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### **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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**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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<p>Keywords:</p>	<p>Cerebral small vessel diseases, Lacunar stroke, white matter hyperintensities, Magnetic Resonance Imaging, Longitudinal studies, Dementia, Cognitive dysfunction, Symptom assessment, Blood-brain barrier, Cerebrovascular circulation</p>
<p>Abstract:</p>	<p><b>Background</b> 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.</p> <p><b>Study Methods/Design</b> 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.</p> <p><b>Discussion</b> 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.</p>

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Manuscripts

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4 Rationale and design of a longitudinal study of cerebral small vessel diseases,  
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7 clinical, and imaging outcomes in patients presenting with mild ischaemic  
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11 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

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 (~~structural, white and grey matter integrity~~) 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.

## 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;

1  
2  
3 n=10/30),(21, 22) while apparently 'silent' acute DWI lesions have been noted in up to 25%  
4  
5 following recent stroke, mostly in small studies (e.g. n<105) that sought typical stroke  
6  
7 symptoms.(16, 23) Thus, knowledge of the extent of clinical correlates of SVD lesions, in  
8  
9 particular, any 'red flag' symptoms or signs that might highlight lesion worsening, remains  
10  
11 limited and may be being clinically overlooked.  
12  
13  
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17

18 SVD is commonly attributed to traditional vascular risk factors, particularly hypertension,  
19  
20 but also smoking and diabetes, yet these factors only account for 2% of WMH variance.(24)  
21  
22 Less is known about potential contributors such as diet, lifestyle and premorbid factors.(25)  
23  
24 Extending the search beyond an individual's current clinical status to early and mid-life  
25  
26 stages is an important target for SVD research.(26) Understanding whether combined risk  
27  
28 factors have a synergistic effect on an individual's risk of developing SVD, as well as  
29  
30 improved recognition of symptoms, would provide better recognition of persons at risk of  
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32 SVD development or progression, providing insight on whether multimodal approaches to  
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34 prevention and treatment should be taken.  
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42 Few studies have comprehensively assessed SVD lesion progression, symptomatology and  
43  
44 wide-ranging risk factors. Hence we describe the protocol for a detailed study to assess the  
45  
46 role of cerebrovascular dysfunctions in combination, symptoms, risk factors including diet,  
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48 sleep, and early life factors, on longitudinal SVD lesion change in patients presenting with  
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50 stroke-related SVD.  
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## 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)  $\leq 2$  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

1  
2  
3 diagnostic MRI or CT scan compatible with the clinical syndrome, or if no visible infarct, no  
4  
5 other lesion explaining the stroke symptoms. Participants with non-lacunar stroke form  
6  
7 controls, since they have similar vascular risk factors and follow similar secondary  
8  
9 prevention, accounting for medication effects on vessel function.  
10  
11

12  
13 We exclude participants with MRI contraindications, major neurological conditions, severe  
14  
15 cardiac and respiratory disease. All participants give written informed consent. The study  
16  
17 was granted ethical approval by Southeast Scotland Regional Ethics Committee (reference  
18  
19 18/SS/0044).  
20  
21  
22

## 23 24 25 26 **Diagnosis**

27  
28 An expert panel of stroke physicians and neuroradiologists reach final stroke diagnosis by  
29  
30 consensus following review of presenting symptoms and signs including motor or sensory  
31  
32 deficit, hemianopia, visuospatial disorder, ataxia, dysphasia, dysarthria, cerebellar or  
33  
34 brainstem symptoms, supplemented by diagnostic brain MRI or CT and other relevant  
35  
36 investigations, as previously.(27, 28) An experienced neuroradiologist (J.M.W.) assesses all  
37  
38 scans for acute ischaemic lesions including recent small subcortical infarcts, prior infarcts or  
39  
40 haemorrhages, WMH, lacunes, PVS, microbleeds, siderosis, atrophy, using standardized  
41  
42 validated scales.(2, 28)  
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50  
51 **Table 1: Study assessments**

52 53 <b>Activity/assessment</b>	54 55 <b>Pre-visit</b>	56 57 <b>V1<sup>a</sup> baseline<sup>a</sup></b>	58 59 <b>V2<sup>b</sup>: 2-3 months*</b>	60 <b>V3<sup>c</sup>: 6 months</b>	<b>V4<sup>d</sup>: 12 months</b>
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

<sup>a</sup>Visit 1: baseline visit within 3 months of index stroke +/- 1 week

<sup>b</sup>Visit 2: ~~2-3 months~~ +/- 1 week

<sup>c</sup>Visit 3: ~~6 months~~ +/- 2 weeks

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2  
3 <sup>d</sup>Visit 4: ~~12 months~~ +/- 3weeks  
4  
5  
6  
7

8 MRI=Magnetic Resonance Imaging, NIHSS=National Institute of Health Stroke Scale,  
9

10 IQCODE=Informant Questionnaire for Cognitive Decline in the Elderly, NPI-Q=Neuropsychiatric  
11

12 Inventory Questionnaire, AES-I=Apathy Evaluation Scale-Informant  
13  
14  
15  
16

### 17 ***Vascular risk factors, past medical history, medications, incident vascular events***

18  
19 Each participant provides a medical history of diagnoses confirmed by a physician,  
20  
21 supplemented by hospital medical records and general practitioner correspondence,  
22  
23 following standard definitions, including diabetes mellitus, hypertension,  
24  
25 hypercholesterolaemia, previous stroke or TIA, peripheral vascular disease, atrial fibrillation,  
26  
27 ischaemic heart disease, valvular defects, heart failure and physician-diagnosed anxiety,  
28  
29 depression or delirium. We record current medications, cross-checking with electronic  
30  
31 medical records. At follow-up we record recurrent stroke, TIA and cardiac events.  
32  
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### 39 ***Subjective symptoms***

40  
41 We use a structured questionnaire to ask open-ended questions about subjective symptoms  
42  
43 experienced prior to, at the time of, and since index stroke diagnosis (see Supplementary  
44  
45 Appendix 1). Participants also answer questions based on previous clinico-radiological  
46  
47 studies regarding symptoms within the past month including subjective memory concerns,  
48  
49 confusional episodes, unsteadiness, falls, dizziness and headaches.(29)  
50  
51

52  
53 Participants self-administer the Fatigue Severity Scale,(30) Generalized Anxiety Disorder-  
54  
55 7,(31) the Center for Epidemiologic Studies-Depression Scale,(32) and an adapted Pittsburgh  
56  
57 Sleep Quality Index.(33)  
58  
59  
60

### ***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

1  
2  
3 (NINDS-CSN) Vascular Cognitive Impairment Harmonization Standards. This battery spans  
4  
5 multiple cognitive domains and includes the Montreal Cognitive Assessment (MoCA),  
6  
7 Hopkins Verbal Learning Test-revised, Controlled Oral Word Association Test, Animal  
8  
9 Naming, Letter Digit Coding, and Trailmaking Tests A+B.  
10  
11

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

13  
14 Participants repeat MoCA and Trailmaking Tests A+B at each visit. We use three different  
15  
16 MoCA versions, randomly assigning a test sequence to each participant to minimize learning  
17  
18 effects on serial test performance.  
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### 28 ***Functional recovery***

29  
30 We administer the modified Rankin Scale(41) at baseline, 6 and 12 months and the Stroke  
31  
32 Impact Scale(42) at 6 and 12 months.  
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### 37 ***Magnetic Resonance Imaging***

38  
39 We scan all participants at diagnosis at 1.5T (General Electric Signa HDxt) or 3T (Siemens  
40  
41 Prisma) MRI or CT with core structural brain MRI sequences at each visit: 3D T1w, T2w, Fluid  
42  
43 Attenuated Inversion Recovery (FLAIR), Susceptibility-weighted (SWI/SWAN/GRE), and  
44  
45 single- or multi-shell diffusion imaging (dMRI). Subsequent full cerebrovascular assessment  
46  
47 and all follow-up imaging is at 3T.  
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51  
52 At 1-3 months post-stroke, participants undergo 3T MRI to measure blood-brain barrier  
53  
54 (BBB) integrity, CVR, cerebral blood flow (CBF) and intracranial vascular and CSF pulsatility  
55  
56 (protocol in Supplementary Appendix 2). We assess BBB integrity using dynamic contrast-  
57  
58 enhanced (DCE-) MRI and gadolinium-based contrast agent (gadobutrol) injection,(11, 43)  
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3 unless eGFR<30ml/min. We assess CVR using a Blood Oxygenation Level Dependent (BOLD)  
4 MRI sequence, during which participants inhale air with intermittent added CO<sub>2</sub> (12-minute  
5 paradigm alternating 2 minutes air and 3 minutes 6% CO<sub>2</sub>) through a tight-fitting facemask,  
6 described previously.(13, 44) Arterial, venous and CSF pulsatility are measured using phase  
7 contrast MRI sequences.(14, 44) We measure CBF using major arterial phase contrast flow  
8 measures obtained during pulsatility measurements (and arterial spin labelling where  
9 feasible).

10  
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12  
13 We process MRI computationally using well-validated methods to assess intracranial  
14 volume, brain, CSF, normal-appearing white and grey matter, WMH volumes, index and  
15 prior stroke lesion volumes, lacunes, microbleeds and perivascular space metrics.(45, 46)  
16 We visually quantify index and prior stroke lesions (location, type), WMH (baseline, change),  
17 lacunes (number, location), perivascular spaces, microbleeds, siderosis, superficial and deep  
18 brain volume loss, according to STRIVE criteria using validated scales.(2, 47-51) See  
19 Supplementary Appendix 2 for image processing methods description including advanced  
20 neuroimaging data.

### 21 22 23 **Retinal imaging**

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

11  
12 We record BP at three standard points during the baseline visit and measure arterial  
13  
14 velocities through pulse wave velocity and pulse wave analysis using a tonometric device  
15  
16 (*SphygmoCor*®, AtCor Medical/Vicorder, Skidmore Medical) held over the carotid and  
17  
18 radial pulses while supine. We provide a 24-hour ambulatory BP monitoring device  
19  
20 (SpaceLabs Medical) to most and encourage all to submit self-monitored blood pressure  
21  
22 recordings. We repeat BP measurements at all study visits.  
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### 32 ***Biochemical, haematological, cardiovascular, imaging investigations***

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34 We document routinely collected index stroke investigation results including serum  
35  
36 haematology and biochemistry, electrocardiogram, echocardiography and carotid Doppler  
37  
38 ultrasound. We collect 18ml venous blood at baseline for inflammatory and endothelial  
39  
40 function markers. We store 20ml urine for inflammatory marker analysis and 5ml for  
41  
42 albumin-to-creatinine ratio.  
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### 50 **Endpoints**

51  
52 The primary endpoint is the proportion of SVD lesions that regress, progress or appear de  
53  
54 novo in the year after stroke. The secondary endpoints are: (a) blood-brain barrier (BBB)  
55  
56 integrity; (b) cerebrovascular reactivity (c) intracranial vascular/CSF pulsatility; (d) WMH, PVS,  
57  
58 lacunes and microbleeds; (e) white matter structural integrity measured with diffusion tensor  
59  
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3 and  $T_1$  parameters; and (f) incidence of reported symptoms including neuropsychiatric and  
4  
5 cognitive symptoms, recurrent stroke, transient ischaemic attacks and cardiac events.  
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## 15 **Statistical analysis**

### 16 ***Sample size calculation***

17  
18 In a previous study with one year of longitudinal imaging follow-up at this centre, 10.6% of  
19  
20 participants had a de novo lesion on MRI at follow-up.(28) In the same study, 65% had a mean  
21  
22 5.5ml WMH volume increase and 35% had a mean 6.6ml decrease.(54) A sample of 250  
23  
24 participants would be required to detect WMH change in the year after stroke, with  
25  
26 significance 0.05 and power 0.90 in univariate analysis. We aim to recruit up to total 300  
27  
28 participants which allows for loss to follow-up.(54)  
29  
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### 35 ***Proposed analyses***

36  
37 In our primary analyses, we will use linear mixed effects models adjusted for age, vascular risk  
38  
39 factors and baseline SVD burden to estimate the effect of cerebrovascular structure/function  
40  
41 on SVD lesion progression and regression.  
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46 Secondly, we will use similar models and other approaches (e.g. stratifying by low vs. high  
47  
48 SVD burden, stroke subtype) to quantify associations of the following factors with lesion  
49  
50 change: cognitive test scores and incident cognitive impairment; functional status; life course  
51  
52 factors; lifestyle factors; blood pressure; systemically measured vascular stiffness; retinal  
53  
54 measures; and inflammatory and endothelial function markers.  
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## 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

1  
2  
3 insight into novel preventative and therapeutic targets by uncovering the nature of and  
4  
5 factors associated with lesion regression. This study will deepen our understanding of SVD,  
6  
7 essential to future prevention and treatment of stroke and vascular dementia.  
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9

10 To date we have recruited 105 participants. The baseline visit lasts 6.5 hours and most are  
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12 willing to attend three further visits, each lasting two hours, with positive participant  
13  
14 feedback, demonstrating the feasibility of applying this design at other centres.  
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### 20 **Conflicting Interests**

21  
22 UC, MS, MJT, GB, AM, OH, CM, FND and JMW hold academic grants from government and  
23  
24 charitable funding agencies, outlined below.  
25

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33  
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39  
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43  
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47  
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49  
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55  
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59  
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Research Fund, and the University of Edinburgh.

### 61 **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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For Peer Review



Mild Stroke Study 3: Initial Data Collection Form

MSS-3 Study Number \_\_\_\_\_

**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

Mild Stroke Study 3: Initial Data Collection Form

MSS-3 Study Number \_\_\_\_\_

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

Mild Stroke Study 3: Initial Data Collection FormMSS-3 Study Number \_\_\_\_\_

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 /

Mild Stroke Study 3: Initial Data Collection Form

MSS-3 Study Number \_\_\_\_\_

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

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3 **Title:** Studies of small vessel diseases: the Mild Stroke Study 3 (MSS-3). The  
4 longitudinal study of cerebral small vessel diseases following mild ischaemic stroke:  
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6 rationale and design.  
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10 **Supplement 2:**

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13 (a) MRI protocol at baseline assessment  
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16 (b) Summary of image analysis methods  
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For Peer Review

## (a) MRI protocol at baseline assessment

	MRA	Flow			quantitative $T_1$			DCE-MRI
<b>Sequence</b>	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°)	T1w 3D sGRE
<b>Voxel size (mm)</b>	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
<b>TR (ms)</b>	20.0	19.60	25.18	21.70	1040	1940	5.4	3.44
<b>TE (ms)</b>	3.51	5.82	8.45	6.59	1.82	1.82	1.82	1.68
<b>TI</b>	-	-	-	-	600	1500	-	-
<b>Flip Angle (°)</b>	20	12	12	12	5	5	2, 5, 12	15
<b>Acquisition Time (mm:ss)</b>	2:45	1:39 approx.	1:55 approx.	2:11 approx.	1:55	3:35	1:36 x 3	21:08
<b>Other</b>		R=2 venc = 70 cm s <sup>-1</sup> NA = 2	R=2 venc = 6 cm s <sup>-1</sup>	R=2 venc = 50 cm s <sup>-1</sup>	R=2	R=2	R=2	32 volumes

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

			$TI=1800$ ms				500 s/mm <sup>2</sup> , 64 × b = 1000 s/mm <sup>2</sup> , 64 × b = 2000 s/mm <sup>2</sup> (3 × b <sub>0</sub> acquired with reversed phase encoding)	3030 (x12)
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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; SACS =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

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2  
3 (<https://surfer.nmr.mgh.harvard.edu/>), LOTS-IM (10) and multispectral Gaussian clustering  
4  
5 optimised using an Expectation-Maximisation algorithm (11) to output the volumes,  
6  
7 probabilistic and binary masks of: 1) venous sinuses, meninges and main venous pathways,  
8  
9 2) cerebrospinal fluid, 3) intense and less-intense WMH, 4) normal-appearing white matter  
10  
11 5) deep grey matter structures, 6) cortical grey matter, 7) stroke lesions and 8) lacunes, total  
12  
13 and per cerebral and cerebellar hemisphere. PVS are segmented in the native T2W space in  
14  
15 the basal ganglia and centrum semiovale regions for each visit as described previously (2,  
16  
17 12), both segmented fully automatically using the output from the main structural pipeline.  
18  
19 Venous pathways and mineral deposition are segmented in the native SWI space using the  
20  
21 minimum intensity projection, phase and magnitude images combined with the T1w  
22  
23 sequence.(13, 14)  
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32 Diffusion data are processed using TractoR version 3.3.(15) DICOM data are converted to  
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34 NIfTI-1 format using 'divest' (16), corrected for susceptibility and eddy current induced  
35  
36 distortions using topup and eddy from FSL version 6.0.1 (17-19), and the brain is masked  
37  
38 using FSL's brain extraction tool. The water self-diffusion tensor is calculated for each brain  
39  
40 voxel, and parametric maps of fractional anisotropy (FA) and mean diffusivity (MD) are  
41  
42 derived from its eigenvalues with TractoR's 'tensorfit' using an iterative weighted least-  
43  
44 squares approach.(20) NODDI parameters (intracellular volume fraction (ICVF), isotropic  
45  
46 volume fraction (ISOVF) and orientation dispersion index (ODI)) will be determined from the  
47  
48 registered multi-shell diffusion MRI data using the NODDI Matlab toolbox  
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51 (<http://mig.cs.ucl.ac.uk/>).  
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## 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 (HARNES) 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 FSLeves 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

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2  
3 flow - min flow) / mean flow). We also extract the pulse waveform delay between the  
4  
5 carotids and other intracranial vessels.(24)  
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10 During the 12 minute cerebrovascular reactivity (CVR) paradigm using a Blood Oxygenation  
11 Level Dependent (BOLD) MRI, we record end-tidal CO<sub>2</sub> (ETCO<sub>2</sub>) while participants  
12  
13 alternately inhale medical air (2 minutes) and air containing 6% carbon dioxide (3 minutes).  
14  
15 Linear regression with a variable CVR delay is used to extract measurements of  
16  
17 cerebrovascular reactivity (% change in BOLD signal per mmHg change in ETCO<sub>2</sub>) and the  
18  
19 delay value in white and grey matter.(25)  
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### 28 **Retinal imaging**

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30 All retinal images are acquired with a SPECTRALIS imaging platform (Heidelberg Engineering,  
31 Heidelberg, Germany) that combines fundus imaging with a scanning laser ophthalmoscope  
32  
33 and simultaneous optical coherence tomography (OCT) imaging. The camera employs  
34  
35 spectral domain OCT which achieves micrometre resolution with very fast scanning times.  
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37 The beam of a super luminescence diode scans across the retina to produce cross-sectional  
38  
39 images, with an infrared wavelength of 870nm.  
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46 The retinal imaging protocol builds on systems successfully established during the Mild  
47  
48 Stroke Study 1 at our centre.(26, 27) Both eyes are imaged during a 25minute protocol that  
49  
50 includes: horizontal and vertical single line scans through the macula, enhanced depth  
51  
52 imaging (EDI) to permit enhanced visualisation and subsequent measurement of the sub-  
53  
54 foveal choroidal thickness, posterior pole multi-line imaging that captures 61 individual  
55  
56 slices inferiorly to superiorly across the retina, circular optic nerve head scan for vascular  
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3 assessment of main vessels and peripapillary retinal nerve fibre layer (RNFL) thickness  
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5 measurements, and multicolour imaging via three colour wavelengths for an assessment of  
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7 maculopathy and retinopathy. Additionally, we conduct optical coherence tomography  
8  
9 angiography (OCTA) to assess the microvasculature, including vessel area density of the  
10  
11 superficial vascular complex which supplies the RNFL and the ganglion cell layer.  
12  
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15  
16 The retinal images are used as input to two themes of analysis: vascular and neuroretinal.

17  
18 Fundus images are processed with the Vascular Assessment and Measurement Platform for  
19  
20 Images of the REtina (VAMPIRE; Web version, Universities of Edinburgh and Dundee:  
21  
22 [vampire.computing.dundee.ac.uk](http://vampire.computing.dundee.ac.uk)), a validated software application for semi-automatic  
23  
24 quantification of retinal vessel properties. RNFL segmentation is undertaken using the  
25  
26 manufacturer's software. Vessel density of the small vessels discerned by OCTA is  
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28 undertaken with bespoke image analysis software. We also administer a short ocular health  
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30 questionnaire, assess visual acuity and measure eye axial length.  
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For Peer Review

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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.

Guarantor: Prof Joanna Wardlaw

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