Rationale and design of a longitudinal study of cerebral small vessel diseases, clinical and imaging outcomes in patients presenting with mild ischaemic stroke: Mild Stroke Study 3

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Abstract:

Background

Cerebral small vessel disease (SVD) is a major cause of dementia and stroke, visible on brain MRI. Recent data suggests SVD lesions may be dynamic, damage extends into normal-appearing brain, and microvascular dysfunctions include abnormal blood-brain barrier leakage, vasoreactivity and pulsatility, but much remains unknown regarding underlying pathophysiology, symptoms, clinical features and risk factors of SVD.

Study Methods/Design

The Mild Stroke Study 3 (MSS-3) is a prospective observational cohort study to identify risk factors for and clinical implications of SVD progression and regression among up to 300 adults with non-disabling stroke. We perform detailed serial clinical, cognitive, lifestyle, physiological, retinal and brain MRI assessments over one year; we assess cerebrovascular reactivity, blood flow, pulsatility, blood-brain barrier leakage on MRI at baseline; we follow up to four years by post and phone. The study is registered ISRCTN 12113543.

Discussion

Factors which influence direction and rate of change of SVD lesions are poorly understood. We investigate the role of small vessel dysfunction using advanced serial neuroimaging in a deeply phenotyped cohort to increase understanding of the natural history of SVD, identify those at highest risk of early disease progression or regression and uncover novel targets for SVD prevention and therapy.

Rationale and design of a longitudinal study of cerebral small vessel diseases, clinical, and imaging outcomes in patients presenting with mild ischaemic stroke: Mild Stroke Study 3 (MSS-3).

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ABSTRACT

Background

Cerebral small vessel disease (SVD) is a major cause of dementia and stroke, visible on brain MRI. Recent data suggests SVD lesions may be dynamic, damage extends into normal-appearing brain, and microvascular dysfunctions include abnormal blood-brain barrier leakage, vasoreactivity and pulsatility, but much remains unknown regarding underlying pathophysiology, symptoms, clinical features and risk factors of SVD.

Study Methods/Design

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Discussion

Factors which influence direction and rate of change of SVD lesions are poorly understood. We investigate the role of small vessel dysfunction using advanced serial neuroimaging in a deeply phenotyped cohort to increase understanding of the natural history of SVD, identify those at highest risk of early disease progression or regression and uncover novel targets for SVD prevention and therapy.

Background

Cerebral small vessel disease (SVD) describes diffuse disease processes affecting the perforating cerebral arterioles, capillaries, venules and consequent damage to the white and deep grey matter.(1) This damage is visible on brain MRI as white matter hyperintensities (WMH), ~~lacunar~~ recent small subcortical infarcts, perivascular spaces, ~~focal cortical thinning~~brain atrophy, and cerebral microbleeds.(2)

SVD causes 20% of ischaemic strokes and almost half of all dementias,(3, 4) contributing to both vascular and Alzheimer's dementia subtypes,(5) its presence more than doubling future risk of stroke, dementia and functional impairment.(6)

Recent advances in neuroimaging have uncovered candidate mechanisms for underlying pathophysiological processes. Furthermore, SVD appears to be more dynamic and global than previously thought, since recent studies show: (a) WMH can regress as well as progress;(7-10) (b) SVD is associated with cerebrovascular dysfunction including diffuse blood-brain barrier failure;(11) (c) with some evidence for other vascular dysfunctions including reduced cerebrovascular reactivity (CVR) and increased intracranial pulsatility;(12-14) and d) acute, apparently 'silent', lesions on Diffusion Weighted Imaging (DWI) may be more frequent than previously thought.(15-17)

Most SVD lesions are thought to develop 'silently'. However, some studies suggest that SVD lesions are associated with subjective cognitive complaints,(18) gait disturbance,(19) mood disorders and apathy.(20) Moreover, subtle symptoms have been associated with acute DWI lesions in a few small cross-sectional studies in non-stroke populations (n=6/649;

n=10/30),(21, 22) while apparently 'silent' acute DWI lesions have been noted in up to 25% following recent stroke, mostly in small studies (e.g. n<105) that sought typical stroke symptoms.(16, 23) Thus, knowledge of the extent of clinical correlates of SVD lesions, in particular, any 'red flag' symptoms or signs that might highlight lesion worsening, remains limited and may be being clinically overlooked.

SVD is commonly attributed to traditional vascular risk factors, particularly hypertension, but also smoking and diabetes, yet these factors only account for 2% of WMH variance.(24) Less is known about potential contributors such as diet, lifestyle and premorbid factors.(25) Extending the search beyond an individual's current clinical status to early and mid-life stages is an important target for SVD research.(26) Understanding whether combined risk factors have a synergistic effect on an individual's risk of developing SVD, as well as improved recognition of symptoms, would provide better recognition of persons at risk of SVD development or progression, providing insight on whether multimodal approaches to prevention and treatment should be taken.

Few studies have comprehensively assessed SVD lesion progression, symptomatology and wide-ranging risk factors. Hence we describe the protocol for a detailed study to assess the role of cerebrovascular dysfunctions in combination, symptoms, risk factors including diet, sleep, and early life factors, on longitudinal SVD lesion change in patients presenting with stroke-related SVD.

Methods

Study design

The Mild Stroke Study 3 (MSS-3: ISRCTN 12113543) is a detailed prospective observational cohort study with clinical and imaging follow-up which aims to recruit up to 300 participants. In addition to a scan at initial stroke diagnosis, the patients undergo a minimum of three study scans over a year; in addition, those with lacunar stroke or moderate to severe white matter hyperintensities are invited for a further one or two scans between baseline and six months. The baseline assessment occurs within a maximum of 3 months of index stroke (Table 1). We invite most participants to attend an interim visit 2-3 months later. All participants return 6 and 12 months respectively after the baseline assessment. Annually thereafter up to four years, we will invite participants to continue annual postal or phone follow-up with another MRI at 3 years. The follow-up questionnaire includes recurrent vascular events, cognition and functional status. MSS-3 benefits from systems established during the MSS-1 and MSS-2 studies(27, 28) and commenced in August 2018.

Study population

Adults >18 years old with mild ischaemic stroke with a modified Rankin Scale (mRS) ≤2 at recruitment presenting to Edinburgh/Lothian stroke services.

Eligibility criteria

We define stroke as previously(27, 28): clinical lacunar stroke syndrome (50%) and control participants with non-lacunar ischaemic stroke syndromes (50%) i.e. partial anterior circulation syndrome or posterior circulation syndrome, with recent infarct visible on diagnostic MRI or CT scan compatible with the clinical syndrome, or if no visible infarct, no other lesion explaining the stroke symptoms. Participants with non-lacunar stroke form controls, since they have similar vascular risk factors and follow similar secondary prevention, accounting for medication effects on vessel function.

We exclude participants with MRI contraindications, major neurological conditions, severe cardiac and respiratory disease. All participants give written informed consent. The study was granted ethical approval by Southeast Scotland Regional Ethics Committee (reference 18/SS/0044).

Diagnosis

An expert panel of stroke physicians and neuroradiologists reach final stroke diagnosis by consensus following review of presenting symptoms and signs including motor or sensory deficit, hemianopia, visuospatial disorder, ataxia, dysphasia, dysarthria, cerebellar or brainstem symptoms, supplemented by diagnostic brain MRI or CT and other relevant investigations, as previously.(27, 28) An experienced neuroradiologist (J.M.W.) assesses all scans for acute ischaemic lesions including recent small subcortical infarcts, prior infarcts or haemorrhages, WMH, lacunes, PVS, microbleeds, siderosis, atrophy, using standardized validated scales.(2, 28)

Table 1: Study assessments

Activity/assessment	Pre-visit	V1[a] baseline[a]	V2[b]: 2-3 months*	V3[c]: 6 months	V4[d]: 12 months
Eligibility	X				
Diagnostic MRI/CT	X				
Consent		X			

Routine blood tests	X				
Electrocardiogram	X				
Carotid doppler ultrasound	X				
Symptom assessment		X	X	X	X
Cognitive tests		X	X	X	X
MRI		X	X	X	X
Retinal imaging		X	X	X	X
Blood pressure		X	X	X	X
Recurrent vascular events		X	X	X	X
NIHSS		X	X	X	X
Modified Rankin Scale		X		X	X
Medical history/vascular risk factors		X		X	X
Demographic/socioeconomic factors		X			
Diet questionnaire		X			
Sleep questionnaire		X			
Mood/fatigue questionnaires		X			
Blood/urine collection		X			
24-hour blood pressure monitoring		X			
Premorbid IQ (education, National Adult Reading Test)		X			
Informant questionnaire: IQCODE, NPI-Q, AES-I		X			X
Pulse wave measures		X			
9-hole peg test		X			X
Timed Up+Go		X			X
Stroke Impact Scale				X	X

[a]Visit 1: baseline visit within 3 months of index stroke +/- 1 week

[b]Visit 2: ~~2-3 months~~+/- 1 week

[c]Visit 3: ~~6 months~~ +/- 2weeks

[d]Visit 4: ~~12 months~~ +/- 3weeks

MRI=Magnetic Resonance Imaging, NIHSS=National Institute of Health Stroke Scale, IQCODE=Informant Questionnaire for Cognitive Decline in the Elderly, NPI-Q=Neuropsychiatric Inventory Questionnaire, AES-I=Apathy Evaluation Scale-Informant

Vascular risk factors, past medical history, medications, incident vascular events

Each participant provides a medical history of diagnoses confirmed by a physician, supplemented by hospital medical records and general practitioner correspondence, following standard definitions, including diabetes mellitus, hypertension, hypercholesterolaemia, previous stroke or TIA, peripheral vascular disease, atrial fibrillation, ischaemic heart disease, valvular defects, heart failure and physician-diagnosed anxiety, depression or delirium. We record current medications, cross-checking with electronic medical records. At follow-up we record recurrent stroke, TIA and cardiac events.

Subjective symptoms

We use a structured questionnaire to ask open-ended questions about subjective symptoms experienced prior to, at the time of, and since index stroke diagnosis (see Supplementary Appendix 1). Participants also answer questions based on previous clinico-radiological studies regarding symptoms within the past month including subjective memory concerns, confusional episodes, unsteadiness, falls, dizziness and headaches.(29)

Participants self-administer the Fatigue Severity Scale,(30) Generalized Anxiety Disorder-7,(31) the Center for Epidemiologic Studies-Depression Scale,(32) and an adapted Pittsburgh Sleep Quality Index.(33)

Informant-reported symptoms

A nominated close friend or relative completes the following prior to the baseline visit: Neuropsychiatric Inventory Questionnaire,(34) behavioural changes since stroke,(35) Apathy Evaluation Scale, Informant version(36) and the Informant Questionnaire for Cognitive Decline in the Elderly,(37) repeated at 12 months.

Family, lifestyle, social, early life factors

We record stroke or dementia family history including age at diagnosis, alcohol consumption and smoking status including quantity and duration. Participants self-administer the EPIC-Norfolk Food Frequency Questionnaire, a comprehensive dietary overview including salt intake.(38, 39)

To assess early life socioeconomic status, we record childhood postal address, number of individuals, rooms and toilets in the property and parental occupations. We note ethnicity, educational duration and attainment,(40) occupation, current postcode and retirement age.

Physical examination

We record presenting and current neurological deficits and stroke severity (NIHSS), blood pressure 3 times, gait (Timed Up and Go), manual dexterity (9-Hole Peg Test), height and weight.

Cognitive assessment

Participants complete the comprehensive 30-minute neuropsychological test protocol based on the National Institute of Neurological Disorders and Stroke–Canadian Stroke Network

(NINDS-CSN) Vascular Cognitive Impairment Harmonization Standards. This battery spans multiple cognitive domains and includes the Montreal Cognitive Assessment (MoCA), Hopkins Verbal Learning Test-revised, Controlled Oral Word Association Test, Animal Naming, Letter Digit Coding, and Trailmaking Tests A+B.

We estimate peak adult intelligence using the National Adult Reading Test.(40)

Participants repeat MoCA and Trailmaking Tests A+B at each visit. We use three different MoCA versions, randomly assigning a test sequence to each participant to minimize learning effects on serial test performance.

Functional recovery

We administer the modified Rankin Scale(41) at baseline, 6 and 12 months and the Stroke Impact Scale(42) at 6 and 12 months.

Magnetic Resonance Imaging

We scan all participants at diagnosis at 1.5T (General Electric Signa HDxt) or 3T (Siemens Prisma) MRI or CT with core structural brain MRI sequences at each visit: 3D T1w, T2w, Fluid Attenuated Inversion Recovery (FLAIR), Susceptibility-weighted (SWI/SWAN/GRE), and single- or multi-shell diffusion imaging (dMRI). Subsequent full cerebrovascular assessment and all follow-up imaging is at 3T.

At 1-3 months post-stroke, participants undergo 3T MRI to measure blood-brain barrier (BBB) integrity, CVR, cerebral blood flow (CBF) and intracranial vascular and CSF pulsatility (protocol in Supplementary Appendix 2). We assess BBB integrity using dynamic contrast-enhanced (DCE-) MRI and gadolinium-based contrast agent (gadobutrol) injection,(11, 43) unless eGFR<30ml/min. We assess CVR using a Blood Oxygenation Level Dependent (BOLD) MRI sequence, during which participants inhale air with intermittent added CO_2 (12-minute paradigm alternating 2 minutes air and 3 minutes 6% CO_2) through a tight-fitting facemask, described previously.(13, 44) Arterial, venous and CSF pulsatility are measured using phase contrast MRI sequences.(14, 44) We measure CBF using major arterial phase contrast flow measures obtained during pulsatility measurements (and arterial spin labelling where feasible).

We process MRI computationally using well-validated methods to assess intracranial volume, brain, CSF, normal-appearing white and grey matter, WMH volumes, index and prior stroke lesion volumes, lacunes, microbleeds and perivascular space metrics.(45, 46) We visually quantify index and prior stroke lesions (location, type), WMH (baseline, change), lacunes (number, location), perivascular spaces, microbleeds, siderosis, superficial and deep brain volume loss, according to STRIVE criteria using validated scales.(2, 47-51) See Supplementary Appendix 2 for image processing methods description including advanced neuroimaging data.

Retinal imaging

We assess vision (Logmar cabinet, Sussex Vision) and use Spectralis OCT2® with Optical Coherence Tomography Angiography (OCTA)(Heidelberg Engineering) at each visit, imaging retinal vessels, retinopathy, nerve fibre layer thickness, choroid OCTA, intra-retinal and sub-retinal fluid. We computationally process retinal and arteriolar widths, branching patterns, complexity,(52) nerve fibre layer thickness and microvessels on OCTA using well-validated tools.(53) See Supplementary Appendix 2 for processing details.

Systemic vascular measures

We record BP at three standard points during the baseline visit and measure arterial velocities through pulse wave velocity and pulse wave analysis using a tonometric device (*SphygmoCor*®, AtCor Medical/Vicorder, Skidmore Medical) held over the carotid and radial pulses while supine. We provide a 24-hour ambulatory BP monitoring device (SpaceLabs Medical) to most and encourage all to submit self-monitored blood pressure recordings. We repeat BP measurements at all study visits.

Biochemical, haematological, cardiovascular, imaging investigations

We document routinely collected index stroke investigation results including serum haematology and biochemistry, electrocardiogram, echocardiography and carotid Doppler ultrasound. We collect 18ml venous blood at baseline for inflammatory and endothelial function markers. We store 20ml urine for inflammatory marker analysis and 5ml for albumin-to-creatinine ratio.

Endpoints

The primary endpoint is the proportion of SVD lesions that regress, progress or appear de novo in the year after stroke. The secondary endpoints are: (a) blood-brain barrier (BBB) integrity; (b) cerebrovascular reactivity (c) intracranial vascular/CSF pulsatility; (d) WMH, PVS, lacunes and microbleeds; (e) white matter structural integrity measured with diffusion tensor and T_1 parameters; and (f) incidence of reported symptoms including neuropsychiatric and cognitive symptoms, recurrent stroke, transient ischaemic attacks and cardiac events.

Statistical analysis

Sample size calculation

In a previous study with one year of longitudinal imaging follow-up at this centre, 10.6% of participants had a de novo lesion on MRI at follow-up.(28) In the same study, 65% had a mean 5.5ml WMH volume increase and 35% had a mean 6.6ml decrease.(54) A sample of 250 participants would be required to detect WMH change in the year after stroke, with significance 0.05 and power 0.90 in univariate analysis. We aim to recruit up to total 300 participants which allows for loss to follow-up.(54)

Proposed analyses

In our primary analyses, we will use linear mixed effects models adjusted for age, vascular risk factors and baseline SVD burden to estimate the effect of cerebrovascular structure/function on SVD lesion progression and regression.

Secondarily, we will use similar models and other approaches (e.g. stratifying by low vs. high SVD burden, stroke subtype) to quantify associations of the following factors with lesion change: cognitive test scores and incident cognitive impairment; functional status; life course factors; lifestyle factors; blood pressure; systemically measured vascular stiffness; retinal measures; and inflammatory and endothelial function markers.

Discussion

The Mild Stroke Study 3 is a detailed prospective observational study which will advance our knowledge of how detailed measures of small vessel dysfunction and changing lesions relate to comprehensive symptom, cognitive, retinal, early life and lifestyle factors, whether some individuals are more vulnerable than others to the effects of small vessel dysfunction and whether a single candidate measure could best differentiate abnormal from normal-appearing brain tissue by stage and severity of SVD.

This study is novel in its concurrent use of advanced neuroimaging techniques to measure CVR, BBB leakage, CBF and vascular/CSF pulsatility; the first time these measures have been performed contemporaneously, alongside an unprecedented comprehensive assessment of symptoms and signs as they relate to these measures and lesion changes across multiple time-points. We build on previous studies,(8, 55) establishing a well-phenotyped profile of the dynamic natural history of SVD, capturing this rich dataset in the subacute post-stroke phase, monitoring the vulnerable brain at risk for early disease accumulation.(16, 56) Our systematic approach is a template for application to future research studies, designed to optimally assess rates of disease progression and regression, translatable to other SVD presentations including mild cognitive impairment.

This study will fill an existing gap of longitudinal imaging studies evaluating symptoms such as apathy, fatigue, anxiety, delirium, sleep disturbance, and emotional lability, contributing to the detection of preclinical SVD states. We will identify ‘red flags’ to the presence and progression of SVD so that we may intercept disease earlier, even before it develops, rather than in patients presenting with overt brain dysfunction e.g. stroke, dementia. We will gain insight into novel preventative and therapeutic targets by uncovering the nature of and factors associated with lesion regression. This study will deepen our understanding of SVD, essential to future prevention and treatment of stroke and vascular dementia.

To date we have recruited 105 participants. The baseline visit lasts 6.5 hours and most are willing to attend three further visits, each lasting two hours, with positive participant feedback, demonstrating the feasibility of applying this design at other centres.

Conflicting Interests

UC, MS, MJT, GB, AM, OH, CM, FND and JMW hold academic grants from government and charitable funding agencies, outlined below.

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Approvals

Ethical approval for this study was obtained from South East Scotland Research Ethics Committee (Ref 18/SS/0044) on 31/05/2018. NHS Lothian Research & Development approved this study on 31/05/2018 (Ref 2018/0084).

Informed consent

Written informed consent is obtained from all subjects before the study.

Trial registration

ISRCTN 12113543

Guarantor

JMW

Authors' contributorship

UC:recruitment, data collection/management, study design, study coordination. DJG, WH:data collection/management, study coordination. IM, MB, MJT, MS, GB, SMM, ES, CM, AGM, IH:advanced neuroimaging techniques advice/design. TM, SW, KH, CH:retinal imaging techniques advice/design, data collection. MCVH, LB, MS, MJT:image analysis techniques advice/design. OH:cognitive test protocol advice, data collection. RB, EB:laboratory processing advice. DJ:data management. CA:advice regarding study design. FC:advice/study design, data management, statistical analysis plan. FD:recruitment, funding, study design, supervision, clinical oversight. JMW:conception, funding, ethics and regulatory approvals, study design, data collection, all supervision and governance, drafting and final editing of text. All authors also prepared, revised and approved the final manuscript.

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Title: Studies of small vessel diseases: the Mild Stroke Study 3 (MSS-3). The longitudinal study of cerebral small vessel diseases following mild ischaemic stroke: rationale and design.

Supplement 1: Symptom questionnaire

OTHER SYMPTOMS

Q45 (a) Other than the symptoms that first brought you to medical attention with your stroke, did you have any *other* symptoms *in the month prior to* the stroke?

Yes / No / Unknown

If "no", go to Q46

(b) If yes, please describe:

Symptom 1____

Symptom 2(optional)
____

Symptom 3(optional)
____

(c) Categorise domain of symptoms under the following:
Symptom 1 Gait / Low mood / Anxiety / Cognition / Apathy / Sleep and fatigue / Urinary / Sensory / Motor / Visual / Speech / Other

Symptom 2 (optional): Gait / Low mood / Anxiety / Cognition / Apathy / Sleep and fatigue / Urinary / Sensory / Motor / Visual / Speech / Other

Symptom 3 (optional): Gait / Low mood / Anxiety / Cognition / Apathy / Sleep and fatigue / Urinary / Sensory / Motor / Visual / Speech / Other

(d) Categorise characteristics of symptoms under the following:
Symptom 1:
Sudden onset / gradual onset /unknown
Duration <24hours / duration >24hours /unknown
Focal / non-focal /unknown
Resolved / ongoing /unknown

Symptom 2 (optional):
Sudden onset / gradual onset /unknown
Duration <24hours / duration >24hours /unknown

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Focal / non-focal /unknown
Resolved / Ongoing /unknown

Symptom 3:
Sudden onset / gradual onset /unknown
Duration <24hours / duration >24hours /unknown
Focal / non-focal /unknown
Resolved / Ongoing /unknown

Q 46 (a) Other than the symptoms that first brought you to medical attention, have you had any *other* symptoms *since* the stroke?

Yes / No / Unknown

If "no", go to Q47

(b) If yes, please describe:

Symptom 1____

Symptom 2(optional)
____

Symptom 3(optional)
____

(c) Categorise domain of symptoms under the following:

Symptom 1 Gait / Low mood / Anxiety / Cognition / Apathy / Sleep and fatigue / Urinary / Sensory / Motor / Visual / Speech / Other

Symptom 2 (optional): Gait / Low mood / Anxiety / Cognition / Apathy / Sleep and fatigue / Urinary / Sensory / Motor / Visual / Speech / Other

Symptom 3 (optional): Gait / Low mood / Anxiety / Cognition / Apathy / Sleep and fatigue / Urinary / Sensory / Motor / Visual / Speech / Other

(d) Categorise characteristics of symptoms under the following:
Symptom 1:
Sudden onset / gradual onset /unknown
Duration <24hours / duration >24hours /unknown
Focal / non-focal /unknown
Resolved / ongoing /unknown

Symptom 2 (optional):
Sudden onset / gradual onset /unknown
Duration <24hours / duration >24hours /unknown

Focal / non-focal /unknown
Resolved / Ongoing /unknown

Symptom 3:
Sudden onset / gradual onset /unknown
Duration <24hours / duration >24hours /unknown
Focal / non-focal /unknown
Resolved / Ongoing /unknown

Q 47 Have you had any previous episodes of delirium?
(Select "No" if the following criteria are met: diagnosis is not recorded anywhere on TRAK correspondence *or* ECS *and* the patient has never been informed by a healthcare professional that they have got the diagnosis that is listed)

Yes /
No /
Unknown

Q 48 Do you have any concerns about your memory?

Yes /
No /
Unknown

Q49 Have you experienced a feeling of "brain fog" or lack of clarity in thinking during the past month?

Yes /
No /
Unknown

Q50 Have you experienced any episodes of confusion or felt confused during the past month?
Yes /
No /
Unknown

Q51 Have you felt unsteady on your feet during the past month?

Yes /
No /
Unknown

Q 52 Have you experienced any light-headedness, dizziness, vertigo, or any combination of the above during the past month

(a)
Yes /

No /
Unknown

(b) If yes, did you experience:
1- light-headedness
2- dizziness
3- vertigo
4- a combination of any of the above

Q53 Have you had any falls in the past month? (Note to interviewer: a fall is defined as an event which results in a person coming to rest inadvertently on the ground or floor or other lower level)

Yes /
No /
Unknown

Q54 During the last 30 days, on how many of these days did you have a headache? (answer 0 if none)

_________/unknown

Title: Studies of small vessel diseases: the Mild Stroke Study 3 (MSS-3). The longitudinal study of cerebral small vessel diseases following mild ischaemic stroke: rationale and design.

Supplement 2:

(a) MRI protocol at baseline assessment

(b) Summary of image analysis methods

(a) MRI protocol at baseline assessment

	MRA	Flow			quantitative T_1			DCE-MRI
Sequence	**TOF**	**2D PC (carotids)**	**2D PC (SACSF)**	**2D PC (sinus)**	**3D IR-sGRE (TI = 600 ms)**	**3D IR-sGRE (TI = 1500 ms)**	**3D sGRE (FA = 2°,5°, 12°)**	***T1*w 3D sGRE**
Voxel size (mm)	0.5x0.7x1.6	1.0x1.0x5.0	0.8x0.8x5.0	0.7x0.7x5.0	1.2x1.2x1.2	1.2x1.2x1.2	1.2x1.2x1.2	2x2x2
TR (ms)	20.0	19.60	25.18	21.70	1040	1940	5.4	3.44
TE (ms)	3.51	5.82	8.45	6.59	1.82	1.82	1.82	1.68
TI	-	-	-	-	600	1500	-	-
Flip Angle (°)	20	12	12	12	5	5	2, 5, 12	15
Acquisition Time (mm:ss)	2:45	1:39 approx.	1:55 approx.	2:11 approx.	1:55	3:35	1:36 x 3	21:08
Other		*R*=2 venc = 70 cm s^{-1} NA = 2	*R*=2 venc = 6 cm s^{-1}	*R*=2 venc = 50 cm s^{-1}	*R*=2	*R*=2	*R*=2	32 volumes

	CVR	T1w	FLAIR	PD	T2w	SWI	dMRI	ASL
Sequence	**2D GE-EPI**	**MPRAGE (3D IR-sGRE)**	**SPACE (3D RARE)**	**3D sGRE**	**SPACE (3D RARE)**	**3D sGRE**	**2D GE-EPI**	**3D pcASL**
Voxel size	2.5x2.5x2.5	1.0x1.0x1.0	1.0x1.0x1.0	1.0x1.0x1.0	0.9x0.9x0.9	0.6x0.6x3.0	2.0x2.0x2.0	3.4x3.5x3.5
TR	1550	2500	5000	6.04	3200	28	4300	4350
TE	30.0	4.37	388	2.44	408	20	74.0	20.98
TI	-	-	1100	1800	-	-	-	-
Flip Angle	67	7	-	2.0	-	9	-	-
Acquisition time	12:30	3:45	5:57	1:57	3:42	4.02	11:16	3:45
Other	*R*=2, *MB*=2	*R*=3	*R*=3	*R*=3	*R*=2x2	*R*=2	*R*=2, *MB*=2 15 × b = 0 s/mm2, 3 × b = 200 s/mm2, 6 × b =	*R*=2 *TI* = 500-

			TI=1800 ms				500 s/mm2, 64 × b = 1000 s/mm2, 64 × b = 2000 s/mm2 (3 x b0 acquired with reversed phase ecoding)	3030 (x12)

CVR =Cerebrovascular reactivity; MPRAGE =Magnetization-prepared rapid acquisition with gradient echo; FLAIR =Fluid-attenuated inversion recovery; PD VIBE =Proton density; SPACE =Sampling perfection with application-optimized contrast using different flip-angle evolution; SWI =Susceptibility weighted imaging; dMRI =Diffusion imaging; pcASL =Pseudo-continuous arterial spin labelling; TOF =Time-of-flight; PC =Phase-contrast; SACSF =Subarachnoid cerebrospinal fluid; IR =Inversion recovery; sGRE =Spoiled gradient recalled echo; TI =Inversion time; FA =Flip angle; DCE =Dynamic contrast-enhanced; TR =Repetition time; TE =Echo time; *R*=parallel imaging acceleration factor; *MB*=multiband acceleration factor; NA = number of averages.

(b) Summary of image analysis methods

Structural and Diffusion Imaging

The index, old and recurrent infarcts and SVD imaging markers (i.e. white matter hyperintensities (WMH), lacunes, perivascular spaces (PVS) and microbleeds are assessed by an expert neuroradiologist using validated visual scores (1-4), and recorded in standard assessment templates (5, 6) as described previously.(7)

All images are converted from DICOM to NIFTI-1 format using dcm2niix (https://github.com/rordenlab/dcm2niix). For each patient, structural tissue/lesion segmentation is performed in the native space of the T2-weighted image acquired at visit 1. Therefore, we linearly align all structural sequences from all visits to this image space using FSL-FLIRT (8). The structural processing pipeline is fully automatic and combines the output from state-of-the-art neuroimaging processing tools: FSL-FAST (9), freesurfer (https://surfer.nmr.mgh.harvard.edu/), LOTS-IM (10) and multispectral Gaussian clustering optimised using an Expectation-Maximisation algorithm (11) to output the volumes, probabilistic and binary masks of: 1) venous sinuses, meninges and main venous pathways, 2) cerebrospinal fluid, 3) intense and less-intense WMH, 4) normal-appearing white matter 5) deep grey matter structures, 6) cortical grey matter, 7) stroke lesions and 8) lacunes, total and per cerebral and cerebellar hemisphere. PVS are segmented in the native T2W space in the basal ganglia and centrum semiovale regions for each visit as described previously (2, 12), both segmented fully automatically using the output from the main structural pipeline. Venous pathways and mineral deposition are segmented in the native SWI space using the minimum intensity projection, phase and magnitude images combined with the T1w sequence.(13, 14)

Diffusion data are processed using TractoR version 3.3.(15) DICOM data are converted to NIfTI-1 format using 'divest' (16), corrected for susceptibility and eddy current induced distortions using topup and eddy from FSL version 6.0.1 (17-19), and the brain is masked using FSL's brain extraction tool. The water self-diffusion tensor is calculated for each brain voxel, and parametric maps of fractional anisotropy (FA) and mean diffusivity (MD) are derived from its eigenvalues with TractoR's 'tensorfit' using an iterative weighted least-squares approach.(20) NODDI parameters (intracellular volume fraction (ICVF), isotropic volume fraction (ISOVF) and orientation dispersion index (ODI)) will be determined from the registered multi-shell diffusion MRI data using the NODDI Matlab toolbox (http://mig.cs.ucl.ac.uk/).

Advanced imaging measures of vessel function

Blood brain barrier permeability

Full details of the DCE-MRI acquisition protocol are available to download at the Harmonizing Brain Imaging Method for Vascular Contributions to Neurodegeneration (HARNESS) website (21): https://harness-neuroimaging.org

In summary, we derive values for the blood plasma volume fraction (v_P) and the capillary permeability-surface area product *PS* for each voxel and region as described in (22).

Cerebrovascular flow, perfusion, and reactivity

The multi-inversion time pseudo-continuous arterial spin labelling data is processed through FSL's BASIL using a 1-compartment model and partial volume correction to obtain cerebral blood flow and arterial transit time (12 equally spaced TIs=500-3030ms, TR/TE=4350/20.98ms with 4 background suppression pulses, bolus duration=1800ms).(23) Further analysis is performed using white and subcortical grey matter regions of interest.

We acquire four phase-contrast scans following manual placement of a 2D slice perpendicular to the following vessels before manual segmentation: internal carotid and vertebral arteries, internal jugular veins, venous sinuses (superior sagittal, straight, and transverse sinuses), subarachnoid CSF at the level of C2-C3 and aqueduct. We manually segment vessel regions of interest (ROIs) using FSLeyes before processing phase-contrast MRI data, using in-house MATLAB code to obtain flow measurements.

We extract velocity on a pixel-by-pixel basis and calculate the blood/CSF flow for each vessel/space, performing aliasing and background corrections where required. We calculate flow across the cardiac cycle for each vessel and estimate the pulsatility index (PI = (max flow - min flow) / mean flow). We also extract the pulse waveform delay between the carotids and other intracranial vessels.(24)

During the 12 minute cerebrovascular reactivity (CVR) paradigm using a Blood Oxygenation Level Dependent (BOLD) MRI, we record end-tidal CO2 (ETCO2) while participants alternately inhale medical air (2 minutes) and air containing 6% carbon dioxide (3 minutes). Linear regression with a variable CVR delay is used to extract measurements of cerebrovascular reactivity (% change in BOLD signal per mmHg change in ETCO2) and the delay value in white and grey matter.(25)

Retinal imaging

All retinal images are acquired with a SPECTRALIS imaging platform (Heidelberg Engineering, Heidelberg, Germany) that combines fundus imaging with a scanning laser ophthalmoscope and simultaneous optical coherence tomography (OCT) imaging. The camera employs spectral domain OCT which achieves micrometre resolution with very fast scanning times. The beam of a super luminescence diode scans across the retina to produce cross-sectional images, with an infrared wavelength of 870nm.

The retinal imaging protocol builds on systems successfully established during the Mild Stroke Study 1 at our centre.(26, 27) Both eyes are imaged during a 25minute protocol that includes: horizontal and vertical single line scans through the macula, enhanced depth imaging (EDI) to permit enhanced visualisation and subsequent measurement of the sub-foveal choroidal thickness, posterior pole multi-line imaging that captures 61 individual slices inferiorly to superiorly across the retina, circular optic nerve head scan for vascular assessment of main vessels and peripapillary retinal nerve fibre layer (RNFL) thickness measurements, and multicolour imaging via three colour wavelengths for an assessment of maculopathy and retinopathy. Additionally, we conduct optical coherence tomography angiography (OCTA) to assess the microvasculature, including vessel area density of the superficial vascular complex which supplies the RNFL and the ganglion cell layer.

The retinal images are used as input to two themes of analysis: vascular and neuroretinal. Fundus images are processed with the Vascular Assessment and Measurement Platform for Images of the REtina (VAMPIRE; Web version, Universities of Edinburgh and Dundee: vampire.computing.dundee.ac.uk), a validated software application for semi-automatic quantification of retinal vessel properties. RNFL segmentation is undertaken using the manufacturer's software. Vessel density of the small vessels discerned by OCTA is undertaken with bespoke image analysis software. We also administer a short ocular health questionnaire, assess visual acuity and measure eye axial length.

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Conflicting Interests

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Approvals

Ethical approval for this study was obtained from South East Scotland Research Ethics Committee (Ref 18/SS/0044) on 31/05/2018. NHS Lothian Research & Development approved this study on 31/05/2018 (Ref 2018/0084)

Informed consent

Written informed consent is obtained from all subjects before the study

Trial registration

ISRCTN 12113543

Guarantor

JMW

Authors' contributorship

UC: recruitment, data collection/management, study design, study coordination. DJG, WH: data collection/management, study coordination. IM, MB, MJT, MS, GB, SMM, ES, CM, AGM, IH: advanced neuroimaging techniques advice/design. TM, SW, KH, CH: retinal imaging techniques advice/design, data collection. MCVH, LB, MS, MJT: image analysis techniques advice/design. OH: cognitive test protocol advice, data collection. RB, EB: laboratory processing advice. DJ: data management. CA: advice regarding study design. FC: advice/study design, data management, statistical analysis plan. FD: recruitment, funding, study design, supervision, clinical oversight. JMW: conception, funding, ethics and regulatory approvals, study design, data collection, all supervision and governance, drafting and final editing of text. All authors also prepared, revised and approved the final manuscript.

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