measurements rejected because of aspects related to data quality during the analysis protocol, with recorded reasons.
3) Overall, the percentage of recruited subjects (healthy controls, MCI patients, VascD patients and AlzD patients) in whom values for the following parameters can be derived:

- % change of CBFv at baseline in response to performance of the ACE-III Cognitive Examination
- Autoregulation index (using the Tiecks model and from the phase, gain and coherence).

7. ELIGIBILITY CRITERIA

7.1 Overall Description of Trial Participants

Patients with MCI, Patients with VascD and Patients with AlzD, together with age-, gender- and BP matched healthy volunteer control subjects (HC) will be recruited to enable the complete data analysis of 11 MCI Patients, 11 VascD Patients, 11 ALzD Patients and 11 HCs.

7.2 Recruitment Process

Patient recruitment at the site will only commence once the research team has ensured that the following approval/essential documents are in place:

- Research Ethics Committee (REC) approval
- HRA approval
- Final Sponsorship approval
- NHS Trust Research and Development Department (R&D) approval
- Signed delegation of duties and responsibilities logs

Suitable MCI patients, AlzD patients and VascD patients will be identified at the specialist memory clinics at Leicester Partnership NHS Trust, and in the stroke and geriatric outpatient clinic at University Hospitals of Leicester NHS Trust, according to a study-specific list of inclusion and exclusion criteria (detailed in next section). We will also be using Join Dementia Research (JDR) as a recruitment tool. This is an online self-registration service that enables volunteers with memory problems or dementia, carers of those with memory problems or dementia and healthy volunteers to register their interest in taking part in research. The purpose of JDR is to allow such volunteers to be identified by researchers as potentially eligible for their studies. Researchers can then contact volunteers, in line with the volunteers' preferred method of contact, to further discuss potential inclusion. Eligibility screening will be conducted by the specialist nurses or consultants who are part of the direct clinical care team. They will only refer potential participants who meet the eligibility criteria and who have capacity to consent to the researcher. The researcher will not access patients' medical records until formal written consent has been obtained. Participants recruited from Join Dementia Research will be screened by the researcher through discussion with the participant and their carers, friends or relatives, or GP surgery

to ensure they meet the study specific inclusion and exclusion criteria. Participants will only be referred to the researcher if the direct care team, based on the patient's cognitive functioning and clinical assessment determine that the patient will have capacity to consent to the study. Capacity will then be assessed formally by the researcher at the initial stage of information provision and at the formal consent process after a cooling off period of up to 7 days. These patients will be introduced to the researcher by the responsible clinical physician or specialist nurse for the provision of further study specific information, in the form of *Participant (Patient) Information Leaflet*. After provision of study specific information, the researcher will assess the patient's capacity in accordance with the Mental Capacity Act 2005. This is a 2 stage process where firstly, there must be a concern that the patient may lack capacity (in this case due to cognitive impairment). When the study information is provided to participants, it will be assessed in 4 steps. Firstly, the ability to understand the information, secondly to weigh up the information, thirdly to retain the information, and finally be able to communicate the decision back to the researcher. This initial discussion and assessment will take place at the outpatient memory clinic and stroke clinic at the LPT and UHL respectively, or through the participant's preferred contact method if recruiting from Join Dementia Research.

Age-, gender-, BP-matched HC will be recruited from University of Leicester departmental volunteers, patients' relatives and friends, and by poster advertisement (using *Research Participant Poster*) displayed in outpatient clinics at the University Hospital of Leicester (UHL) NHS Trust. Care will be taken to ensure that the HC are matched to the patients in terms of age, gender, and medical co-morbidities. HC will be provided with a volunteer specific information leaflet *(Participant (Volunteer) Information).*

After study specific information has been provided to the patients or volunteers, the researcher's contact details will be provided at the end of the PIS and the patient or volunteer will be able to contact the researcher directly if they wish to participate in the study.

Participants who contact the researcher to be included in the study will be made an appointment to attend the Cerebral Haemodynamics in Ageing and Stroke Medicine (CHIASM) laboratory at the Leicester Royal Infirmary to undergo formal consent using the Participant (Patient or Volunteer) Consent Form. At this point, the researcher will then explain the nature and purpose of the research again and answer any questions that the

participant, may have. Capacity will be re-assessed at this time using the process described above and in accordance with the principles of the Mental Capacity Act 2005.

Formal consent will then be obtained as detailed in Section 8 and the participant will be enrolled into the study.

All participants will have their travel expenses reimbursed.

If a patient or volunteer expresses an interest in the study at the initial stage but does not make contact with the researcher within 7 days, a follow-up reminder letter (*Patient Follow-up Letter*) will be sent to the patient through the direct care team with the researcher's contact details.

7.3 Inclusion Criteria

- Informed volunteer consent, patient consent
- Male or female, aged between 18 and 100 years of age
- Able (in the Investigator's opinion) and willing to comply with all study requirements
- Willing to allow his or her General Practitioner (GP) to be notified of participation in the study
- Good understanding of written and verbal English

Healthy Controls-specific Inclusion Criteria

- No evidence of subjective or objective memory impairment on cognitive testing
- No major medical co-morbidity (outlined in detail in the exclusion criteria) or medication use that could adversely affect cognition

MCI Patient-specific Inclusion Criteria

Clinical diagnosis of MCI made by a specialist* in a patient who fulfils the established clinical consensus criteria for MCI [NIA/AA 2011] specifically:

- Concern regarding a change in cognition compared to the person's previous level, by the patient and/or informant

- Objective evidence of impairment of one or more cognitive domains, greater than expected for age, and educational background, over time if repeated measures are available.
- Preserved independence of functional abilities and minimal to no impairment on complex instrumental functions
- Not demented

Vascular Dementia Specific Inclusion Criteria

Clinical diagnosis of VascD made by a specialist* in a patient who fulfils the NINDS-AIREN criteria for VascD, specifically:

- Cerebrovascular disease defined by the presence of focal signs on neurological examination consistent with stroke and evidence of cerebrovascular disease on brain imaging.
- One or more of:
    - Onset of dementia within 3 months of a diagnosed stroke
    - Abrupt deterioration in cognitive function
    - Fluctuating, stepwise progression of cognitive deficits

Alzheimer's Dementia Specific Inclusion Criteria

Clinical Diagnosis of AlzD made by a specialist* in a patient who fulfils the NIA/AA criteria for Probable AlzD, specifically:

- Meets the criteria for dementia
    - The memory impairment and cognitive deficits cause significant impairment in social or occupational functioning, and represent a significant decline from a previous level of functioning, not explained by a delirium or a major psychiatric disorder
    - Impairment of at least two cognitive domains
- Insidious or gradual onset
- Clear history of worsening cognition by report or observation

- The initial and most prominent cognitive deficits are evident on history and examination in one of the following domains:
    - Amnestic: impaired learning and recall of recently learned information
    - Non amnestic: language/visuospatial/executive dysfunction
- No evidence of substantial cerebrovascular disease, core features of dementia with lewy bodies, features of frontotemporal dementia, prominent features of semantic variant primary progressive aphasia, evidence of active neurological disease, a non-neurological co-morbidity or medication that could affect cognition

**A specialist being defined as a psychiatrist or a geriatrician, or a specialist mental health nurse with a specific interest or expertise in cognitive disorders.*

7.4 Exclusion Criteria

- Male or Female, 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
- Major co-morbidity likely to affect cerebral autoregulation; severe respiratory disease, carotid artery stenosis, atrial fibrillation, severe cardiac failure (left ventricular ejection fraction <20%), extreme frailty or multi-morbidity.

8. STUDY PROCEDURES

8.1 Informed Consent and Assessment of Capacity

Following provision of study specific information, the potential research participant (Volunteer or Patient) will be able to contact the researcher with the contact details provided to confirm willingness to participate in the study. At this time an appointment will be made to attend the Leicester Royal Infirmary to undergo formal consent and capacity assessment as outlined below.

Participant consent form must be in place before the undertaking of any research project-specific assessments, using the current version (*Participant (Volunteer or Patient) Consent Form)* which has been approved by the National Research Ethics Service and the local Trust Research and Development Department. Written and verbal versions of the current approved Participant (Volunteer or Patient Information Leaflet, Participant (Patient or Volunteer) Consent Form or 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.

At the time of consent, the researcher will re-assess the patient's capacity in accordance with the Mental Capacity Act 2005. This is a 2 stage process where firstly, there must be a concern that the patient may lack capacity (in this case due to cognitive impairment). When the study information is provided to participants, it will be assessed in 4 steps. Firstly, the ability to understand the information, secondly to weigh up the information, thirdly to retain the information, and finally be able to communicate the decision back to the researcher. This initial discussion and assessment will take place at the outpatient memory clinic and stroke clinic at the LPT and UHL respectively.

The person who obtains consent and performs the capacity assessment must be suitably qualified and experienced, and have been authorised to do so by the Chief/Principal Investigator (CI/PI), as detailed on the Delegation of Authority and Signature log for the study. The original signed Informed Consent form will be retained at the study site within the Investigator Site File (ISF). A copy of the signed Informed Consent form will be given to

participants and a copy retained in the participant's medical notes For HC volunteers, the original signed copy will be retained within the ISF, and a copy provided to the volunteer.

A letter will be sent to the volunteer's and patient's GP informing them of their participation in the study *(GP Information Leaflet (Volunteer or Patient)).*

Process for Consent in Volunteers

We will only invite volunteers who have capacity to give us consent in this research study. All potential volunteers will be recruited from departmental volunteers, patient's relatives and friends and by poster advertisement in the outpatient clinics at the University Hospitals of Leicester (UHL) NHS Trust. The researcher will explain the details of the research study and provide a Participant (Volunteer) Information Leaflet. The researcher will answer any questions or concerns that the volunteer has regarding participation in the study.

Volunteers will be allowed up to seven days to consider the information, and the opportunity to question the Investigator, their GP 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.

Prior to the study, we will also ensure that the volunteer is aware that he/she is free to withdraw from the study at any time for any reason. However, should this situation occur the research team would like to keep all the health information and data that has been collected so far for the final analysis. This will be clearly stated in both the Participant (Volunteer) Information Leaflet and Participant (Volunteer) Consent Form.

Process for Consent in Patients

We will only invite patients who have capacity to give us consent in this research study. All potential participants will be approached initially by the responsible clinical physician, who will undertake the eligibility screening and subsequently introduced to the research team member(s). The research team member(s) will explain the details of the research study and

provide a Participant (Patient) Information Leaflet. The researcher will answer any questions or concerns that the Participant (Patient) has regarding participation in the study.

The Participant (Patient) will be provided with the researcher's contact details, to allow up to 7 days to consider the information, and have the opportunity to question the Investigator, their GP or other independent parties to decide whether they wish to 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. Capacity will be assessed as described above in accordance with the Mental Capacity Act 2005. If the patient expressed an interest in the study at the initial stage but did not make contact with the researcher, a follow-up reminder letter (*Patient Follow-up Letter*) will be sent to the patient through the direct care team with the researcher's contact details.

Prior to the study, we will ensure that the participant is aware that he/she is free to withdraw from the study at any time for any reason. However, should this situation occur we would like to keep all the health information and data that has been collected so far for the final analysis. This will be clearly stated in both the Participant (Patient) Information Leaflet and Participant (Patient) Consent Form.

8.2 TCD window insonation check

Once a consent form has been signed, and before collecting baseline information on demographics, medical history, medications, and handedness, 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, Bos et al 2007, Marinoni et al 1997].

8.3 Collection of demographic data

Baseline demographic data will be collected from the participants as detailed below:

Healthy controls

- Demographics

Date of birth, gender, height, weight, and ethnicity will be recorded

- Medical History

Details of any relevant history of disease or surgical interventions will be recorded

Smoking status and weekly alcohol intake will be recorded

- Current Medications

Details of any prescription or over the counter medications will be recorded

- Handedness

Determined using the Edinburgh Handedness Inventory

Patients with MCI, VascD and AlzD

- Demographics

Date of birth, gender, height, weight, and ethnicity will be recorded.

- Medical History

Details of any relevant history of disease or surgical interventions will be recorded

Smoking status and weekly alcohol intake will be recorded

Any family history of dementia will be recorded

- Current Medications

Details of prescription medication or over the counter medications will be recorded, including the use of any anti-dementia drugs

- Handedness

Determined using the Edinburgh Handedness Inventory [Oldfield et al, 1971]

In addition, the patient's medical records will be reviewed to extract the following information:

- Results and significant findings from Computerised Tomography (CT) head/Brain Magnetic Resonance Imaging (MRI), if undertaken
- Results and details of any previous cognitive testing
- Date of diagnosis of MCI, VascD or AlzD

8.4 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 for four hours prior to attending the research laboratory. The subject will sit on an examination couch. Baseline casual BP will be calculated as a mean of three supine brachial BP readings 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 non-dominant hand. R-R interval will be recorded using a 3-lead ECG. 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. Simultaneous bilateral insonation of the middle cerebral arteries (MCAs) will be performed using TCD with 2MHz probe using a Viasys Companion III, with the subject either sitting or lying supine on a couch (in the case of difficult to insonate windows). The vessel will be located via the temporal bone window, and identified as the MCA by the waveform, its depth, velocity, and the 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 patient sits quietly with their eyes open
2) A recording during which the participant undertakes the Attention, Memory, and Fluency tasks of the ACE-III Cognitive Examination.
3) A recording during which the participant undertakes the language tasks of the ACE-III Cognitive Examination.
4) A recording during which the participant undertakes the visuospatial ability, and final memory tasks, of the ACE-III Cognitive Examination.

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. Recordings 2, 3 and 4 will each begin and end with a period of 1 minute baseline recording where the

participant sits quietly with their eyes open. An event marker will be used to mark the start of each individual question of the ACE-III, so that these can be identified within the recordings. Each individual question will be followed by a period of 1 minute rest, to allow CBFv to return to baseline levels. In order to ensure that visually impaired participants are not placed at a disadvantage during the cognitive examination, enlarged versions of the pictures contained within the ACE-III will be provided on A4 laminated cards.

8.5 Follow-up measurements

A sub-set of 11 Participants will be invited to return after 3 months to repeat the study measurement to assess for reproducibility. This will be stated explicitly on the consent form (*Volunteer and Patient consent forms*), but participants can decline to be contacted at this time, and can withdraw from the study at any time.

8.6 Definition of End of Trial

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

At the end of the study, both healthy controls and patients will receive a written letter, in plain English, summarising the study findings and conclusions.

8.7 Discontinuation/Withdrawal of Participants from Study Treatment

Each participant has the right to withdraw from the study at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care that participant receives. 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
- Consent withdrawn
- Loss of capacity

In the case of withdrawal in the healthy control group, they will be replaced to ensure that there are 11 healthy control subjects with complete data analysis.

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

Prior to the study, we will ensure that the participant is aware that he/she is free to withdraw from the study at any time for any reason. However, should this situation occur we would like to keep all the health information and data that has been collected so far for the final analysis. This will be clearly stated in both the Participant (Patient and Volunteer) Information Leaflet and Participant (Patient and Volunteer) Consent Form.

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.

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).

All documents will be stored safely in confidential conditions. On all study-specific documents, other than the signed consent, the participant will be referred to 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 in a research-dedicated lab, and the Department of Medical Physics, University of Leicester is responsible for the service and maintenance of the equipment.

9.2 Clinical Care

Throughout the study, patients with MCI, VascD and AlzD will continue to receive standard clinical care.

9.3 Incidental Findings

Any unexpected or incidental findings in Volunteers of Patients, such as, high blood pressure, abnormal heart rhythm, undiagnosed cognitive impairment (volunteers), cerebral blood flow velocity suggestive of intracranial pathology or carotid artery stenosis will be reported to the patient or volunteer's GP for further investigation. This will only be with the patient or volunteer's permission and written consent (*Participant (Patient or Volunteer) Consent Form*), and is expressly stated in the *Participant (Patient or Volunteer) Information Sheet.*

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

Serious adverse events are common in dementia. For a full list of expected SAE that are not subject to expedited reporting, investigators should refer to Appendix A.

Suspected Unsuspected Serious Adverse Events/Reactions (SUSAR)

A serious adverse event, the nature or severity of which is not consistent with a diagnosis of dementia.

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 treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the participant must undergo an end of study assessment and be given appropriate care under medical supervision until symptoms cease or the condition becomes stable.

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, must be reported to the Sponsor within one working day 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.

Only SAEs that occur during the fTCD study will be reported to the sponsor. After this, the Patient or Volunteer, ceases to be involved in the study.

11. STATISTICS

11. 1Statistical analysis

The null hypothesis being tested is that:

(a) cerebral haemodynamic parameters, including CBFv and ARI, and any alterations related to the performance of the ACE-III Cognitive Examination are not significantly different between MCI, VascD, AlzD patients and HC subjects.

All normally distributed continuous variables will be described as mean (SD) and continuous variables with skewness as median (IQR). Comparison between baseline data for MCI patients, VascD patients, AlzD patients and HC will be made using Student t-tests for normally distributed data, or by appropriate non-parametric test, with Bonferroni correction applied to multiple comparisons. Repeated measures ANOVA will be adopted to test for the effect of performance of the ACE-III Cognitive Examination on CBFv and ARI between MCI patients, VascD patients, AlzD patients and HC subjects.

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.

However, to detect a change of 2 units in autoregulation Index (ARI) and to calculate the required sample size we followed the technique as previously described by others (Brodie FG et al, 2009). For this study a sample of 11 patients per intervention group will allow the detection of a difference between groups (MCI patients, VascD patients, AlzD patents and HC subjects) in the ARI of 2 units with 80% power at the 5% significance level.

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 PROCEDURE

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

The University of Leicester, as the study Sponsor, operates a risk based monitoring system to which this study will be subject.

14. CODES OF PRACTICE AND REGULATION

14.1 Ethics

All informed consent taken from MCI patients, VascD patients, AlzD patients and HC subjects will be taken following GCP guidelines.

JDR is funded by Department of Health working in partnership with the charities Alzheimer Scotland, Alzheimer's Research UK and Alzheimer's Society and is Health Research Authority (HRA) endorsed. The online service and all associated documentation, methods of contacting volunteers and handling of data, were reviewed by a specially convened HRA committee which included experts in research ethics, data protection and information governance. Formal endorsement was issued by the HRA in a letter dated 20 May 2014.

14.2 Sponsor Standard Operating Procedures

All relevant Sponsor Standard Operating Procedures 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 2008).

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 GCP.

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), 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 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 participant's 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

15.1Data Extraction

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

Other information which will be extracted from the medical notes and recorded on the CRF include results and details of any previous cognitive testing, and the date of diagnosis of MCI, VascD or AlzD.

15.2Data Analysis

All other parameters recorded, including CBFv, heart rate, BP, and $ETCO_2$ will be simultaneously recorded onto a computer system (PHYSIDAS), providing data for subsequent analyses. Off-line analyses will be undertaken using software designed by the University of Leicester's Medical Physics Group.

15.3 Data Management

All parameters (signals) that are collected during the measurement will be saved using a coded filename. The name and other identifying detail will NOT be included in any study data electronic file.

All files will be encrypted and stored on a password secured computer/laptop, which will have restricted access by members who are authorised on the authorisation log.

16. STUDY GOVERNANCE

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

17. FINANCING AND INSURANCE

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

In this study, the extra assessments on patients 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. No other patient related costs will be incurred by the project in addition to the routine care provided from the University Hospitals of Leicester NHS Trust or Leicestershire Partnership Trust.

18. PUBLICATION POLICY

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

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20. APPENDIX A: EXPECTED EVENTS NOT SUBJECT TO EXPEDITED REPORTING

Acute Coronary Syndromes

Agitation

Angina

Anorexia

Anxiety

Constipation

Delirium

Depression

Dysphagia

Electrolyte disturbance

Fall

Fatigue

Gastrointestinal disturbance

Hallucinations

Incontinence, faecal

Incontinence, urinary

Infections

Institutionalisation / Admission to care home

Intracerebral Haemorrhage

Loss of ability to function or care for self

Myocardial infarction

Nausea

Personality change

Peripheral Vascular Disease

Renal impairment

Sedation

Seizure

Sexual dysfunction

Sleep disturbance

Stroke

Transient ischemic attack

Urinary retention

Urinary tract infection

Violent behaviour

Visual loss

Vomiting

Weakness

Weight loss

21. APPENDIX B: ADDENBROOKE'S COGNITIVE EXAMINATION-III

ADDENBROOKE'S COGNITIVE EXAMINATION – ACE-III
English Version A (2012)

Name: Date of Birth: Hospital No. or Address:	Date of testing: ___/___/___ Tester's name:_____ Age at leaving full-time education:_____ Occupation: _____ Handedness: _____

ATTENTION

➢ Ask: What is the	Day _____	Date _____	Month _____	Year _____	Season _____	**Attention** [Score 0-5] ☐
➢ Ask: Which	No./Floor _____	Street/Hospital _____	Town _____	County _____	Country _____	**Attention** [Score 0-5] ☐

ATTENTION

- ➢ Tell: "I'm going to give you three words and I'd like you to repeat them after me: lemon, key and ball." After subject repeats, say "Try to remember them because I'm going to ask you later".
- ➢ Score *only* the first trial (repeat 3 times if necessary).
- ➢ Register number of trials: _____

Attention [Score 0-3] ☐

ATTENTION

- ➢ Ask the subject: "Could you take 7 away from 100? I'd like you to keep taking 7 away from each new number until I tell you to stop."
- ➢ If subject makes a mistake, do not stop them. Let the subject carry on and check subsequent answers (e.g., 93, 84, 77, 70, 63 – score 4).
- ➢ Stop after five subtractions (93, 86, 79, 72, 65): ____ ____ ____ ____ ____

Attention [Score 0-5] ☐

MEMORY

- ➢ Ask: 'Which 3 words did I ask you to repeat and remember?' _____ _____ _____

Memory [Score 0-3] ☐

FLUENCY

- ➢ **Letters**

Say: "I'm going to give you a letter of the alphabet and I'd like you to generate as many words as you can beginning with that letter, but not names of people or places. For example, if I give you the letter "C", you could give me words like "cat, cry, clock" and so on. But, you can't give me words like Catherine or Canada. Do you understand? Are you ready? You have one minute. The letter I want you to use is the letter "P".

Fluency [Score 0 – 7] ☐

				≥ 18	7
				14-17	6
				11-13	5
				8-10	4
				6-7	3
				4-5	2
				2-3	1
				0-1	0
				total	correct

- ➢ **Animals**

Say: "Now can you name as many animals as possible. It can begin with any letter."

Fluency [Score 0 – 7] ☐

				≥ 22	7
				17-21	6
				14-16	5
				11-13	4
				9-10	3
				7-8	2
				5-6	1
				≤5	0
				total	correct

MEMORY

➢ Tell: "I'm going to give you a name and address and I'd like you to repeat the name and address after me. So you have a chance to learn, we'll be doing that 3 times. I'll ask you the name and address later."

Score only the third trial.

Memory [Score 0 – 7] ☐

	1st Trial	*2nd Trial*	*3rd Trial*
Harry Barnes 73 Orchard Close Kingsbridge Devon	_____ _____ ___ _____ _____ _____ _____	_____ _____ ___ _____ _____ _____ _____	_____ _____ ___ _____ _____ _____ _____

MEMORY

➢ Name of the current Prime Minister.....
➢ Name of the woman who was Prime Minister .....
➢ Name of the USA president.....
➢ Name of the USA president who was assassinated in the 1960s.....

Memory [Score 0 – 4] ☐

LANGUAGE

➢ Place a pencil and a piece of paper in front of the subject. As a practice trial, ask the subject to "**Pick up the pencil and then the paper.**" If incorrect, score 0 and do not continue further.

➢ If the subject is correct on the practice trial, continue with the following three commands below.
- Ask the subject to "**Place the paper on top of the pencil**"
- Ask the subject to "**Pick up the pencil but not the paper**"
- Ask the subject to "**Pass me the pencil after touching the paper**"

Note: Place the pencil and paper in front of the subject before each command.

Language [Score 0-3] ☐

LANGUAGE

➢ Ask the subject to write two (or more) complete sentences about his/her last holiday/weekend/Christmas. Write in complete sentences and do not use abbreviations. Give 1 point if there are two (or more) complete sentences about the one topic; and give another 1 point if grammar and spelling are correct.

Language [Score 0-2] ☐

LANGUAGE

➢ Ask the subject to repeat: '**caterpillar**'; '**eccentricity**; '**unintelligible**'; '**statistician**'

Score 2 if all are correct; score 1 if 3 are correct; and score 0 if 2 or less are correct.

Language [Score 0-2] ☐

LANGUAGE

➢ Ask the subject to repeat: '**All that glitters is not gold**'

Language [Score 0-1] ☐

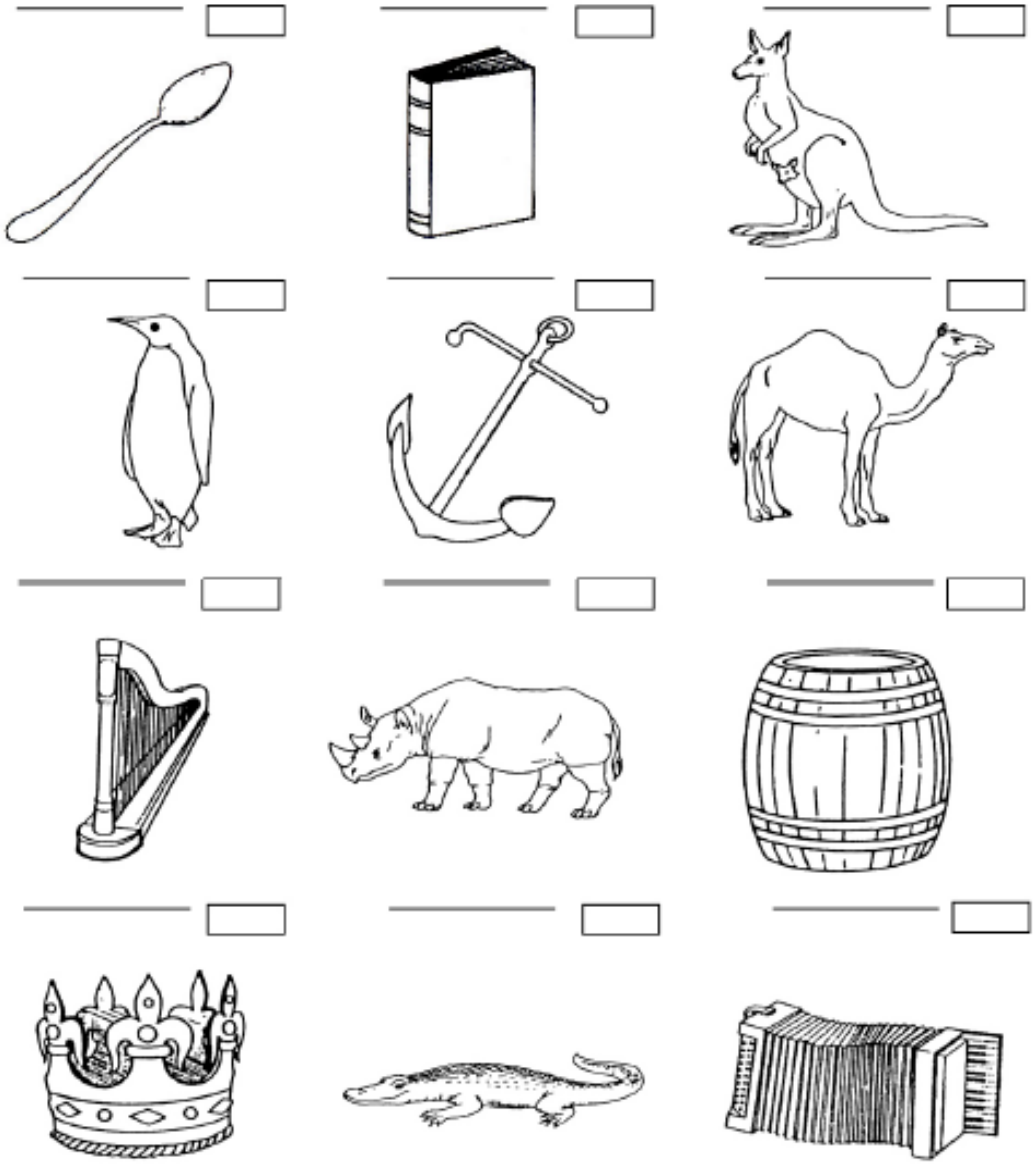
➢ Ask the subject to repeat: '**A stitch in time saves nine**'

Language [Score 0-1] ☐

LANGUAGE

➢ Ask the subject to name the following pictures:

Language [Score 0-12] ☐



LANGUAGE

➢ Using the pictures above, ask the subject to:

- Point to the one which is associated with the monarchy .....
- Point to the one which is a marsupial .....
- Point to the one which is found in the Antarctic .....
- Point to the one which has a nautical connection .....

Language [Score 0-4] ☐

LANGUAGE

- Ask the subject to read the following words: (Score 1 only if all correct)

Language [Score 0-1] ☐

sew
pint
soot
dough
height

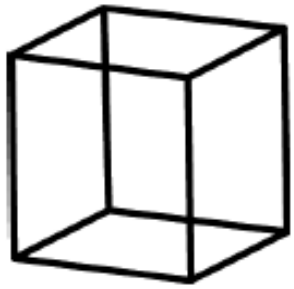
VISUOSPATIAL ABILITIES

- Infinity Diagram: Ask the subject to copy this diagram

Visuospatial [Score 0-1] ☐

- Wire cube: Ask the subject to copy this drawing (for scoring, see instructions guide).

Visuospatial [Score 0-2] ☐



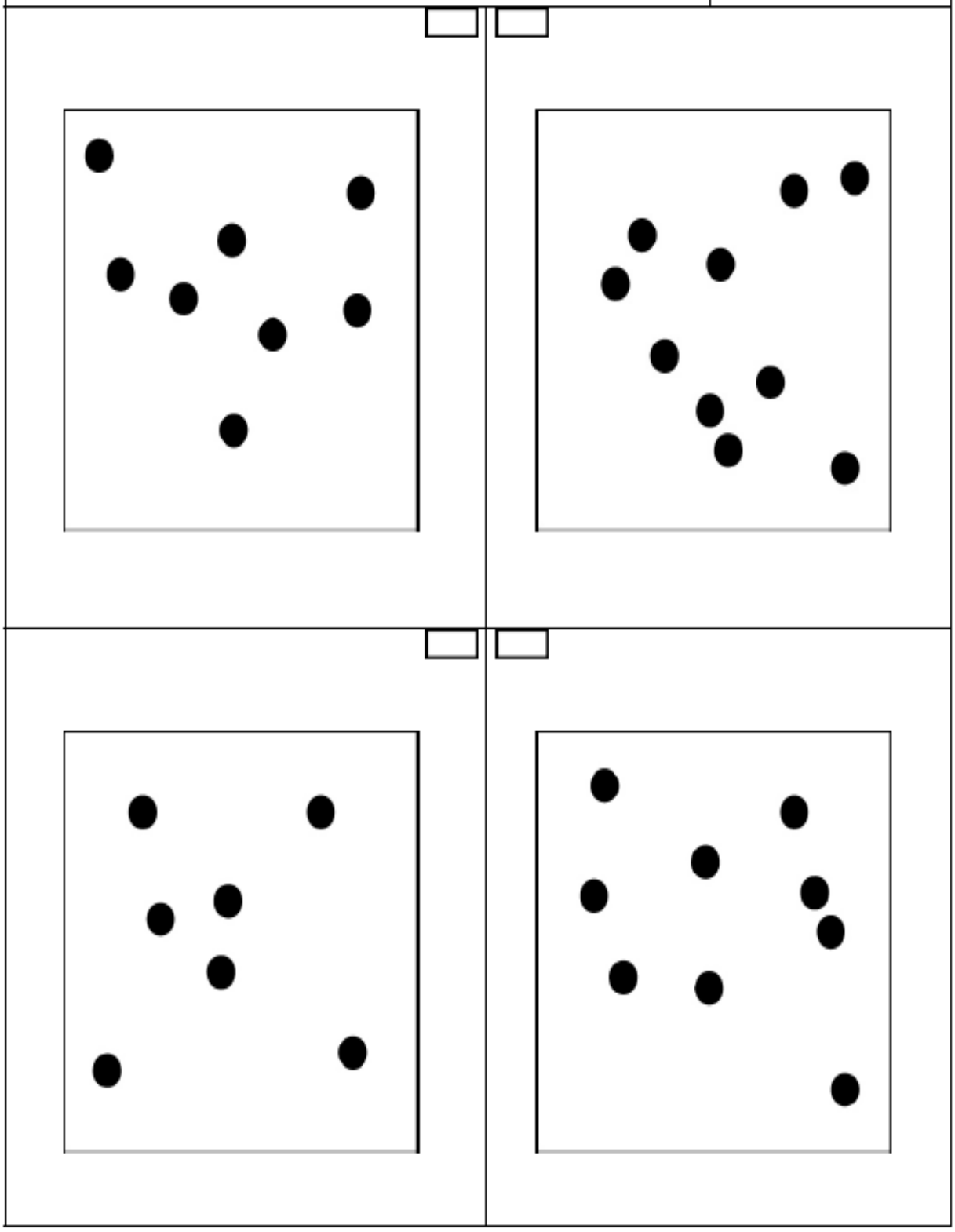
- Clock: Ask the subject to draw a clock face with numbers and the hands at ten past five. (For scoring see instruction guide: circle = 1, numbers = 2, hands = 2 if all correct).

Visuospatial [Score 0-5] ☐

VISUOSPATIAL ABILITIES

- Ask the subject to count the dots without pointing to them

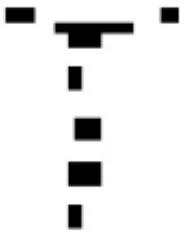
Visuospatial
[Score 0-4]



VISUOSPATIAL ABILITIES

➢ Ask the subject to identify the letters

Visuospatial [Score 0-4] ☐



MEMORY

➢ Ask "Now tell me what you remember about that name and address we were repeating at the beginning"

Harry Barnes 73 Orchard Close Kingsbridge Devon	…… …… …… ……	**Memory** [Score 0-7] ☐

MEMORY

➢ This test should be done if the subject failed to recall one or more items above. It all items were recalled, skip the test and score 5. If only part was recalled start by ticking items recalled in the shadowed column on the right hand side; and then test not recalled items by telling the subject "ok, I'll give you some hints: was the name X, Y or Z?" and so on. Each recognised item scores one point, which is added to the point gained by recalling.

Memory [Score 0-5] ☐

Jerry Barnes	☐	Harry Barnes	☐	Harry Bradford	☐	recalled	☐
37	☐	73	☐	76	☐	recalled	☐
Orchard Place	☐	Oak Close	☐	Orchard Close	☐	recalled	☐
Oakhampton	☐	Kingsbridge	☐	Dartington	☐	recalled	☐
Devon	☐	Dorset	☐	Somerset	☐	recalled	☐

SCORES

TOTAL ACE-III SCORE	/100
Attention	/18
Memory	/26
Fluency	/14
Language	/26
Visuospatial	/16

22. APPENDIX C: 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)		