THE ROLE OF GLYCERYL TRINITRATE, A NITRIC OXIDE DONOR, IN ACUTE STROKE

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ABSTRACT

Nitric oxide donors (e.g. glyceryl trinitrate (GTN)) are candidate treatments for acute stroke with haemodynamic, reperfusion, and neuroprotective properties. In order to establish the safety and efficacy of NO donors in acute stroke a Cochrane systematic review and meta-analysis was performed. Transdermal GTN was assessed in 5 trials (n=4197) in acute stroke. Overall, GTN was safe and lowered blood pressure but did not influence clinical outcome. However, early treatment with GTN within 6 hours (n=312) improved clinical outcomes across a variety of domains (death and dependency, death, disability, cognition, mood, and quality of life).

Using data from the large Efficacy of Nitric Oxide in Stroke (ENOS) trial, the haemodynamic properties of GTN were explored. GTN lowered BP and its derivatives including BP variability. Further, increased BP variability was associated with poor functional and cognitive outcomes and increased death at day 90.

The safety and efficacy of transdermal GTN in important subgroups in acute stroke was assessed. GTN was safe in the context of blood markers of dehydration with no precipitous drops in BP seen in such patients. GTN in the context of ipsilateral or bilateral carotid stenosis was safe and may improve outcome in severe ipsilateral carotid stenosis. Although GTN was safe, it did not improve outcome in patients with lacunar syndromes either overall or within 6 hours of onset. Baseline imaging markers of

small vessel disease and 'brain frailty' were associated with functional and cognitive outcomes 90 days after stroke.

In summary, this thesis has confirmed the safety of transdermal GTN in acute stroke both overall and in important subgroups. Mechanistic data suggest that GTN may reduce BP variability, which seems to be more strongly associated with outcome than absolute BP or trend in BP. Transdermal GTN is safe to be administered in acute stroke patients with elevated BP prior to blood markers of dehydration or carotid stenosis status being known. Baseline imaging markers of SVD and 'brain frailty' predict clinical outcome and should be used as minimisation criteria in future acute stroke trials and may help guide future clinical decision-making.

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CONTRIBUTORSHIP

The work presented in this thesis was performed by the author with supervision, advice and guidance from Prof. Philip Bath and Prof. Nikola Sprigg from the University of Nottingham. The roles of other contributors are listed by chapter below.

Chapter 2: The author performed the searches as detailed in the methods and appendices, performed data extraction of individual patient data for meta-analysis, performed meta-analysis and updated the text. Dr. Kailash Krishnan checked data and analyses for accuracy. Prof. Philip Bath is guarantor for this Cochrane systematic review and meta-analysis.

Chapter 3: The author collated, analysed and interpreted the data, wrote the chapter and has presented the work nationally. Prof. Peter Rothwell (University of Oxford) provided critical review of the resulting manuscript along with members of the ENOS trial steering committee.

Chapter 4: Dr. Charlotte Billington collected the blood sample data for these analyses. The author collated, analysed and interpreted the data, wrote the chapter and has presented the work nationally and internationally. The author and Dr. Charlotte Billington are joint first authors on the resulting manuscript.

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The author confirms that all work in this thesis is original and their own work, unless otherwise stated.

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Publications contributing to this chapter:

Appleton JP, Sprigg N, Bath PM. Blood pressure management in acute stroke. Stroke and vascular neurology 2016;1(2):72-82

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1.1 STROKE

1.1.1 Epidemiology

Stroke can be defined as a focal neurological deficit of sudden onset, which lasts for at least 24 hours or results in death, and is presumed to be of vascular origin.(1) This can be due to either blockage (ischaemic stroke (IS)), or rupture (intracerebral haemorrhage (ICH)), of an artery supplying the brain. Stroke has a global incidence of 15 million people per year, is the third leading cause of death and is the most common cause of disability in the western world.(2) Stroke accounts for 5% of the total UK NHS budget with societal costs estimated at £8.9 billion per year.(3) The incidence of this common, life-threatening condition is only going to increase as the population ages. In turn, the associated costs and impact upon society are predicted to increase. This has led to calls for both investment and research devoted to the management of stroke.(3, 4)

1.1.2 Stroke type

Ischaemic stroke is the commonest type of stroke accounting for 85% of all strokes, whilst ICH accounts for around 15-20%.(5)

1.1.2.1 Ischaemic Stroke (IS)

IS occurs when blood flow to the brain is disturbed and brain tissue undergoes ischaemia. If this reduction in blood flow is prolonged, then death of brain cells can occur leading to permanent damage (infarction) and resultant neurological impairment. If, however, blood flow is restored quickly, the brain may recover producing the clinical syndrome of transient ischaemic attack (TIA).

IS can be caused be a number of processes, which can be classified using Trial of ORG 10172 in Acute Stroke Treatment (TOAST) subtyping:(6)

- Cardioembolic: arrhythmias (e.g. atrial fibrillation), left ventricular hypokinesis, and valvular disorders can result in cardioembolic stroke.
- Large artery disease (50%): atherosclerosis of the major arteries supplying the brain can lead to infarction by either reducing blood flow distal to the area of stenosis or as a result of distal embolisation of atherosclerotic material.
- Small vessel disease (25%): damage to the small blood vessels of the brain leads to injury of the brain tissue they supply, which can result in infarction. These processes manifest as cerebral small vessel disease (SVD), which is the underlying cause of the majority (90%) of lacunar ischaemic strokes.(7)

- Unclear aetiology: unknown cause.

1.1.2.2 Intracerebral Haemorrhage (ICH)

ICH is bleeding into the brain tissue, which occurs as a result of rupture of an artery within the brain. Causes of ICH can be categorised as follows:(8)

- Abnormalities of brain vasculature: cerebral amyloid angiopathy; arteriovenous malformations; dural arteriovenous fistulae.
- Haemostatic abnormalities: ICH resulting from clotting or platelet abnormalities including medication such as anticoagulation or thrombolysis.
- Hypertension: chronic high blood pressure (BP) is associated with ICH in known sites including the basal ganglia, pons and sub-cortical white matter.
- Small vessel disease: as outlined in the IS section above, damaged small blood vessels can also result in both micro- and macro-haemorrhages.
- Tumour-related haemorrhage: primary or secondary tumours.

1.1.3 Pathophysiology

1.1.3.1 Ischaemic Stroke (IS)

Cardioembolic

Distant thrombus from the heart can travel through the arterial tree, occluding a cerebral artery resulting in a cardioembolic IS. The most common cause is arrhythmias, of which atrial fibrillation is the most prevalent cause of stroke. Turbulent blood flow results from irregular contraction of the atria, which can cause a thrombus to form with resultant embolisation to the brain. Alternative causes include left ventricular systolic dysfunction, left ventricular thrombus following myocardial infarction, dilated cardiomyopathy, valvular disorders including mechanical prosthetic valves, atrial myxoma and infective endocarditis.(6)

Large artery disease

Atherosclerosis is a chronic inflammatory process in which plaques form in the walls of arteries and is the major cause of large artery strokes. Atherosclerotic plaques are composed of cholesterol, inflammatory cell infiltrate and calcifications. Narrowing or stenosis of the carotid arteries through atherosclerosis, particularly where the common carotid artery bifurcates into the external and internal carotid arteries, is an important risk factor for both first and recurrent stroke.(9) Severe stenosis can result in reduced blood flow to the brain distal to the site of stenosis and/or rupture of the atherosclerotic plaque with embolisation of atherosclerotic material resulting in ischaemic stroke. The nature of the atherosclerotic plaque itself – its composition, volume, and features such

as plaque haemorrhage – may influence its stability and therefore likelihood of plaque rupture, thrombosis and embolisation.(10, 11)

Traditional risk factors for atherosclerosis can be classified as follows:

- Modifiable risk factors:(12)
    - Smoking
    - Obesity
    - High blood pressure
    - Hypercholesterolaemia: high serum levels of low-density lipoprotein (LDL) and low serum levels of high-density lipoprotein (HDL) cholesterol
    - Diabetes mellitus
    - Hyperhomocysteinaemia: increased plasma homocysteine levels have been associated with atherosclerosis(13)
- Non-modifiable risk factors:(12)
    - Increasing age
    - Male sex
    - Family history / genetic conditions

Small vessel disease

SVD is characterised by injury to deep grey and white matter structures of the brain. Damage to the perforating arterioles and capillaries of the brain through inflammation(14) and breakdown of the blood brain barrier(15) leads to these blood vessels becoming stiff and less able to vasodilate when needed. Such diseased blood vessels can lead to damage to the brain tissue they supply. In addition, these vessels are

predisposed to thrombosis, which can result in infarction.(16) Such processes manifest as cerebral SVD, which is the underlying cause of the majority of lacunar ischaemic strokes. In addition to clinically evident lacunar ischaemic stroke, SVD can present as intracerebral haemorrhage, cognitive impairment or vascular dementia, late-onset depression, or gait or bladder dysfunction.(7)

1.1.3.2 Intracerebral Haemorrhage (ICH)

ICH can occur in the cerebral lobes, basal ganglia, thalamus, brainstem and cerebellum. Spread of blood into the ventricular system (intraventricular haemorrhage) typically occurs with large, deep haematomas. ICH was previously considered to be monophasic in nature with bleeding stopping as a result of clotting and tamponade by surrounding tissues. However, it is now clear that the haematoma expands over time. Haemodynamic instability and/or local clotting deficits are likely to have important roles in haematoma expansion.(8) The presence of blood initiates the process of fluid pooling around the haematoma (oedema), which can persist for between 5 and 14 days. Disruption of the blood-brain barrier and neuronal death follow, compounded by vasogenic and cytotoxic oedema, which lead to further neuronal injury.(8)

1.1.4 Diagnosis

Stroke is a clinical diagnosis made on the basis of history and examination. The Oxfordshire Community Stroke Project (OCSP) classification provides a clinically useful way to classify stroke syndromes based on an individual's signs and symptoms:(17)

- Total anterior circulation syndrome (TACS): higher cerebral dysfunction (dysphasia, visuospatial disorder); and homonymous visual field defect; and motor and/or sensory deficit affecting two areas of face, arm, and leg.
- Partial anterior circulation syndrome (PACS): two of the three components listed for TACS i.e. higher cerebral dysfunction, and homonymous visual field defect or motor and/or sensory deficit OR homonymous visual field defect and motor and/or sensory deficit.
- Lacunar syndrome (LACS): pure motor, pure sensory or sensorimotor features involving at least two regions of face, arm and leg; ataxic hemiparesis; with no visual field or higher cerebral features.
- Posterior circulation syndrome (POCS): visual disturbances including isolated visual field defect or cranial nerve palsies; incoordination; bilateral weakness; or swallowing problems.

A commonly used tool to assess stroke severity, in both research and clinical practice, is the National Institute of Health Stroke Scale (NIHSS), which ranges from 0 to 42, with higher scores indicating more severe neurological deficits.(18)

Imaging can aid the clinician to reach a stroke diagnosis, confirm the type of stroke, and establish the aetiology of the stroke including underlying disease processes. Computed tomography (CT) is used to assess patients presenting acutely with presumed stroke, and should be performed within 1 hour of admission according to recent guidelines.(4) The main focus is to rule out ICH in patients presenting within the time window for potential treatment with intravenous thrombolysis (see section 1.1.5). In IS, CT imaging is often normal within the first few hours of stroke onset. Signs of early ischaemia can be detected and include loss of grey-white matter differentiation, loss of the insular ribbon and blurring of the border of the lentiform nucleus. In addition, clot within an artery (typically the middle cerebral artery (MCA)) can be visualised as hyperdense. CT angiography uses intravenous contrast injection to visualise the arterial tree in more detail and is now recommended in patients where a proximal vessel occlusion is suspected, as a screening and diagnostic tool for potential clot removal termed endovascular therapy or thrombectomy.(19)

Magnetic resonance imaging (MRI) provides more detailed resolution of both anatomy and pathology in the context of stroke. A variety of MRI sequences are of interest in stroke and associated conditions:

- Diffusion weighted imaging is able to detect acute infarctions;
- T1, T2 and fluid-attenuated inversion recovery (FLAIR) sequences are sensitive to stroke evolution;
- Gradient echo (GRE) and susceptibility weight imaging (SWI) are sensitive to blood products;

- Time of flight (TOF) and contrast-enhanced magnetic resonance angiography (MRA) are useful for assessing vasculature.

Neuroimaging can also be helpful in detecting features of cerebral SVD, which include lacunar strokes, lacunes (lakes of cerebrospinal fluid), periventricular white matter disease, cerebral microbleeds, cerebral atrophy and perivascular spaces.(20) The incidence of SVD increases with age, such that some radiological features are present in 10% of people in their 7^{th} decade, rising to 90% of people in their 9^{th} decade.(21) However, the location of these features is important in determining the impact upon the individual; small lesions in primary motor or sensory pathways are more likely to be symptomatic compared with those in the frontal white matter, for instance.(22)

1.1.5 Management

1.1.5.1 Pre-hospital stroke management

Due to recent advances in acute stroke management and increased public awareness, stroke is gaining momentum as a medical emergency that demands rapid assessment and transport to a hospital with acute stroke services. The face-arm-speech-test (FAST) is used in the pre-hospital setting to aid the public and ambulance crews in recognising potential stroke patients.(23) At present, there are no specific treatments that can be administered in the ambulance setting prior to hospital admission for acute stroke care.

1.1.5.2 Hyperacute stroke management

The few evidence-based treatments for the management of acute stroke are here-in discussed.

Intravenous thrombolysis

Within 4.5 hours of IS onset, intravenous thrombolysis is recommended after ruling out ICH using imaging.(4) In an individual patient data meta-analysis under the auspices of the Stroke Thrombolysis Trialists' Collaborative Group, data from 9 randomised trials (n=6756) assessing alteplase compared with placebo/open control found that alteplase given within 4.5 hours of stroke onset significantly improved the odds of a good outcome, with earlier treatment being associated with more benefit (within 3 hours: odds ratio [OR] 1.75, 95% confidence interval [CI] 1.35-2.27; between 3 and 4.5 hours: OR 1.26, 95% CI 1.05-1.51).(24) Alteplase significantly increased the odds of symptomatic ICH and fatal

ICH within 7 days, an effect more likely to be seen in those with more severe strokes. There was an average absolute increased risk of early death from ICH of 2%, but by 3-6 months this risk was negated by an absolute increase in good functional outcome of 10% in those who received alteplase within 3 hours, and 5% in those treated between 3 and 4.5 hours.(24)

Endovascular therapy / thrombectomy

A proportion of patients presenting with acute IS have a blockage of a main cerebral artery, termed proximal vessel occlusion. In people with proximal vessel occlusions affecting the anterior cerebral circulation five randomised trials published in 2015 have shown that restoring blood flow to the brain through removal of the clot using stent retriever devices improves functional outcome.(19) An individual patient data meta-analysis of these five trials (n=1287) comparing endovascular therapy/thrombectomy versus standard medical therapy including individual thrombolysis found that endovascular thrombectomy was associated with significantly reduced disability at 90 days compared with control (OR 2.49, 95% CI 1.76-3.53).(19) The number of people needed to treat (NNT) with endovascular thrombectomy to reduce disability by at least one level of the modified Rankin Scale (mRS) in one person was 2.6. Like with thrombolysis, this treatment is time-dependent with earlier treatment being associated with improved functional outcome. Patients with acute IS should be considered for endovascular thrombectomy if they have confirmed proximal vessel occlusion on imaging (e.g. CT

angiography) causing a significant neurological deficit (NIHSS ≥6) and the procedure can start within 5 hours of symptom onset.(4)

Blood pressure management

Blood pressure management in acute stroke is discussed in detail in section 1.2.

1.1.5.3 Acute stroke management

Stroke Units

An organised stroke unit provides care for stroke patients who are exclusively managed by a dedicated multidisciplinary team on a ward dedicated to stroke patients, with a mobile stroke team or within a mixed rehabilitation ward. A Cochrane systematic review involving 5855 participants from 28 trials found that stroke unit care was consistently associated with improved clinical outcomes including death (OR 0.81, 95% CI 0.69-0.94), death or institutionalisation (OR 0.78, 95% CI 0.68-0.89), and death or dependency (OR 0.79, 95% CI 0.68-0.90) compared with non-stroke unit care.(25) Thus, all acute stroke patients should be admitted to a stroke unit.(4)

Aspirin

Antiplatelet therapy in the form of aspirin (orally or via nasogastric tube or rectally if swallowing impaired) given within 48 hours of onset of IS reduced the risk of early recurrent IS and death or dependency end of follow-up (OR 0.95, 95% CI 0.91-0.99) compared with control in 41483 participants in 8 trials. Aspirin was associated with a small excess of

symptomatic ICH, which was negated by the benefit seen in reducing recurrent IS.(26)

Decompressive hemicraniectomy

Life-threatening brain swelling or oedema following large infarctions affecting the MCA territory occur in 1-10% of patients with IS and are termed malignant MCA infarctions. Mortality associated with this complication is 80%. A pooled analysis of 3 trials of decompressive surgery (removal of a large piece of the skull to decompress the swollen brain (hemicraniectomy)) versus control (n=93) found that surgery within 48 hours of stroke onset was associated with less death (NNT of 2 for survival) and increased the proportion of people with a favourable functional outcome (mRS ≤3).(27)

Intermittent pneumatic compression

Deep vein thrombosis (DVT) and pulmonary embolism (PE) are frequent complications of strokes causing immobility. Intermittent pneumatic compression (IPC) using a device which sequentially inflates cuffs around the legs to promote venous flow in immobile stroke patients for 30 days after stroke reduced the occurrence of proximal DVT and improved survival, but did not positively influence functional outcome.(28) As such, IPC is recommended in immobile patients after stroke and should be started within three days of admission and continued for 30 days or until the patients is mobile or discharged, if sooner.(4)

In acute IS, intravenous thrombolysis,(24) thrombectomy(19) and decompressive hemicraniectomy(27) each have high efficacy but low utility (a relatively small proportion of stroke patients are eligible for each treatment). In contrast, aspirin has high utility but low efficacy.(26) Managing patients with all stroke types in stroke units has very high utility with medium-level efficacy.(25)

Evidence-based treatments for ICH in the hyperacute and acute setting are lacking, although intensive blood pressure lowering is recommended within 6 hours of symptom onset.(4)

1.1.5.4 Rehabilitation

Rehabilitation involves a broad range of healthcare professionals working in a multidisciplinary team: physiotherapists, occupational therapists, speech and language therapists, psychologists, dieticians, specialist nurses and rehabilitation physicians. The majority of impairment and restriction of activities of daily living are present at stroke onset. Some can recover completely over a short timeframe, whilst others persist for weeks, months and years. Rehabilitation can occur in a number of settings including the acute stroke unit, stroke rehabilitation units, early supported discharge teams and other community-based rehabilitation services. Appropriately resourced early supported discharge teams have been shown to reduce long-term death or dependency in patients with mild to moderate disability (OR 0.80, 95% CI 0.67-1.0) and shorten hospital length of stay.(29) Delivery of rehabilitation tends to be focused

in the first months after stroke and unfortunately insufficiently manages the long-term and evolving needs of people affected by stroke.(4)

1.1.5.5 Secondary prevention

Lifestyle interventions

Reducing weight, high alcohol, salt and saturated fat intake, and smoking cessation are all effective lifestyle changes that reduce the long-term risk of stroke recurrence.

Antiplatelet therapy

The majority of patients with non-cardioembolic IS and TIA require antiplatelet therapy to prevent recurrent events. Clopidogrel is recommended for long-term secondary prevention of stroke and TIA.(30) Long-term dual antiplatelet therapy with aspirin and dipyridamole reduced recurrence by 23% compared with either drug in isolation, whilst dual aspirin and clopidogrel was no more effective than either drug alone but did lead to increased bleeding compared with clopidogrel only.(31, 32) In a meta-analysis of small studies, dual antiplatelet therapy reduced the risk of recurrence more than monotherapy when started within 72 hours of the index event.(33) The large "clopidogrel in high-risk patients with acute nondisabling cerebrovascular events" (CHANCE) trial found that dual aspirin and clopidogrel reduced stroke recurrence compared with aspirin alone when started within 24 hours of minor IS or TIA in the Chinese population.(34, 35) Similarly, the platelet-oriented inhibition in new TIA and minor ischaemic stroke (POINT) trial found that aspirin and clopidogrel in combination started within 12 hours of symptom onset

reduced the risk of major ischaemic events, but increased the risk of major haemorrhage at 90 days.(36) Triple antiplatelet therapy given within 48 hours of stroke/TIA onset did not reduce stroke recurrence but did cause more, and more severe, bleeding than guideline therapy in the triple antiplatelets for reducing dependency after ischaemic stroke (TARDIS) trial.(37)

Anticoagulation

Cardioembolic causes of IS are often, unless contraindicated, treated with anticoagulation. The vitamin K antagonist, warfarin is commonly prescribed for this indication. However, it requires regular monitoring using the international normalised ratio (INR) blood level to ensure appropriate anticoagulation is established and maintained. More recently, direct oral anticoagulants (DOACs), which do not require such monitoring, have been shown to be non-inferior to warfarin in preventing recurrent stroke in non-valvular atrial fibrillation and are becoming more widely used in clinical practice. DOACs significantly reduced all-cause mortality and ICH, but increased gastrointestinal bleeding in comparison to warfarin in a large meta-analysis.(38)

Blood pressure

High BP or hypertension after stroke is common. Treatment with angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor antagonists (ARA) – so-called 'A' drugs – is recommended as first line in patients under 55 years of age. For those aged over 55 years or of black race, calcium channel antagonists (CCA) are first line ('C' drugs). Second

line medications include an 'A' or 'C' drug if first line was a 'C' or 'A' drug respectively. Third line medications are diuretics. Target BP after stroke or TIA is 130/80.(39) Although these medications are used in the long-term management of hypertension to reduce cardiovascular events, including stroke, there is limited evidence regarding their use in acute stroke (see section 1.2).

Statins

Statins are 3-hydroxy 3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors that lower LDL cholesterol and triglyceride levels and increase HDL cholesterol. Statin therapy protects against stroke in terms of both primary and secondary prevention: two meta-analyses have shown a 20% relative risk (RR) reduction in IS for each 1 mmol/L reduction in LDL cholesterol;(40, 41) in those with a history of stroke or TIA statin therapy was associated with a reduction in recurrent stroke, major cardiovascular events or death compared with placebo.(42, 43) There is no evidence to support the use of statins for the prevention or treatment of vascular dementia.(44)

Carotid endarterectomy

Surgical removal of atherosclerotic plaque and blood clots within the carotid artery is termed carotid endarterectomy. This procedure is beneficial for those with 50-99% stenosis of the offending carotid artery who have had a recent TIA or non-disabling IS by reducing recurrent stroke. The absolute risk reduction of ipsilateral IS within 5 years was 4.6% and 16% in those with 50-69% and 70-99% stenosis respectively.

This procedure should be performed within 2 weeks of the index vascular event.(45)

1.1.6 Prognosis

Death from stroke is unfortunately common; 14% at 1 month, rising to 27% by 1 year.(46) Mortality within the first few days after stroke is usually due to direct complications of stroke (e.g. brain swelling or compression) and is more commonly seen in more severe strokes and ICH. Later complications such as infection (e.g. pneumonia, urinary tract infection), deep vein thrombosis, and pulmonary embolism can also result in death. Survivors can be left with significant impairments that can impact upon their activities of daily living, affecting their lives in a myriad of ways.

1.2 BLOOD PRESSURE AND ACUTE STROKE

1.2.1 Background

High BP is the leading modifiable risk factor for both ischaemic and haemorrhagic stroke (47) affecting 1 billion people worldwide.(48) In acute stroke, 75% of patients have high BP and 50% of those have a prior history of hypertension.(49, 50) Although BP spontaneously falls in two thirds of patients in the first week following stroke,(49) one third remain hypertensive and have an increased risk of a poor outcome.(51) Data from the first International Stroke Trial demonstrated a U-shaped relationship between baseline systolic BP (SBP) and outcome, such that both high and low SBP were independently associated with increased early death and late death or dependency.(52) In addition, high SBP is associated with an increased risk of early stroke recurrence.(52, 53) Post-hoc analyses from several acute stroke clinical trials suggest that as well as increased SBP, other haemodynamic variables including higher peak SBP, mean arterial pressure (MAP), pulse pressure and increased SBP variability, are each associated with poor functional outcome,(54) early neurological deterioration,(55) recurrent stroke, and death.(56)

The acute hypertensive response seen in stroke has numerous potential causes including: fluctuations in, or elevation of, pre-existing hypertension; infection; pain, e.g. due to urinary retention; stress related to hospitalisation; activation of cortisol, natriuretic peptide, renin-angiotensin-aldosterone and sympathetic neuroendocrine systems; impaired cardiac baroreceptor sensitivity; and raised intracranial

pressure (Cushing's reflex).(57-60) Although low BP is far less common in acute stroke, it is associated with a poor outcome.(52) Potential causes include sepsis, cardiac arrhythmias, heart failure and ischaemia, hypovolaemia and aortic dissection.(61)

Normal cerebral autoregulation, which maintains cerebral blood flow (CBF) despite fluctuations in cerebral perfusion pressure (CPP) between 50 and 150 mmHg, is impaired in acute stroke resulting in cerebral perfusion having a linear relationship with CPP and therefore MAP.(62) Rapid, large falls in BP could reduce CBF leading to extension of cerebral infarction,(63) or perihaematomal ischaemia.(64) Equally, with higher BP there is increased risk of haematoma expansion in ICH, haemorrhagic transformation in animal models of IS, and cerebral oedema, in both types of stroke.(51, 65)

The debate surrounding whether high blood pressure should or should not be treated in the context of acute stroke commenced over 30 years ago (66, 67) and despite large clinical trials the answer remains largely unclear.

1.2.2 Class action

A variety of BP modulating agents have been assessed in the context of acute stroke according to mode of pharmacological action (Table 1.1).(68)

Table 1.1: BP modulation by class action

Trial	Stroke type	Drug	Time given (hours)	BP effect	CBF effect	Clinical outcome
α2 adrenoreceptor agonist					Increase (rats)	Neutral
Lisk 1993 (69)	IS	Clonidine	<72	Mean reduction: SBP 13.6; DBP 2.1 mmHg		
ACEi					Maintain/increase	Neutral
CHIPPS 2009 (70)	All	Lisinopril (PO/SL)	<36 (mean 19)	Mean reduction: SBP 14mmHg; DBP 7mmHg		
PIL-FAST 2013 (71)	All	Lisinopril (SL/PO)	<3			
ARA					Neutral/reduce	Neutral/poor
ACCESS 2003 (72)	IS	Candesartan (PO)	<36 (mean 29)	No difference		
PRoFESS 2009 (73)	IS	Telmisartan (PO)	<72 (mean 58)	SBP: 6-7mmHg DBP: 2-4mmHg		
SCAST 2011 (74)	All	Candesartan (PO)	<30 (mean 18)	Mean difference at 7 days: SBP 5mmHg; DBP 2mmHg		
VENTURE 2015 (75)	IS	Valsartan (PO)	<24 (mean 12)	Mean difference at 7 days: SBP 4mmHg; DBP 2mmHg		
α & β-Blocker					Neutral	Neutral
CHIPPS 2009 (70)	All	Labetalol (PO/IV)	<36 (mean 19)	Mean reduction: SBP 7mmHg DBP increase 0.6mmHg		
β-Blockers					?Reduce	Poor
BEST 1988 (76)	Unknown	Propranolol (PO), atenolol (PO)	<48	Reduction: 6-9% vs. 2% (placebo)		
CCA					Reduce	Poor
INWEST 1994 (77)	IS	Nimodipine (IV) 1mg/hr (low dose), 2mg/hr (high dose)	<24	SBP low dose: 6.6%; high dose 11.4%; placebo 2.1% DBP low dose 7.7%; high 14.1%; placebo 1.7%		
VENUS 2001 (78)	All	Nimodipine (PO)	<6	No difference		

Systematic review (Horn 2001) (79)	IS					Poor
Diuretics					Neutral	Neutral
Eames 2005 (80)	IS	Bendroflumethiazide (PO)	<96	No difference		
Magnesium					Increase	Neutral
IMAGES 2004 (81)	IS	Magnesium sulphate IV bolus and infusion	<12 (median 7)	BP difference at 24: 4/3 mmHg vs. placebo		
FAST-MAG 2015 (82)	All	Magnesium sulphate IV bolus and infusion	<2 (median 45 mins)	SBP difference at 24: 3 mmHg		
NO donors					Increase	Neutral ?early effect
RIGHT 2013 (83)	All	GTN 5mg topical patch	<6 (median 55 mins)	SBP difference at 2: 18 mmHg		
ENOS 2015 (84)	All	GTN 5mg topical patch	<48 (median 26)	Mean reduction at 24: SBP 7 mmHg; DBP 3 mmHg		
Pressors						
Hillis 2003 (85)	IS	IV Phenylephrine	<1 week	No data	Increase	Unknown
Sprigg 2007 (86)	IS	PO Amphetamine	3-30 days	SBP at 90 mins increased by 11 mmHg	Neutral	Neutral/poor (83)
Saxena 1999 (87)	IS	IV DCLHb	<72	MAP at 2 increased by 21 mmHg		Poor

ACEi: Angiotensin converting enzyme inhibitors; ARA: angiotensin receptor antagonists; BP: blood pressure; CBF: cerebral blood flow; CCA; calcium channel antagonists; DBP: diastolic blood pressure; DCLHb: diaspirin cross-linked haemoglobin; GTN: glyceryl trinitrate; ICH: intracerebral haemorrhage; IS: ischaemic stroke; iv: intravenous; po: per oral; NO: nitric oxide; SBP: systolic blood pressure

1.2.2.1 Alpha-2-adrenoreceptor agonists

The alpha-2-adrenoreceptor agonist, clonidine, was tested in a small randomised controlled trial (RCT), which allocated 16 participants with MCA infarction within 72 hours of onset and high baseline BP (SBP 170-220 mmHg, diastolic BP [DBP] 95-120 mmHg) to nicardipine 20 mg, captopril 12.5 mg, clonidine 0.1 mg, or placebo given every 8 hours for 3 days.(69) BP fell in all groups but there was no significant difference in BP between the two main groups and no difference in stroke outcome, measured using the NIHSS, over the 3 days of treatment. To date, no large RCTs have assessed the use of alpha-2-adrenoreceptor agonists in acute stroke.

1.2.2.2 Angiotensin converting enzyme inhibitors (ACEi)

In three small RCTs of acute IS oral perindopril,(88) lisinopril(89) and captopril (69) independently reduced BP, whilst preserving CBF, although no differences in neurological impairment (NIHSS) or functional outcome (mRS) were seen between groups.(89)

The Controlling Hypertension and Hypotension Immediately Post-Stroke (CHHIPS) trial (70) randomised 179 patients with either IS or ICH within 36 hours of ictus and SBP >160 mmHg to oral labetalol (50 mg), lisinopril (5 mg), or placebo in those without dysphagia, or intravenous labetalol (50 mg), sublingual lisinopril (5 mg), or placebo in those with dysphagia. Dose escalation occurred if participants did not reach target SBP (145-155 mmHg or 15 mmHg reduction) at 4 and 8 hours post-randomisation. Lisinopril reduced mean BP by 14 / 7 mmHg compared

with placebo between randomisation and 24 hours. Following 14 days of treatment there was no difference in functional outcome (mRS >3) between treatment and control (relative risk (RR) 1.03, 95% CI 0.8-1.33, p=0.82), although lisinopril was safe with no increased reporting of serious adverse events.

In the pre-hospital environment the Paramedic Initiated Lisinopril For Acute Stroke Treatment (PIL-FAST) study randomised 14 patients with new unilateral arm weakness within 3 hours of onset and SBP >160 mmHg to either sublingual lisinopril (5 mg) or placebo for a total of 7 days.(71) BP fell in the lisinopril group compared to control by hospital admission and persisted for the duration of treatment. As a feasibility trial it was successful but was not powered to assess efficacy.

1.2.2.3 Angiotensin receptor antagonists (ARA)

The Acute Candesartan Cilexetil Therapy in Stroke Survivors (ACCESS) study,(72) randomised 339 participants with IS and elevated BP (≥180/105 mmHg) to 7 days of oral candesartan or placebo within 36 hours of admission. Mortality at 12 months and cardiovascular events (secondary outcome) were significantly reduced in the candesartan arm, although there was no significant effect on functional outcome (Barthel index (BI), primary outcome) at 3 months, or on BP throughout the 12 months of the trial.

A *post-hoc* subgroup analysis of the multinational Prevention Regimen for Effectively Avoiding Second Strokes (PRoFESS) trial (73) examined the

effect of adding telmisartan versus placebo to standard antihypertensive management in 1360 patients with mild IS recruited within 72 hours of ictus. Telmisartan lowered SBP and DBP by 6-7 mmHg and 2-4 mmHg respectively compared with placebo and was safe with no excess of adverse events. However, telmisartan did not influence functional outcome (mRS at day 30, primary outcome) or death, stroke recurrence and cardiovascular events at days 7, 30 or 90. In contrast to the ACCESS study, PRoFESS participants had lower BP at randomisation, milder strokes, enhanced antihypertensive therapy, were recruited later (58 vs. 29 hours) and had a longer period of treatment (30 months vs. 7 days), which may account for the dissimilar findings between the trials.

Following ACCESS, the Scandinavian Candesartan Acute Stroke Trial (SCAST) (74) recruited 2029 participants with acute stroke (IS and ICH) within 30 hours of onset and SBP ≥140 mmHg. Patients were randomised to either candesartan 4 mg with dose escalation up to 16 mg, or placebo for 7 days. BP fell in both groups over the treatment period and was significantly lower in the candesartan arm compared to placebo (day 7 mean BP: 147/82 mmHg vs. 152/84 mmHg). Co-primary endpoints were measured at 6 months: a composite of vascular death, myocardial infarction and stroke was neutral; and functional outcome measured by a shift in mRS suggested a higher risk of poor outcome in those randomised to candesartan, but was not statistically significant given the two primary outcomes (adjusted common odds ratio [acOR] 1.17, 95% CI 1.00-1.38, p=0.048). A pre-specified subgroup analysis of those with ICH (n=274) also found that candesartan was associated with

an increased risk of poor outcome (acOR 1.61, 95% CI 1.03-2.50, p=0.036).(90)

Several smaller, underpowered trials have assessed candesartan,(91) irbesartan,(92) telmisartan,(75) and valsartan(93) in acute IS. Telmisartan did not alter CBF or BP acutely.(75) The Valsartan Efficacy oN modesT blood pressUre Reduction in acute ischaemic stroke (VENTURE) trial,(93) randomised 393 South Korean acute IS patients within 24 hours of onset and SBP 150-185 mmHg to oral valsartan 80 mg daily with dose escalation, or placebo for 7 days. Valsartan significantly reduced mean DBP at day 7 compared with placebo (83.1 vs. 84.8 mmHg), whilst SBP was not significantly reduced. The primary outcome of death or dependency at 90 days (mRS >3) was neutral, but early neurological deterioration within 7 days was significantly higher in the valsartan group (16.6% vs. 6%, OR 2.43, 95% CI 1.25-4.73, p=0.008); mainly due to stroke progression in those with large artery atherosclerosis as the cause of their stroke and angiographically confirmed large-artery stenosis or occlusion.

These neutral and negative findings may indicate that ARAs have undesirable properties in acute stroke or that gradual and late treatment of BP is 'too little too late', thus reducing cerebral perfusion and increasing brain injury.

1.2.2.4 Beta-blockers

The single-centre β blocker stroke (BEST) trial (76) randomised 302 patients with clinically diagnosed strokes within 48 hours of onset to oral propranolol, atenolol or placebo. There was a greater fall in mean BP in the first 24 hours of treatment (6-9% vs. 2%) and an increase in early and later death in those assigned to β-blockers compared to placebo. The negative inotropic effects of β-blockers may worsen cerebral perfusion in acute stroke and thus explain this finding, although pathophysiological trial data are lacking (Table 1.2).

Table 1.2: Multimodality of BP modulating agents in acute stroke

Agent	Potential beneficial effects				Potential detrimental effects		
	Anti-inflammation	*Smooth muscle cell anti-proliferation*	*Cerebral vasodilatation*	*Neuroprotection*	*Antiplatelet**	*Negative inotrope*	*Stress hormone attenuation*
α2-adrenoreceptor agonist (94-96)				+		-	
α & β blocker (94)						-	-
ACEi (97, 98)	+	+	+	+			-
ARA (99, 100)	+	+		+			-
β-blocker (101, 102)					-	-	-
CCA (102)					-	-	
Magnesium (103-106)			+	+	-		
NO donor (107-110)	+	+	+	+	- (SNP)		

Broad categories of other potential effects of BP modulating agents, with over-arching beneficial and detrimental groups.
'+'=Beneficial effects, '-'=Detrimental effects, '*'=In the context of ICH, antiplatelet properties are potentially detrimental.
ACEi: angiotensin converting enzyme inhibitors; ARA: angiotensin receptor antagonists; CCA: calcium channel antagonists; NO: nitric oxide; SNP: sodium nitroprusside

In those randomised to labetalol (a mixed alpha and beta adrenergic antagonist) in the CHHIPS trial,(70) SBP fell by 7 mmHg at 24 hours. In contrast to the BEST trial, labetalol was safe with no increase in serious adverse events, early neurological deterioration or death. Overall, the active treatment group (labetalol and lisinopril combined) had reduced 90-day mortality compared to the placebo group (hazard ratio [HR] 0.40, 95% CI 0.2-1.0, p=0.05) but the study was not powered for this outcome.

1.2.2.5 Calcium channel antagonists (CCA)

Early studies showed significant drops in BP in patients who received nimodipine (111) or nicardipine,(69) with the latter suggesting that large drops in BP due to nicardipine were associated with reduced regional CBF to infarcted tissue. Contrary to this, other small trials reported positive results of oral (112, 113) and intravenous nimodipine (114) on long-term recovery in acute IS, prompting the need for a larger RCT.

The Intravenous Nimodipine West European Stroke Trial (INWEST) (77) randomised 295 patients with acute IS within 24 hours of onset to intravenous nimodipine at 1mg/hour (low dose) or 2 mg/hour (high dose) for 5 days then 120 mg daily (orally) for a total of 21 days, or placebo. Recruitment was stopped early due to statistically significant unfavourable functional outcomes (BI and Orgogozo neurological impairment scale) in the nimodipine groups compared with placebo at both 21 days and 6 months. Over the first two days, mean BP significantly fell from baseline in the treatment arms compared with

placebo.(115) In a subsequent analysis, DBP reduction in the high dose treatment arm was associated with a poor functional outcome at day 21, whilst those who received high dose nimodipine and had a large (≥20%) fall in DBP had an increased risk of death or dependency and death at day 21.(115) A similar but unpublished trial in the USA had comparable results.(116)

A further trial of oral nimodipine recruited 454 patients within 6 hours of stroke ictus in primary care.(78) At 24 hours there was no significant difference in BP between the nimodipine and control groups, and the primary outcome of death or dependency (mRS >3) at 3 months was neutral (RR 1.2, 95% CI 0.9-1.6). This trial was stopped early because a Cochrane systematic review involving 7665 patients from 29 trials of CCA in acute IS revealed no treatment effect on functional outcome or death at the end of follow-up.(117) Interestingly, a subgroup analysis of unpublished and methodologically-sound trials yielded a statistically significant unfavourable treatment effect indicative of publication bias (RR 1.14, CI 95% 1.0-1.3); overall, good quality trials produced a statistically significant negative treatment effect (RR 1.09, 95% CI 1.02-1.16).(79) Unfortunately, much of the drive to test CCA, especially nimodipine, was driven by early positive data.(112-114, 118)

1.2.2.6 Diuretics

There is limited data on diuretics in acute stroke.(68) One small RCT randomised 37 hypertensive patients with acute IS within 96 hours of onset to bendroflumethiazide (a thiazide-like diuretic) 2.5 mg daily or

placebo for 7 days.(80) Although mean SBP was lower in the treatment group compared with placebo within 70 hours of randomisation (156 vs. 176 mmHg), there was no difference in BP between the arms at day 7. Measures of CBF and cardiac baroreceptor sensitivity showed no significant change between groups at either time point in the trial, suggesting that bendroflumethiazide is an ineffective agent for use in acute stroke patients.

1.2.2.7 Magnesium

A systematic review of several small pilot studies assessing magnesium in acute stroke reported a non-significant reduction in death or disability in patients treated with magnesium (OR 0.73, 95% CI 0.38-1.41).(119) A large RCT allocated 2589 patients with acute IS within 12 hours of onset to intravenous magnesium sulphate slow bolus (16 mmol) followed by infusion (65 mmol) over 24 hours, or placebo.(81) Although BP fell by 4 / 3 mmHg between baseline and 24 hours in the magnesium group compared with placebo, the only significant difference in BP was during the initial infusion. The primary outcome of death and disability at day 90 (BI <95 and mRS >1 combined) was neutral, but there was a trend towards increased mortality in the magnesium group (HR 1.18, 95% CI 0.97-1.42, p=0.098). In a pre-specified subgroup of non-cortical strokes, magnesium significantly reduced death and disability (OR 0.75, 95% 0.58-0.97, p=0.011); a finding supported by a *post-hoc* analysis where those with lacunar stroke had reduced death and disability at day 90 (OR 0.70, 95% 0.53-0.92, p=0.0046). Patients who received magnesium

within 3 hours of onset had a tendency towards a better outcome (OR 0.66, 95% 0.25-1.7, p=0.46).

The Field Administration of Stroke Therapy-Magnesium (FAST-MAG) trial (82) sought to assess magnesium in this shorter time window by recruiting 1700 patients with presumed stroke within 2 hours of ictus to intravenous magnesium bolus followed by infusion, or placebo. SBP fell in both groups over the first 48 hours but those on treatment had a significantly lower SBP (~3 mmHg difference) at the end of the bolus dose and from 20 to 32 hours after starting the maintenance infusion. Although pre-hospital initiation of magnesium was safe, there was no significant shift in mRS at day 90 (primary outcome).

1.2.2.8 Nitric oxide (NO) donors

The effects of the NO donor glyceryl trinitrate (GTN) on blood pressure and clinical outcomes in acute stroke are discussed in section 1.3 below.

1.2.2.9 Pressor therapy

Several small studies have assessed the role of pressor therapy in acute IS.(68, 120) One trial assessed intravenous phenylephrine versus conventional management in 15 patients with acute IS within one week of ictus, >20% diffusion-perfusion mismatch on magnetic resonance imaging (MRI) and normotension (SBP <140 mmHg).(85) Phenylephrine was titrated to increase MAP by 10-20% and maintained for up to 72 hours. NIHSS and cognitive scores, and volume of hypoperfused tissue

on MRI, improved in the treatment group with no significant adverse events, but there was no assessment of functional outcome. The aforementioned CHHIPS trial had a pressor arm, which sought to assess phenylephrine in hypotensive IS patients, but grossly under-recruited (one participant only, who received placebo).(121) Similarly, an unpublished trial of dobutamine only managed to recruit three patients.

Diaspirin cross-linked haemoglobin (DCLHb), a cell-free haemoglobin-based oxygen-carrying solution that scavenges NO, (122) was compared with saline in 85 patients with acute IS within 18 hours of onset.(87) DCLHb caused a rapid rise in BP and more serious adverse events, disability (BI), death and poor functional outcome (mRS) at 3 months than control. In a small RCT of 33 patients within 1 month of IS,(86) amphetamine raised BP and heart rate but had no impact on motor function or functional outcome. Although amphetamine was associated with a trend to improved motor function after IS in a systematic review, there was a non-significant increase in death, raising doubts over its safety.(123) Other potential agents including norepinephrine (noradrenaline), epinephrine (adrenaline) and dopamine have no significant evidence-base.(120)

1.2.3 Targeting BP in acute stroke

An alternative avenue of research has focused on whether aiming for a BP target in acute stroke, regardless of the agent(s) used, improves outcome.

1.2.3.1 Ischaemic stroke

The China Antihypertensive Trial in Acute Ischaemic Stroke (CATIS) (124) recruited 4071 acute IS patients with raised SBP (140-220 mmHg) within 48 hours of onset and randomised them to either BP lowering (SBP 10-25% reduction within 24 hours and BP <140/90 mmHg within 7 days) or control (no antihypertensive medication). Although a specific BP-lowering regimen was not being assessed they suggested first-line (intravenous ACEi), second-line (oral CCA), and third-line (oral diuretic) medications. Mean SBP fell by 13% within 24 hours of randomisation in the treatment group, compared with 7% in the control population. At 7 days, mean SBP in the treatment and control arms was 137 and 147 mmHg respectively. The primary outcome of mRS ≥3 at 14 days or hospital discharge and secondary outcome of mRS at day 90 were neutral. A subgroup analysis of time to treatment found that those randomised to BP lowering 24 hours or longer after ictus had a significant reduction in death or dependency at 3 months (OR 0.73, 95% CI 0.55-0.97, p=0.03).(124)

There are several points to mention. First, the recruits had minor strokes (median NIHSS 4) resulting in 66% of the control population being independent at 2 weeks and therefore reducing the potential for the

intervention to show benefit. Second, patients with large vessel carotid disease were omitted from the trial. And last, patients receiving thrombolysis were excluded, further limiting the trial's generalisability. (124)

1.2.3.2 Intracerebral haemorrhage

In a small feasibility study of ICH patients within 8 hours of symptom onset, aggressive BP lowering (MAP <110 mmHg) was safe with no difference in rates of early neurological deterioration, haematoma expansion or cerebral oedema.(125) The concern surrounding whether aggressive BP lowering compromises perihaematomal CBF was addressed in the Intracerebral Haemorrhage Acutely Decreasing Arterial Pressure Trial (ICH-ADAPT).(126) Seventy-five patients with spontaneous ICH within 24 hours of onset and high BP (SBP ≥150 mmHg) were randomised to a target SBP of <150 mmHg or <180 mmHg within 1 hour of randomisation. Two hours after recruitment CT perfusion imaging revealed reduced CBF and cerebral blood volume within the perihaematoma region compared with the contralateral homologous area in all patients. There was no significant difference in relative CBF between the groups, indicating that aggressive BP reduction in ICH did not, at least in this study, precipitate perihaematomal ischaemia.(126)

Early BP lowering in the Intensive Blood Pressure Reduction in Acute Cerebral Haemorrhage Trial (INTERACT)(127) involving 404 patients was safe, feasible, and seemed to reduce haematoma growth. Similarly, BP reduction within 6 hours in the Antihypertensive Treatment of Acute

Cerebral Haemorrhage (ATACH)(128) study was safe. The magnitude of SBP lowering was associated with less haematoma expansion and improved functional outcome.(129) The largest trial of intensive BP lowering in ICH, INTERACT-2,(130) recruited 2839 patients within 6 hours of onset with high SBP (150-220 mmHg) and randomised them to guideline therapy (target SBP <180 mmHg) or intensive therapy (target SBP <140 mmHg within one hour) for 7 days using oral or intravenous agents at the discretion of the local investigator. At one hour, a third of patients in the intensive arm achieved the target SBP of <140 mmHg with a mean SBP of 150 mmHg, compared with 164 mmHg in the guideline group. The primary outcome of death or major disability (mRS ≥3 at 90 days) was neutral, but a pre-specified ordinal shift analysis of mRS revealed a favourable shift in those randomised to intensive BP lowering (OR 0.87, 95% CI 0.77-1.00, p=0.04). In addition, better outcomes were seen in those with larger BP reductions within one hour of randomisation.(131) Intensive BP reduction was safe with no difference in death or other serious adverse events between groups.(130)

The use of mannitol in 62% of INTERACT2 participants is unclear given that the overall 24 hour median haematoma volume was 20 mL, making intracranial hypertension unlikely. The myriad combinations of antihypertensive agents used in the trial included one rarely used in the West (Urapidil) and others with potentially negative or harmful effects, which may have confounded the BP-lowering effect.(132)

In contrast, intensive BP lowering (110-139 mmHg) vs. standard BP lowering (140-179 mmHg) with nicardipine within 4.5 hours of stroke was neutral in the ATACH-2 trial (n=1000).(133) It is important to note that INTERACT-2 and ATACH-2 have crucial differences. Firstly, nicardipine has mild antiplatelet action, so the BP lowering effect of attenuating haematoma expansion may have been negated by its antiplatelet effects. Second, BP eligibility for inclusion differed between the trials (INTERACT-2: >=150 <220 mmHg; ATACH-2 >=180 mmHg) with pre-randomisation lowering of BP allowed in ATACH-2 not INTERACT-2. Third, the more aggressive approach to BP lowering in both arms of ATACH-2 resulted in the guideline group having a similar on-treatment BP to the intensive group in INTERACT-2. As such, a lower target of 110-139 mmHg is perhaps too low in acute ICH and that a less aggressive target of 140 mmHg is appropriate.

Whilst INTERACT2 did not show any change in haematoma expansion with aggressive BP lowering, a meta-analysis of four of the above trials (125-127, 130) found that intensive BP lowering in acute ICH appeared safe with a tendency towards improved functional outcome; an effect which may have been mediated through attenuation of haematoma expansion observed at 24 hours in both unadjusted and adjusted models.(134) Furthermore, a *post-hoc* analysis of INTERACT2 revealed that intensive BP lowering with greater SBP reduction prevented haematoma growth at 24 hours.(135)

1.2.4 Issues

1.2.4.1 To treat or not to treat?

Guidelines suggest that BP lowering in acute stroke should be postponed for days or even weeks unless BP is grossly elevated (>220/120 mmHg), or >200/100 with concomitant evidence of acute kidney injury, aortic dissection, cardiac ischaemia, hypertensive encephalopathy or pulmonary oedema.(136-138)

Thrombolysis for hyper-acute ischaemic stroke

In the context of thrombolysis in acute IS, BP should be <185/110 mmHg prior to administration of alteplase, and <180/105 mmHg for the following 24 hours; suggested methods involve using intravenous labetalol, nicardipine or nitroprusside.(136) Unfortunately there is a paucity of evidence and this recommendation is based on expert opinion with extrapolation from trials of thrombolysis in myocardial infarction.(139, 140) Observational data from the Safe Implementation of Thrombolysis in Stroke (SITS) register (141, 142) revealed that a higher SBP post-thrombolysis is associated with symptomatic ICH and poor outcome. A U-shaped relationship was seen between SBP 2 to 24 hours after thrombolysis and major disability and death, with the most favourable outcomes occurring in those with SBP 141-150 mmHg.(142) The on-going Enhanced Control of Hypertension and Thrombolysis in Stroke Study (ENCHANTED)(143) will provide insight into whether acute intensive lowering of BP (target SBP 130-140 mmHg) has superior efficacy and lower risk of ICH than guideline management (SBP <180 mmHg).

Intracerebral haemorrhage

Following publication of INTERACT-2, both American(144) and European(4, 145) guidelines were updated recommending early and intensive BP lowering to 140 mmHg within six hours of onset. However, since the publication of ATACH-2, some North American guidelines have changed to dissuade intensive BP lowering in ICH.(146, 147) Due to the differences between these two trials discussed above, these updated guidelines should be interpreted with caution.(148)

1.2.4.2 Time to treatment

In a recent Cochrane review, lowering BP in 15432 patients with acute stroke did not improve outcome regardless of stroke type, or drug class and BP target used.(68) However, in those who received treatment within 6 hours of stroke onset (INTERACT-2 and rapid intervention with glyceryl trinitrate in hypertensive stroke trial (RIGHT)), there was a tendency towards a shift to less death or dependency, and improved quality of life.(83, 130) All drug classes described above lowered BP, with greater reductions seen in ICH than acute IS patients (-11.8/-5.1 vs. -7/-3.1 mmHg). Smaller BP changes occurred in patients recruited after 48 hours of onset, whilst the largest BP reduction was seen in those recruited earliest. Importantly, large falls in BP (>20%), especially in acute IS, were associated with a poor outcome.(68)

A subsequent subgroup analysis of the large efficacy of nitric oxide in stroke (ENOS) trial of patients randomised to glyceryl trinitrate (GTN)

within 6 hours adds weight to the argument for early treatment with reduced death or dependency, less death and improved cognition, disability, mood and quality of life.(149) It is unclear whether this may represent a generic effect of early BP lowering or a specific effect of GTN. In contrast, other interventions namely ARA, β-blockers and CCA may be detrimental (Table 1.2).(74, 76, 79, 90, 117)

Time is important: ultra-acute treatment of BP (intensive BP lowering or use of an appropriate agent) within the first few hours of symptoms in the pre-hospital setting is a vital avenue to explore further. Non-oral routes of administration, such as transdermal, sublingual and intravenous, would be preferable in this context, given the need for a swallowing assessment to rule out dysphagia. Of these, transdermal GTN,(83) sublingual lisinopril,(71) and intravenous magnesium,(82) have been assessed in the pre-hospital environment and found to be safe. Whilst transdermal preparations can be easily applied and removed according to clinical need, intravenous administration of BP lowering agents require intensive monitoring. On-going (RIGHT-2: ISRCTN26986053; MR-ASAP: ISRCTN99503308) trials of transdermal GTN in ultra-acute stroke are assessing efficacy in the field.

1.2.4.3 Race and Ethnicity

Demographics are important and especially relevant in a cosmopolitan global community. For example, Chinese stroke patients tend to be younger, smoke more, have increased intracranial atherosclerosis, less cervical atherosclerosis and a higher risk of ICH than their Caucasian

counterparts.(150, 151) Hence, demographic similarities and differences should be considered in both future trials and individual patient data meta-analyses.

1.2.4.4 BP management in carotid disease and large vessel occlusion

High BP is commonly seen in patients with acute IS due to carotid artery stenosis.(152) Concerns regarding dysfunctional cerebral autoregulation are two-fold: a higher systemic BP will result in a higher cerebral perfusion pressure increasing the risk of cerebral oedema and potential for haemorrhagic transformation; whilst lowering BP may reduce CBF resulting in infarct extension.(51)

A pre-specified subgroup analysis from SCAST of patients with carotid imaging (n=993 (57%)), revealed that those with severe unilateral stenosis (≥70%) who received candesartan had a trend towards increased risk of stroke progression and poor functional outcome, although the CI were wide.(153) Whether this was due to a specific effect of candesartan, or to BP lowering *per se* remains unclear. Of the 2038 participants in ENOS with carotid imaging data, GTN was safe with no evidence of harm across all levels of ipsilateral carotid stenosis.(84) Patients with bilateral severe carotid stenosis pose another dilemma. A meta-analysis of three trials found that in patients with bilateral severe stenosis (≥70%), a lower BP was associated with higher stroke recurrence (SBP <130 mmHg: HR 5.97, 95% CI 2.43-14.68, p<0.001).(152) Although bilateral carotid stenosis is uncommon, caution

regarding BP lowering in this group seems warranted pending further data.

With the advent and proven effectiveness of endovascular intervention for proximal anterior circulation vessel occlusions in acute IS,(19) numerous questions remain unanswered, including how BP should be managed before, during and after thrombectomy. At present this is an evidence-free zone. Prospective research in this area should prove illuminating.

1.2.4.5 Continue or stop pre-stroke antihypertensives

Whether to temporarily stop or continue existing antihypertensive agents early after a patient's stroke is a common clinical question. The Continue or Stop Post-Stroke Antihypertensives Collaborative Study (COSSACS)(154) randomised 763 patients within 48 hours of stroke to either stop or continue their pre-existing antihypertensive medication for 2 weeks. Those who continued their medication had a lower BP at 2 weeks compared with those who stopped (mean difference 13 / 8 mmHg). Death or dependency at 2 weeks (mRS >3, primary outcome), death, major cardiovascular events and serious adverse events at 6 months did not differ between the 2 arms.(154)

The partial-factorial ENOS trial (84) enrolled 2097 patients within 48 hours of stroke onset to continue or stop their pre-stroke antihypertensive drugs for 7 days. Although there was no effect on functional outcome (mRS) at day 90, continuation of pre-stroke BP drugs

increased the risk of pneumonia (perhaps due to aspiration), worsened BI and increased cognitive impairment at 90 days.

When pooled data from COSSACS and ENOS were reviewed, continuation of antihypertensives was associated with worse disability (BI) and quality of life but no change in functional outcome (mRS).(68) This incongruity is perplexing, but may represent chance, outcome bias, or be real. If the latter is true and continuing medication is detrimental, what is the mechanism? First, giving medication to dysphagic patients without appropriate enteral access could lead to aspiration and resultant pneumonia.(84) Second, as ACEi, ARA and β-blocker drugs attenuate stress hormones, are common preparations used prior to stroke, and are associated with harm when given acutely after stroke (ARA and β-blockers),(74, 76) it may be that continuing them in the acute phase is potentially hazardous. It therefore seems reasonable to pause existing antihypertensive medication during the acute phase of stroke until patients have suitable enteral access and are medically and neurologically stable.(68)

1.2.5 Summary of BP in acute stroke

Despite the recent publication of several large clinical trials and systematic reviews, there are no definitive recommendations that can be drawn regarding BP modulation in acute IS. BP should be lowered acutely in patients with ICH. Although stroke is more common in the older population, trials to date have mostly involved patients with a mean age of ≤75 years. Despite this, there is no suggestion that older patients should not have their BP lowered.(130) In addition to age, further evidence is needed on whether time of onset, stroke subtype, severity, drug choice (dose, route and timing), and BP variability influence response to changes in BP. Individual patient data meta-analysis is warranted to aid patient selection by identifying groups who are more or less likely to benefit and to establish whether a certain drug class, dose, route or BP target is optimal.

In summary, antihypertensives should be withheld after stroke until they can be given safely in patients who are neurologically stable. Both early intensive lowering of BP in ICH and early nitrate use in all stroke subtypes are safe and associated with improved functional outcome. Whether these effects are mediated through BP reduction or specific pharmacological effects incorporating neuroprotection and/or reperfusion is unclear. Time seems to be a key factor and so on-going and future hyper-acute and ultra-acute trials are pivotal in testing this hypothesis.

1.3 NITRIC OXIDE (NO) AND ACUTE STROKE

1.3.1 Introduction

Nitric oxide (NO) is a diatomic highly reactive gas. It is an obligate molecule in health and disease with a multitude of actions. NO has vasodilatory, pro-endothelial, anti-proliferative (vascular smooth muscle cell),(155) antiplatelet,(156, 157) anti-leucocyte,(108) anti-inflammatory, neuroprotective, neurotransmitter and neuromodulator properties.(158, 159) It has roles in modulating blood brain barrier integrity, CBF, auto- and chemo-regulation,(160-162) and inhibition of apoptosis.(163) NO is an endogenous inorganic soluble gas synthesised from L-arginine by three forms of NO synthase (NOS): endothelial (eNOS); inducible (iNOS); and neuronal (nNOS).(164, 165) The second messenger cyclic guanosine monophosphate (cGMP), which is broken down by phosphodiesterase (PDE), is the main mediator of downstream signalling of NO. NO is broken down via oxidation to nitrite and ultimately nitrate, and it is now apparent that NO may be made by reduction of nitrite and nitrate. Up-regulation of the L-arginine/nitrite-NO-cGMP pathway can be achieved by a number of means: increased substrate, administration of NO gas, induction of NO synthase activity; administration of NO donors; or inhibition of PDE.(166)

Pre-clinical experimental studies in models of ischaemia have demonstrated the role of NO in a time-dependent manner. Models of focal ischaemia have shown that NO production is increased, through nNOS activation, for up to half an hour after middle cerebral artery

(MCA) occlusion.(167, 168) During the first minutes following arterial occlusion eNOS and nNOS activity increases, then falls thereafter.(169) Up regulation of iNOS occurs from 12 hours after the onset of ischaemia and persists for up to seven days,(170) whilst NO within brain tissue is undetectable during this period.(167) L-arginine administered intravenously following MCA occlusion in a rat model improved ischaemic penumbral blood flow and reduced infarct size and volume.(171) This effect was not seen in eNOS-deficient mice who developed smaller penumbral regions, larger infarcts and absent angiogenesis leading to further post-ischaemic injury.(172-174) Therefore, eNOS and eNOS-derived NO are neuroprotective in focal ischaemia, whilst nNOS- and iNOS-derived NO have deleterious effects on tissue survival with resultant poor neurological outcomes.(168, 175) Although neurotoxic in acute stroke, iNOS and nNOS are involved in neurogenesis following stroke.(176, 177) In therapeutic studies, NO donors reduced infarct size in both permanent and transient models of ischaemia, and increased CBF in permanent models, but only if administered soon after stroke induction.(178)

Due to the myriad effects of NO described above and the low levels of endogenous NO seen in both IS and ICH,(179, 180) supplementation through administration of NO donors might be beneficial. In contrast, diaspirin cross-linked haemoglobin (DCLHb) reduces vascular NO levels(122) and was associated with poor neurological outcome in acute IS. Hence, lowering NO can lead to worse outcomes in acute stroke, whilst increasing NO may be beneficial.(87)

1.3.2 NO donors and acute stroke

NO donors can be broadly categorized into organic (e.g. GTN) and inorganic (e.g. sodium nitroprusside) nitrates although there are many subtypes.(181) Transdermal GTN has been administered as a patch to patients with acute and subacute stroke in three phase II trials; GTN lowered BP (peripheral and central), 24 hour BP, peak SBP, pulse pressure and pulse pressure index; increased heart rate; improved vascular compliance; and did not alter CBF and velocity, or induce cerebral steal or increase intracranial pressure (Table 1.3).(110, 182-185) While intravenous sodium nitroprusside has antiplatelet properties,(109) GTN had no such impact on platelet function and can therefore be administered in patients with ICH.(110) None of these earlier studies were powered for efficacy; this was assessed in the large ENOS trial.(84)

Table 1.3: Effects of GTN in acute/subacute stroke

	GTN-1 2001 (110)	**GTN-2 2003** (182)	**GTN-3 2006** (183)	**RIGHT 2013** (83)	**ENOS 2015** (84)	**GTN 1-2** (185)	**GTN 1-3** (184)
Systolic BP (mmHg)	↓ 13 (7.8%)		↓ 23 (14%)	↓ 21	↓ 7	↓ 9.4	↓ 9.8
Diastolic BP (mmHg)	↓ 5.2 (5.4%)		↓ 4 (3%)	↓ 6	↓ 3.5	↓ 4.8	↓ 4.4
Heart rate (bpm)	No change	No change	No change	No change	↑ 1.7	↑ 4.1	↑ 3.9
MAP (mmHg)		↓ 6.2%				↓ 5.0	↓ 6.4
PP (mmHg)		↓ 3.9		↓ 16			↓ 6.1
PPI							↓ 0.03
RPP (mmHg.bpm)				No change			↓ 323
Augmentation index		Improved		Improved			
Cerebral blood flow velocity		No change	No change				
Cerebral blood flow			No change				
Zero flow pressure			No change				
Platelet function	No change						
GTN supplier (5 mg)	Schwarz Pharma	Schwarz Pharma (and 10mg)	Novartis (Transiderm-Nitro)	MSD Schering-Plough (NitroDur)	UCB Pharma (Deponit-5) 38% of sites (29% †) Novartis (Transiderm-Nitro) 28% of sites (34% †) MSD/Schering-Plough (NitroDur) 25% of sites (19% †) Meda/3M Healthcare (Minitran) 10% of sites (3% †) Wuhan Jianmin 1% of sites (2% †)		

†=% of sites for ENOS-early (within 6 hours)
BP: blood pressure; bpm: beats per minute; GTN: glyceryl trinitrate; MAP: mean arterial pressure; PP: pulse pressure; PPI: pulse pressure index; RPP: rate pressure product

ENOS enrolled 4011 participants with acute stroke (within 48 hours of onset) and raised systolic BP (140-220 mmHg) and randomised these to transdermal GTN patch (5 mg) or no patch. Overall, there was no significant shift in functional outcome measured using the mRS at day 90 (primary outcome, adjusted common odds ratio [acOR] 1.01, 95% confidence intervals [CI] 0.91-1.13) or of any secondary outcomes; further, GTN was safe with no increased reporting of serious adverse events.(84) GTN lowered BP by 7.0 / 3.5 mmHg compared with control at day 1. In a pre-defined subgroup (n=312), those who received GTN within six hours of stroke onset (ENOS-early) had a favourable shift in mRS (acOR 0.51, 95% CI 0.32-0.80), less death, less disability (Barthel index), less mood disturbance (Zung depression scale), and improved cognition (telephone Mini-Mental State Examination) and quality of life (EuroQol health utility status and visual analogue scale).(149) The small ambulance-based Rapid Intervention with Glyceryl trinitrate in Hypertensive stroke Trial (RIGHT) also found that transdermal 5 mg GTN, given in the pre-hospital setting by paramedics within four hours of ictus, improved mRS at day 90.(83)

An individual patient data meta-analysis using data from the five completed GTN trials (GTN-1/2/3, ENOS, RIGHT, n=4197) supported the findings that treatment with GTN within six hours of onset (n=312), but not later, improved functional outcome and secondary outcomes across a range of domains: cognition; death; disability; mood; and quality of life (Table 1.4).(186) The time-dependent effect on functional outcome was seen in both IS and ICH; a finding supported by a subgroup analysis of

participants with ICH from the ENOS trial.(187) Those with ICH who received GTN within 6 hours of onset had significant improvements in functional outcome, cognition, disability, mood and quality of life at 90 days compared with those who did not receive GTN.(187) In the aforementioned meta-analysis, those with IS who received thrombolysis – given either before or after randomisation – had a significant shift to less death or dependency in the presence of GTN. A tendency to improved outcome was also seen in those with IS who did not receive thrombolysis.(186)

Further trials are needed to confirm whether GTN is efficacious when given early in patients with acute stroke; one study, the Rapid Intervention with Glyceryl trinitrate in Hypertensive stroke Trial-2 (RIGHT-2, right-2.ac.uk), is assessing transdermal 5 mg GTN patch versus sham in 1050 patients with presumed stroke within four hours of onset, SBP >120 mmHg and FAST score 2 or 3; consent, recruitment and treatment are performed by paramedics in the pre-hospital setting.(188)

Table 1.4: Clinical outcomes with GTN in acute stroke

	All GTN	Randomisation ≤6hours
Patients (%)	4197	312 (7.4)
End of treatment		
Death	1.15 (0.79, 1.68)	0.94 (0.23, 3.81)
Neurological deterioration	1.29 (1.00, 1.66)	0.58 (0.28, 1.23)
Stroke recurrence	1.39 (0.88, 2.18)	0.46 (0.14, 1.54)
SSS (/58)*	0.33 (-0.26, 0.91)	2.33 (-0.42, 5.09)
NIHSS* †	-0.28 (-0.70, 0.14)	**-2.07 (-3.81, -0.34)**
Day 7		
Non-oral feeding †	0.97 (0.82, 1.15)	0.59 (0.32, 1.08)
Hospital		
Length of stay (days)*	0.07 (-1.30, 1.44)	0.02 (-4.59, 4.63)
Physiotherapy †	0.94 (0.79, 1.12)	0.90 (0.40, 2.05)
Occupational therapy †	1.01 (0.72, 1.41)	1.15 (0.36, 3.68)
Speech therapy †	0.95 (0.84, 1.08)	1.01 (0.44, 2.29)
Day 90		
Modified Rankin Scale	0.99 (0.89, 1.10)	**0.52 (0.34, 0.78)**
Death	0.87 (0.71, 1.07)	**0.32 (0.14, 0.78)**
Barthel Index*	1.73 (-0.08, 3.55)	**9.64 (3.19, 16.09)**
Quality of life (HUS)*	0 (-0.02, 0.02)	0.05 (-0.02, 0.13)
Quality of life (EQ-VAS)*	0.69 (-1.06, 2.43)	5.97 (-0.30, 12.24)
Mood (ZDS)*	-0.38 (-1.80, 1.04)	**-8.34 (-13.32, -3.36)**
Cognition (t-MMSE)*	0.34 (-0.16, 0.84)	**2.09 (0.65, 3.54)**
Cognition (TICS-M)*	0.16 (-0.55, 0.88)	**3.56 (1.20, 5.91)**
Cognition (animal naming)*	-0.06 (-0.60, 0.47)	1.63 (-0.13, 3.39)

Data are number (%), mean difference, or odds ratio with 95% confidence intervals. Comparisons by binary logistic regression, ordinal logistic regression or multiple linear regression, with adjustment for trial; or unadjusted † using Mantel-Haenszel random effects model. *multiple linear regression. Significant ($p<0.05$) results in bold.(186)
BI: Barthel index; EQ-VAS: Euro-Quality of life visual analogue scale; HUS: health utility status (derived from EQ-5D); NIHSS: National Institutes of Health stroke scale; SSS: Scandinavian Stroke Scale; TICS-M: telephone interview cognition scale; t-MMSE: telephone mini mental state examination; ZDS: Zung depression scale

1.3.3 Mechanisms of action of glyceryl trinitrate (GTN) in acute stroke

If transdermal GTN is found to be beneficial in the management of acute stroke there are several potential mechanisms through which its actions may be mediated. First, BP lowering may reduce early recurrent events in IS (53) and haematoma expansion in ICH.(51) The ability of GTN to lower BP without reducing CBF or cerebral perfusion pressure, or increasing intracranial pressure, may be due to its vasodilatory effect thus increasing blood flow exiting the cranium.(183) Second, in addition to high SBP being associated with poor clinical outcomes in acute stroke, other haemodynamic variables including higher peak SBP, MAP, pulse pressure, pulse pressure index and increased SBP variability, are independently associated with worse functional outcome, death, recurrent stroke, and early neurological deterioration.(54-56) GTN's published effects on haemodynamic measures in acute stroke are detailed in Table 1.3. Pre-specified secondary analysis of haemodynamic parameters in ENOS is awaited.(189)

Third, the time-dependency of early treatment with GTN is akin to that seen in both thrombolysis and endovascular therapy, so-called reperfusion treatments.(24, 190) As described, GTN is a potent vasodilator, which may have effects in different parts of the vascular tree: large cerebral arteries, increasing 'front door' and peri-lesional perfusion without inducing cerebral steal;(183) and surface pial arteries, increasing collateral ('back door') perfusion.(191) Fourth, in

the context of thrombolysis, GTN may be synergistic through vasodilatation of the occluded or partially occluded artery, which may allow exogenous and endogenous thrombolytic compounds better access to clot. GTN also appears to prepare patients for thrombolysis by lowering systolic BP below the licensed threshold of 185 mmHg. A non-significant increase in both rates of thrombolysis and earlier treatment were seen with GTN in RIGHT.(83) Last, GTN's neuroprotective effects mediated via NO may prevent cell death from ischaemia.(163, 171)

1.4 THESIS AIMS

This thesis aims to explore the safety and efficacy of transdermal GTN in acute stroke both overall and in important patient subgroups, whilst exploring the mechanistic properties of this nitric oxide donor.

The following hypotheses will be explored in this thesis:

1. Transdermal GTN lowers BP in acute stroke.
2. Transdermal GTN is safe in acute stroke.
3. Transdermal GTN improves clinical outcomes when given within 6 hours of stroke onset.
4. Raised BP and its derivatives, including variability, are associated with poor clinical outcomes after acute stroke.
5. Transdermal GTN lowers BP and its derivatives, including variability in acute stroke.
6. Transdermal GTN increases heart rate in acute stroke.
7. Transdermal GTN is safe in the setting of acute stroke patients with blood markers of dehydration.
8. BP lowering with transdermal GTN is safe in acute stroke patients with ipsilateral carotid stenosis.
9. Transdermal GTN is safe in acute stroke patients with lacunar strokes and small vessel disease.
10. Transdermal GTN improves clinical outcomes when given within 6 hours of stroke onset in patients with lacunar strokes and small vessel disease.

CHAPTER 2:

NITRIC OXIDE DONORS (NITRATES), L-ARGININE, OR NITRIC OXIDE SYNTHASE INHIBITORS FOR ACUTE STROKE: A COCHRANE SYSTEMATIC REVIEW AND META-ANALYSIS

Publications contributing to this chapter:

Bath PM, Krishnan K, Appleton JP. Nitric oxide donors (nitrates), L-arginine, or nitric oxide synthase inhibitors for acute stroke. Cochrane Database Syst Rev 2017;4:CD000398

Presentations contributing to this chapter:

Nitric oxide donors (nitrates, L-arginine, or nitric oxide synthase inhibitors) for acute stroke: a Cochrane systematic review and meta-analysis. European Stroke Organisation Conference, Barcelona, Spain (May 2016)

Nitric oxide donors (nitrates, L-arginine, or nitric oxide synthase inhibitors) for acute stroke: a Cochrane systematic review and meta-analysis. British Hypertension Society Annual Scientific Meeting, Dun Laoghaire, Dublin, Ireland (September 2016)

Nitric oxide donors (nitrates, L-arginine, or nitric oxide synthase inhibitors) for acute stroke: a Cochrane systematic review and meta-analysis. UK Stroke Forum, Liverpool UK (November 2016)

2.1 ABSTRACT

Background

Nitric oxide (NO) has multiple effects that may be beneficial in acute stroke, including lowering blood pressure, and promoting reperfusion and cytoprotection. Some forms of nitric oxide synthase inhibition (NOS-I) may also be beneficial. However, high concentrations of NO are likely to be toxic to brain tissue. This is an update of a Cochrane review first published in 1998, and last updated in 2002.

Objectives

To assess the safety and efficacy of NO donors, L-arginine, and NOS-I in people with acute stroke.

Methods

We searched the Cochrane Stroke Group Trials Register (last searched February 2017), EMBASE (1980 to June 2016), MEDLINE (1966 to June 2016), ISI Science Citation Indexes (1981 to June 2016), Stroke Trials Registry (searched June 2016), International Standard Randomised Controlled Trial Number (ISRCTN, searched June 2016), Clinical Trials registry (searched June 2016) and International Clinical Trials Registry Platform (ICTRP, searched June 2016) for randomised controlled trials comparing nitric oxide donors, L-arginine, or NOS-I versus placebo or open control in patients within one week of onset of confirmed stroke. Two review authors independently applied the inclusion criteria, assessed trial quality and risk of bias, and extracted data. The review authors cross-checked data and resolved issues

through discussion. We obtained published and unpublished data, as available. Data were reported as mean difference (MD) or odds ratio (OR) with 95% confidence intervals (CI).

Results

We included five completed trials, involving 4197 participants; all tested transdermal glyceryl trinitrate (GTN), an NO donor. The assessed risk of bias was low across the included studies; one study was double-blind, one open-label and three were single-blind. All included studies had blinded outcome assessment. Overall, GTN did not improve the primary outcome of death or dependency at the end of trial (modified Rankin Scale (mRS) > 2, OR 0.97, 95% CI 0.86 to 1.10, 4195 participants, high-quality evidence). GTN did not improve secondary outcomes, including death (OR 0.78, 95% CI 0.40 to 1.50) and quality of life (MD -0.01, 95% CI -0.17 to 0.15) at the end of trial overall (high-quality evidence). Systolic/diastolic blood pressure (BP) was lower in people treated with GTN (MD -7.2 mmHg (95% CI -8.6 to -5.9) and MD -3.3 (95% CI -4.2 to -2.5) respectively) and heart rate was higher (MD 2.0 beats per minute (95% CI 1.1 to 2.9)). Headache was more common in those randomised to GTN (OR 2.37, 95% CI 1.55 to 3.62). We did not find any trials assessing other nitrates, L-arginine, or NOS-I.

Conclusions

There is currently insufficient evidence to recommend the use of NO donors, L-arginine or NOS-I in acute stroke, and only one drug (GTN) has been assessed. In people with acute stroke, GTN is safe, reduces

blood pressure, increases heart rate and headache, but does not alter clinical outcome.

2.2 INTRODUCTION

NO is one of the most versatile molecules in animal and human biology with diverse roles in both physiology and pathophysiology: it is a vasodilator with anti-smooth muscle cell activity;(155) protects endothelium; inhibits platelet adhesion and aggregation(156, 157) and leucocyte adhesion and chemotaxis,(108) and has other anti-inflammatory properties. Further, it is a neurotransmitter and neuromodulator,(158, 159, 192) and is involved in cerebral blood flow auto- and chemo-regulation.(160-162)

With the weight of evidence for NO-mediated neuroprotection post-stroke, several therapeutic strategies have been proposed: replacement or supplementation of NO deficiency through administration of NO, NO donors or precursors; enhancement of eNOS activity to modulate or increase endogenous NO production;(193) and direct or indirect inhibition of NOS.

Vascular [NO] levels are low in acute stroke(179, 180) and so replacement might be beneficial. NO, however delivered, may have multiple effects that could improve outcome after stroke: BP lowering; reperfusion; and cytoprotection. NO donors could also have adjunctive properties enhancing the effects of existing reperfusion therapies: by lowering BP, NO donors may prepare people with hyper-acute ischaemic stroke for thrombolysis; by dilating cerebral arteries around occluding clot, NO donors may enhance access to the clot by both endogenous and exogenous fibrinolysis.

Evidence-based treatments for acute stroke are limited. In hyper-acute ischaemic stroke, there are interventions with high efficacy but limited utility (intravenous thrombolysis(24)), thrombectomy(19) and decompressive hemicraniectomy(27)), and those with low efficacy but high utility (aspirin (26)). In comparison, there are no definitive treatments for ICH other than early BP lowering.(130) All patients with stroke should have access to stroke unit care.(25)

We aimed to assess the safety and efficacy of NO donors, L-arginine, or NOS-I in people with acute stroke.

2.3 METHODS

Hypotheses tested in this chapter

- Transdermal GTN lowers BP in acute stroke.
- Transdermal GTN is safe in acute stroke.
- Transdermal GTN improves clinical outcomes when given within 6 hours of stroke onset.

2.3.1 Criteria for considering studies for this review

Published and unpublished randomised controlled trials (RCTs) of NO donors, L-arginine, or NOS-I by any route of administration versus placebo or open control including:

- Randomisation within one week of ictus
- IS or ICH

Studies of people with subarachnoid haemorrhage, uncontrolled studies and confounded controlled studies - where two or more active interventions were compared - were excluded.

The primary outcome was end of trial death or dependency, defined as the mRS >2. Secondary outcomes were as follows:

- First blood pressure and heart rate measurements after randomisation
- Early case fatality (end of treatment)
- Late case fatality (end of trial)

- Early neurological deterioration by end of treatment defined as a decrease in the Scandinavian Stroke Scale (SSS) by >5 points or a decrease in the consciousness part of the SSS by >2 points
- Headache on treatment
- Treatment stopped early
- NIHSS at end of treatment derived from SSS(194)
- Late dependency or disability defined as end of trial Barthel index (BI)
- Mood end of trial using the short-form Zung Depression Scale(195)
- Quality of life end of trial using health utility status derived from European Quality of life-5 dimensions-3 levels (EQ5D3L) and EQ-visual analogue scale (EQ-VAS)
- Cognition end of trial using telephone Mini-Mental State Examination (t-MMSE, subscore derived from MMSE), telephone interview cognition scale (TICS) and verbal fluency (animal naming)
- Physiotherapy during hospital stay
- Occupational therapy during hospital stay
- Speech and language therapy during hospital stay
- Feeding route (non-oral feeding at day 7)
- Length of stay (days)

2.3.2 Search methods

We searched the Cochrane Stroke Group Trials Register (last searched in February 2017), EMBASE (Ovid) (1980 to June 2016) (Appendix 10.1), MEDLINE (Ovid) (1966 to June 2016) (Appendix 10.1), Science Citation Index (ISI, Web of Science, 1900 to June 2016) (Appendix 10.1), Stroke Trials Registry (www.strokecenter.org/trials) (searched June 2016), International Standard Randomised Controlled Trial Number (ISRCTN) registry (www.controlled-trials.com) (searched June 2016), Clinical Trials registry (www.clinicaltrials.gov) (searched June 2016) and International Clinical Trials Registry Platform (ICTRP) (www.apps.who.int/trialsearch/) (searched June 2016).For the 2002 version of this review we contacted pharmaceutical companies who make NO donors (Schwarz Pharma, Schering-Plough) or NOS-I (GlaxoWellcome) and researchers in the field to help identify relevant trials.

We extracted data using a standard proforma, which one review author (JA) entered into Review Manager 5 and another review author (KK) checked. One author (JA) screened the outputs from the electronic searches, excluding irrelevant studies. We obtained full paper copies of the remaining studies and two review authors (JA and PB) selected studies based on the aforementioned inclusion criteria. Any disagreements were resolved through discussion.

We assessed risk of bias in the included studies using Cochrane's 'Risk of bias' tool, which is based on the following domains:

- Random sequence generation.
- Allocation concealment.
- Blinding of participants and personnel.
- Blinding of outcome assessment.
- Incomplete outcome data.
- Selective reporting.
- Other sources of bias.

Each domain includes one or more specific entries in a 'Risk of bias' table. Within each entry, the first part of the tool describes what was reported to have happened in the study in sufficient detail to support a judgement on the risk of bias. The second part assigns a judgement relating to the risk of bias: 'Low risk' of bias, 'High risk' of bias, or 'Unclear risk' of bias.

The weighted estimate of the typical treatment effect across trials was calculated using RevMan 5.3. Odds ratios (OR) were calculated using the Mantel-Haenszel random-effects model for binary data, and mean difference (MD) using the random-effects inverse variance method for continuous data, all with 95% confidence intervals (CI).

Since many scales include a value for people who have died (e.g. mRS =6, health utility status =0, Barthel index =-5), extreme worst values were assigned for death for mood (short-form Zung depression scale(195)) =102.5; EQ-VAS =-1; t-MMSE =-1; TICS =-1; animal naming =-1. Where secondary outcomes were not assessed, trials were excluded from analysis of that particular outcome.

Extensive attempts were made to find missing data including utilising unpublished data from authors. Heterogeneity between RCT results was calculated using the I^2 statistic based upon the DerSimonian-Laird formula. Heterogeneity was considered to be significant if I^2 was greater than 50%; if present, potential reasons were sought, e.g. different trial designs.

The primary and secondary outcomes were reviewed in the following pre-specified subgroups:

- Intervention type
- Type of stroke: IS, ICH
- Time from stroke onset to randomisation: ≤6 hours; 6.1-12 hours; 12.1-24 hours; 24.1-36 hours; and >36 hours
- Baseline systolic blood pressure (SBP): ≤160 mmHg; 160.1-180 mmHg; 180.1-200 mmHg; >200 mmHg

Since ordinal or continuous analyses are more sensitive to treatment effects than dichotomous measures,(196) comparison of the primary outcome, mRS, was assessed as MD in addition to OR. Unfortunately, ordinal analysis (a more appropriate statistical approach) is not available in Revman software.

2.4 RESULTS

Figure 2.1 shows the PRISMA study flow diagram. Of the 4772 records identified and screened, we excluded 4750. After full-text review of the remaining 22 studies, we excluded 16 studies including four ongoing studies that did not meet eligibility criteria. One study met inclusion criteria but is currently ongoing. Therefore, we included five trials, which are completed and summarised in Characteristics of included studies (Tables 2.1).

We identified five trials that fulfilled the inclusion criteria.(83, 84, 110, 182, 183) All 5 trials involved administration of transdermal glyceryl trinitrate (GTN) 5-10 mg daily via a patch. Individual patient data were provided for all five studies totaling 4,197 participants. All studies recruited participants with mixed stroke (IS and ICH). One of the trials was multicentre and international,(84) whilst the remainder were UK-based single centre studies.

Figure 2.1: PRISMA study flow diagram

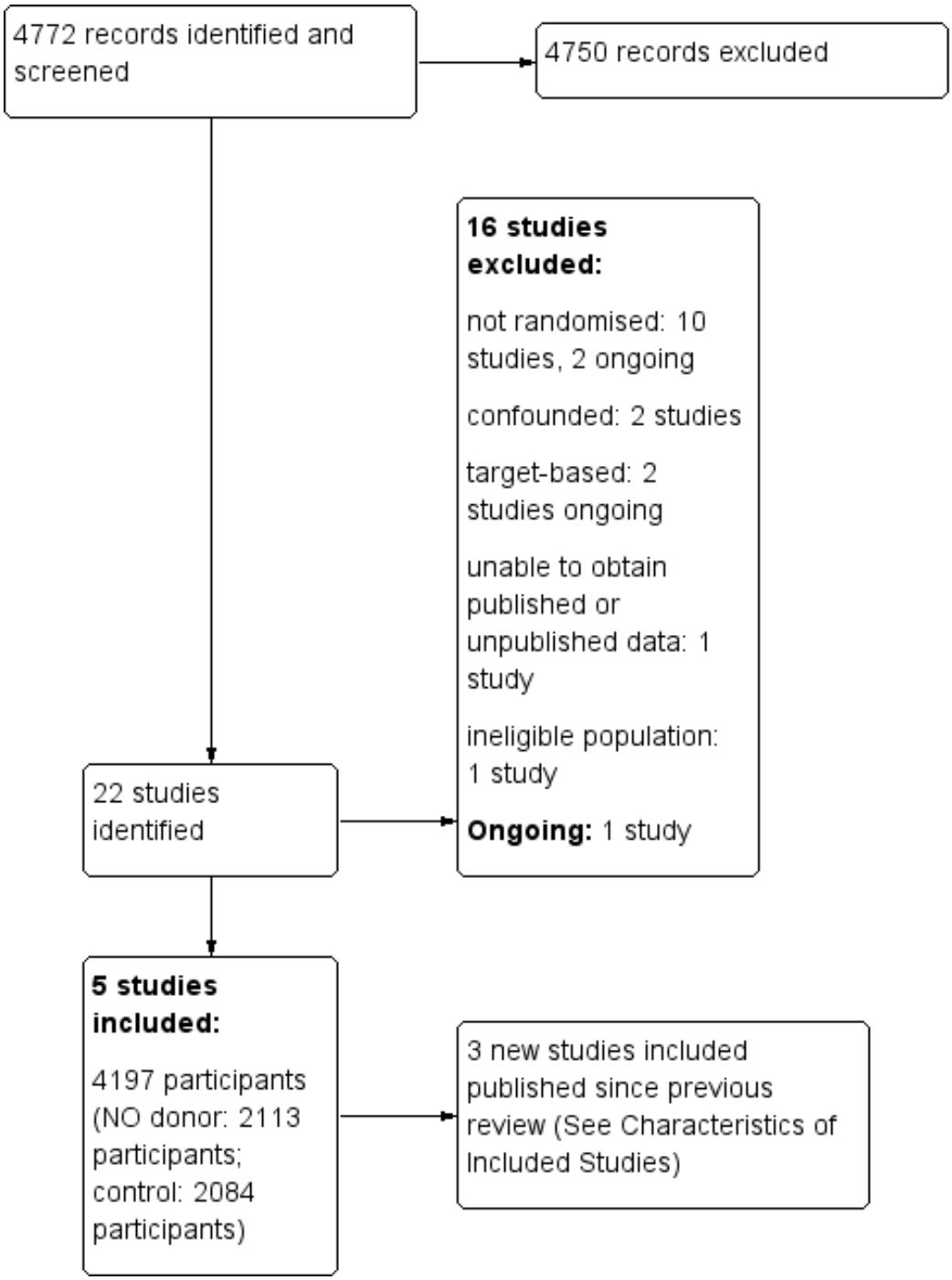


Table 2.1: Characteristics of included studies

	GTN-1	GTN-2	GTN-3	ENOS	RIGHT
Methods	Double-blind, placebo-controlled Randomisation by computer (minimisation by age, mean arterial BP, baseline SSS, hours from onset, presence of stroke lesion on CT)	Open-label, blinded endpoint dose comparison controlled trial Randomisation by computer (minimisation by age, gender, SSS, mean arterial pressure)	Participant- and measurement-blinded RCT Randomisation by computer (2:1, minimisation by age, sex, baseline systolic BP, baseline SSS, time from onset, presence of stroke lesion on CT)	Single-blind, parallel-group, partial factorial study Randomisation via password-protected, data-encrypted website (minimisation by age, sex, stroke severity, time to treatment and total anterior circulation syndrome)	Single-blind, blinded endpoint Randomisation (1:1)
Participants	UK, single centre 37 participants: treatment 16; control 21 Age: treatment 76 years; control 72 years Male: treatment 6 (38%); control; 12 (57%) Inclusion: IS or ICH Time to randomisation: within five days of onset	UK, single centre 90 participants: treatment 60; control 30 Age: treatment 70.8 years; control 73.9 years Male: treatment 28 (46.7%); control 13 (43.3%) Inclusion: IS or ICH Time to randomisation: within 72 hours of onset	UK, single centre 18 participants: treatment 12; control 6 Age: treatment 69 years; control 70 years Male: treatment 2 (16.7%); control 3 (50%) Inclusion: IS or ICH; previously independent; clinical stroke syndrome and limb weakness Time to randomisation: within 5 days of onset	International (23 countries), multicentre (173 sites) 4011 participants: treatment 2000; control 2011 Age: treatment 70 years; control 70 years Male: treatment 1147 (57%); control 1150 (57%) Inclusion: IS or ICH; motor deficit in armor leg, or both; systolic BP 140 to 220 mmHg Time to randomisation: within 48 hours of onset	UK, single centre 41 participants: treatment 25; control 16 Mean age: treatment 79 years; control 81 years Male: treatment 15 (65%); control 7 (43.8%) Inclusion: FAST positive test; systolic BP _ 140 mmHg; age > 40 years for men and > 55 years for women Time to randomisation: within 4 hours of onset
Interventions	Treatment: transdermal GTN patch 5 mg once daily	Treatment: transdermal GTN patch 5 mg for 10 days; 5 mg for 4 days, then 10 mg for	Treatment: transdermal GTN patch 5 mg once daily Control: no patch	• Treatment: transdermal GTN patch 5 mg once daily	Treatment: transdermal GTN patch 5 mg once daily Control: no patch

	Control: matching placebo Duration: 12 days	6 days; 10 mg for 10 days Control: no patch Duration: 10 days	(Blinding with gauze dressing over patch/equivalent area of skin) Duration: 7 days	• Control: no GTN (blinding with gauze dressing over patch/equivalent area of skin) In appropriate participants: • Treatment: continue pre-stroke antihypertensive medication • Control: stop pre-stroke antihypertensive medication Duration: 7 days	(Blinding with gauze dressing over patch/equivalent area of skin) Duration: 7 days
Outcomes	Primary: BP on day 1 (24 hour ambulatory BP measured 3 times per hour during the day and hourly at night at days 0, 1, 8) Secondary: death at end of treatment; death or dependency (mRS > 2), death, and BI at 3 months	Primary: BP on day 1 (24 hour ambulatory BP measured 3 times per hour during the day and hourly at night at days 0, 1, 4, 5, 10) Secondary: death at end of treatment; death or dependency (mRS > 2), death, BI and QoL at 3 months	Primary: BP (immediately before the baseline Xenon-CT and immediately after the posttreatment scan), cerebral blood flow (Xenon-CT and transcranial doppler) and cerebral perfusion pressure (transcranial doppler) Secondary: • days 1 to 7: BP and HR • day 7: SSS, recurrent stroke, death, neurological deterioration	Primary: mRS at day 90 Secondary: • days 1 to 7: BP and HR • day 7: recurrent stroke • discharge: length of hospital stay; disposition • day 90: death or dependency (mRS > 2); BI; EQ-5D, EQ-VAS; MMSE, TICSM; animal naming; Zung Depression Scale	Primary: systolic BP at 2 hours Secondary: • 15 minutes: systolic BP, diastolic BP, HR • Day 7: SSS; recurrent stroke; death; hypotension; neurological deterioration (5-point decrease in SSS) • Day 90: mRS; BI; EQ-5D, EQ-VAS; MMSE; Zung Depression Scale

			• day 90: mRS, BI, EQ-5D, EQ-VAS, MMSE, Zung Depression Scale		
Notes	Exclusion: taking part in another trial	Exclusion: systolic BP > 230 or < 100 mmHg; diastolic BP > 130 or < 60 mmHg; HR > 130 or < 50 bpm; mild stroke; coma; premorbid dependence (mRS _ 3); requirement for or contraindication to nitrate therapy; presence of illness that could confound neurological or functional evaluation Any antihypertensive medication was stopped on admission and recommenced after 10 days	Exclusion: requirement for or contraindication to nitrate therapy; definite need for previous antihypertensive or vasoactive drugs; unable to cooperate with scanning Prior antihypertensive medication was stopped on admission	Exclusion: GCS < 8; pure sensory stroke; preceding dependency (mRS 3 to 5); confounding neurological or psychiatric illness; stroke mimic; severe liver or renal dysfunction; severe comorbidity; pregnant or breastfeeding; planned surgical intervention; previous participation in ENOS; contraindication to or definite need for nitrates and/ or prestroke antihypertensive medication	Exclusion: requirement for or contraindication to nitrate therapy; GCS <8; blood glucose <2.5 mmol/L; non-ambulatory prior to symptom onset

BI: Barthel Index; bpm: beats per minute; BP: blood pressure; CT: computed tomography; DBP: diastolic blood pressure; ENOS: Efficacy of Nitric Oxide in Stroke trial; EQ-5D: European Quality of life - 5 Dimensions; EQ-VAS: European Quality of life - Visual Analogue Scale; GCS: Glasgow Coma Scale; GTN: glyceryl trinitrate; HR: heart rate; ICH: intracerebral haemorrhage; IS: ischaemic stroke; MMSE: Mini Mental State Examination; mRS: modified Rankin Scale; QoL: quality of life; RIGHT: Rapid Intervention with Glyceryl trinitrate in hypertensive stroke trial; SBP: systolic blood pressure; SSS: Scandinavian Stroke Scale; TICS-M: Modified Telephone Interview Cognition Scale

Trial recruitment occurred at different time intervals following stroke:

- Within 4 hours of onset: RIGHT (83)
- Within 48 hours of onset: ENOS(84)
- Within 72 hours of onset: GTN-2(182)
- Within 168 hours of onset: GTN-1 and 3(110, 183)

The duration of the intervention period varied:

- 7 days: RIGHT, ENOS, GTN-3(83, 84, 183)
- 10 days: GTN-2(182)
- 12 days: GTN-1(110)

Four studies used transdermal GTN patch 5 mg once daily(83, 84, 110, 183), whilst Rashid et al(182) adopted a different strategy with three treatment groups: transdermal GTN patch 5 mg once daily for 10 days; 5 mg for 4 days then 10 mg for 6 days; and 10 mg for 10 days. For this review, as with a previous version (2002),(197) we combined the three treatment arms into one group. All studies detailed the equipment used and patient posture when blood pressure recordings were performed.

Randomisation can be summarised as follows:

1. Simple randomisation 1:1(83)
2. Computer minimisation 1: 1(84, 110, 182)
3. Computer minimisation 2: 1(183)

Participants and investigators were blinded to treatment as follows:

- Double-blind (participant and investigator): one trial(110)
- Single-blind (participant): three trials(83, 84, 183)
- Open-label: one trial(182)

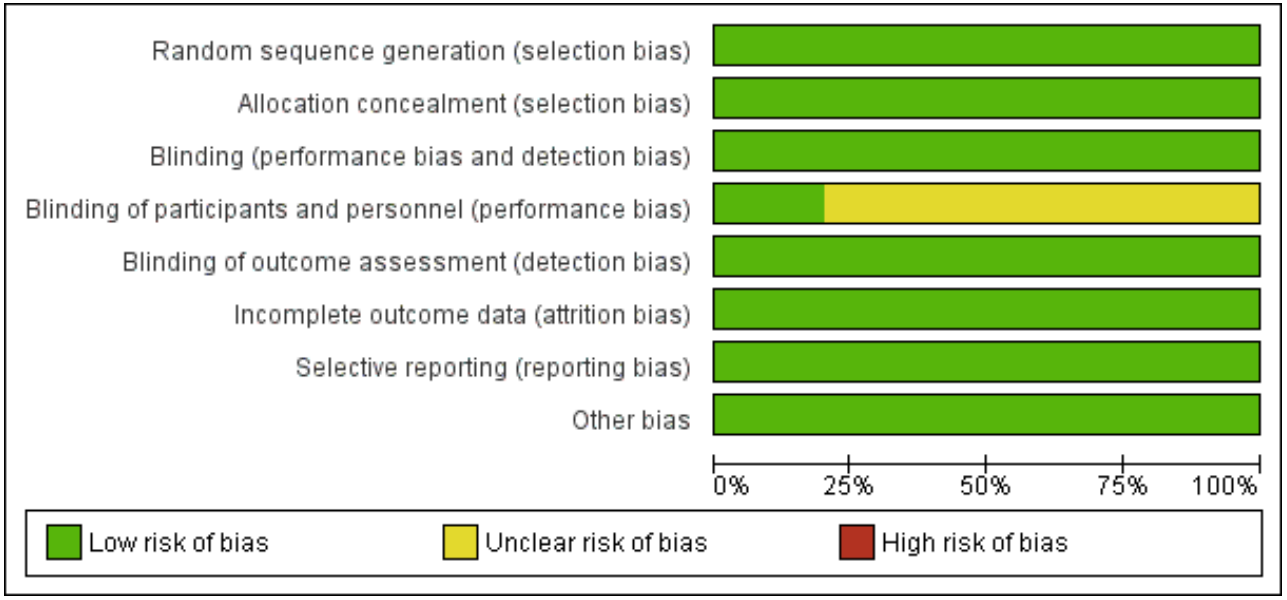
All five included trials were analysed by intention-to-treat. Risk of bias in the included studies is summarised in Table 2.2 and Figure 2.2.

Table 2.2: Risk of bias in included studies

	GTN-1	GTN-2	GTN-3	ENOS	RIGHT
Random sequence generation (selection bias)	Low risk: Computer randomisation with minimisation	Low risk: Computer randomisation with minimisation	Low risk: Computer randomisation with minimisation	Low risk: Central computer randomisation with minimisation	Low risk: Simple randomisation. Equal distribution between trial groups
Allocation concealment (selection bias)	Low risk: Computer randomisation with minimisation	Low risk: Computer randomisation with minimisation	Low risk: Computer randomisation with minimisation	Low risk: Central website-based	Low risk: Opaque envelope containing a gauze dressing +/- GTN patch was only opened after consent was obtained
Blinding (performance bias and detection bias)	Low risk: Matching treatment and placebo patches	Low risk: GTN was given single-blinded (participant), whilst outcomes were assessed blinded to treatment group	Low risk: GTN was given single-blinded (participant), whilst outcomes were assessed blinded to treatment group	Low risk: GTN was given single-blinded (participant), whilst outcomes were assessed blinded to treatment group	Low risk: GTN was given single-blinded (participant), whilst outcomes were assessed blinded to treatment group
Blinding of participants and personnel (performance bias)	Low risk: Matching treatment and placebo patches	Unclear risk: Open-label as no matching treatment and placebo patches were available	Unclear risk: GTN was given single-blinded as no placebo patches were available. A gauze dressing was placed over the GTN patch or equivalent area of skin out of sight. As a result, participants were blinded whilst the treating clinician was unblinded	Unclear risk: GTN was given single-blinded as no placebo patches were available. A gauze dressing was placed over the GTN patch or equivalent area of skin out of sight. As a result, participants were blinded whilst the treating clinician was unblinded	Unclear risk: GTN was given single-blinded as no placebo patches were available. A gauze dressing was placed over the GTN patch or equivalent area of skin out of sight. As a result, participants were blinded whilst the treating clinician was unblinded
Blinding of outcome	Low risk: Outcomes assessed blinded to treatment group	Low risk: Outcomes assessed blinded to treatment group	Low risk: Outcomes assessed blinded to treatment group	Low risk: Outcomes assessed centrally, blinded to	Low risk: Outcomes assessed blinded to treatment group

assessment (detection bias)				treatment group	
Incomplete outcome data (attrition bias)	Low risk: No differences between trial groups	Low risk: No differences between trial groups	Low risk: All participants accounted for	Low risk: No differences between trial groups	Low risk: All participants accounted for. No differences between trial groups
Selective reporting (reporting bias)	Low risk: All prespecified outcomes reported	Low risk: All prespecified outcomes reported	Low risk: All prespecified outcomes reported	Low risk: All prespecified outcomes reported	Low risk: All prespecified outcomes reported
Other bias	Low risk: None found	Low risk: None found	Low risk: None found	Low risk: None found	Low risk: None found

Figure 2.2: Risk of bias graph of included studies



Primary outcome

Death or dependency was assessed as mRS >2 at day 90 in all 5 included trials totaling 4195 participants (Table 2.3). There was no significant difference between GTN and control (OR 0.97, 95% CI 0.86 to 1.10, Figure 2.3), and no evidence of heterogeneity (I^2 = 0%). When assessed as mean mRS, there was no significant difference between GTN and control (MD -0.08, 95% CI -0.52 to 0.36, I^2 = 47%). There was no differential effect by stroke type. Those patients randomised to GTN within 6 hours of stroke onset (2 trials, n=312) had a tendency toward less death and dependency (OR 0.65, 95% CI 0.41 to 1.02; p=0.06; I^2 = 0%). Randomisation at more than 6 hours after ictus did not influence the primary outcome (Figure 2.4). In the sensitivity analysis, mean mRS was lower in those randomised to GTN within 6 hours of onset as compared with control participants (MD -0.79, 95% CI -1.35 to -0.23; p=0.005); an effect not seen in any other time period from randomisation although the interaction between time and mRS was significant (p=0.05) (Figure 2.5). There was no differential effect by baseline systolic BP.

Figure 2.3: Forest plot of primary outcome (mRS >2, end of trial) GTN vs. no GTN

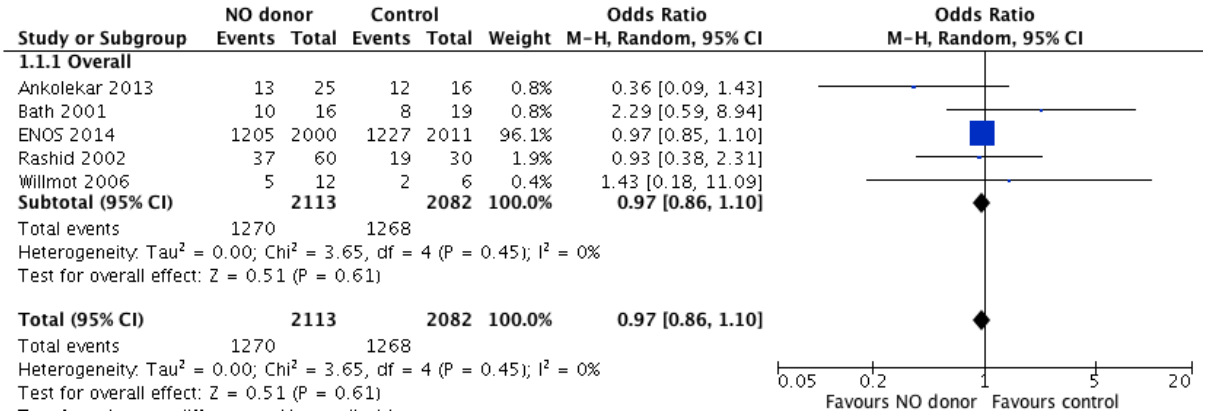


Figure 2.4: Forest plot of primary outcome (mRS >2, end of trial) GTN vs. no GTN, by time to randomisation

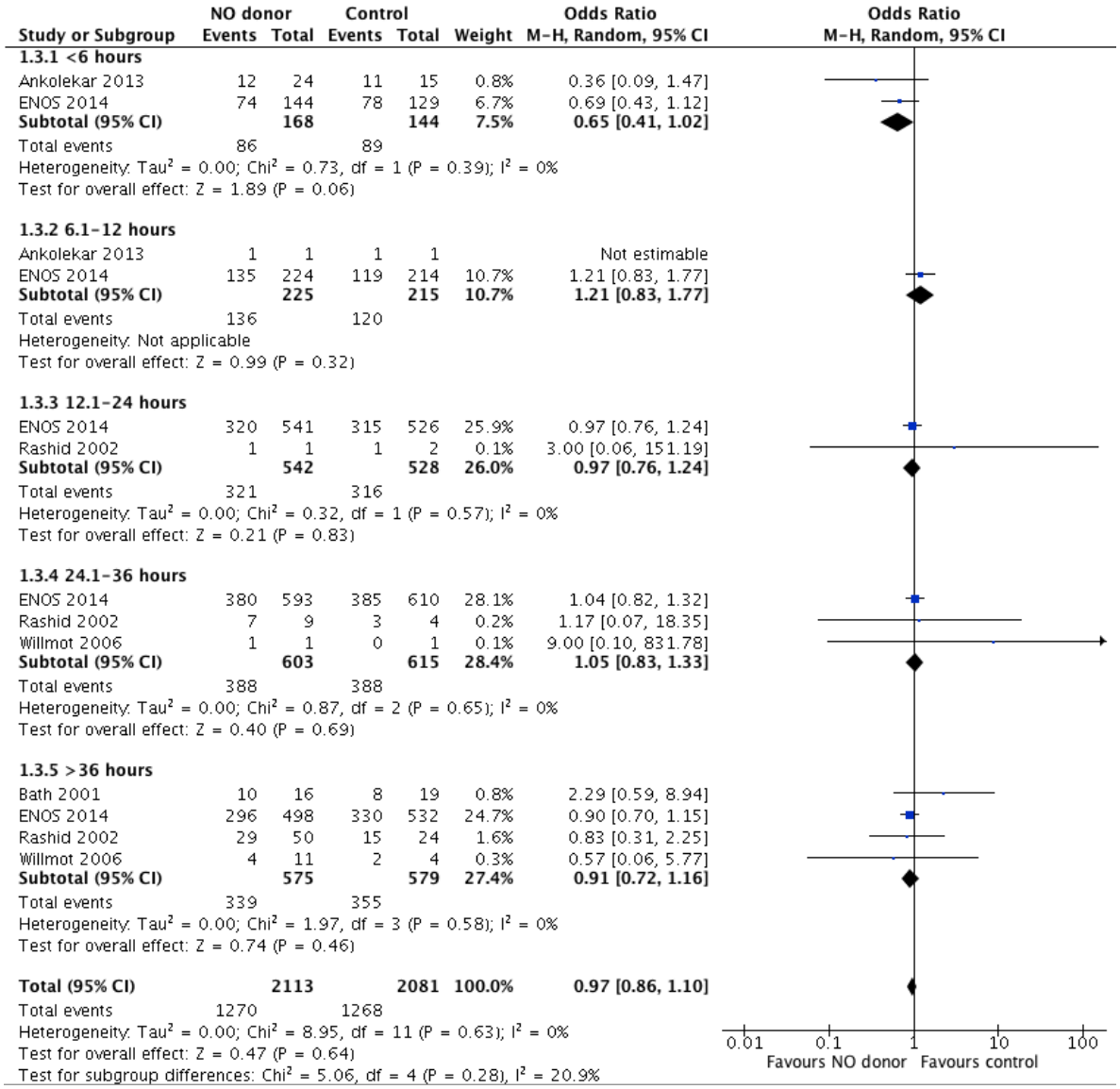
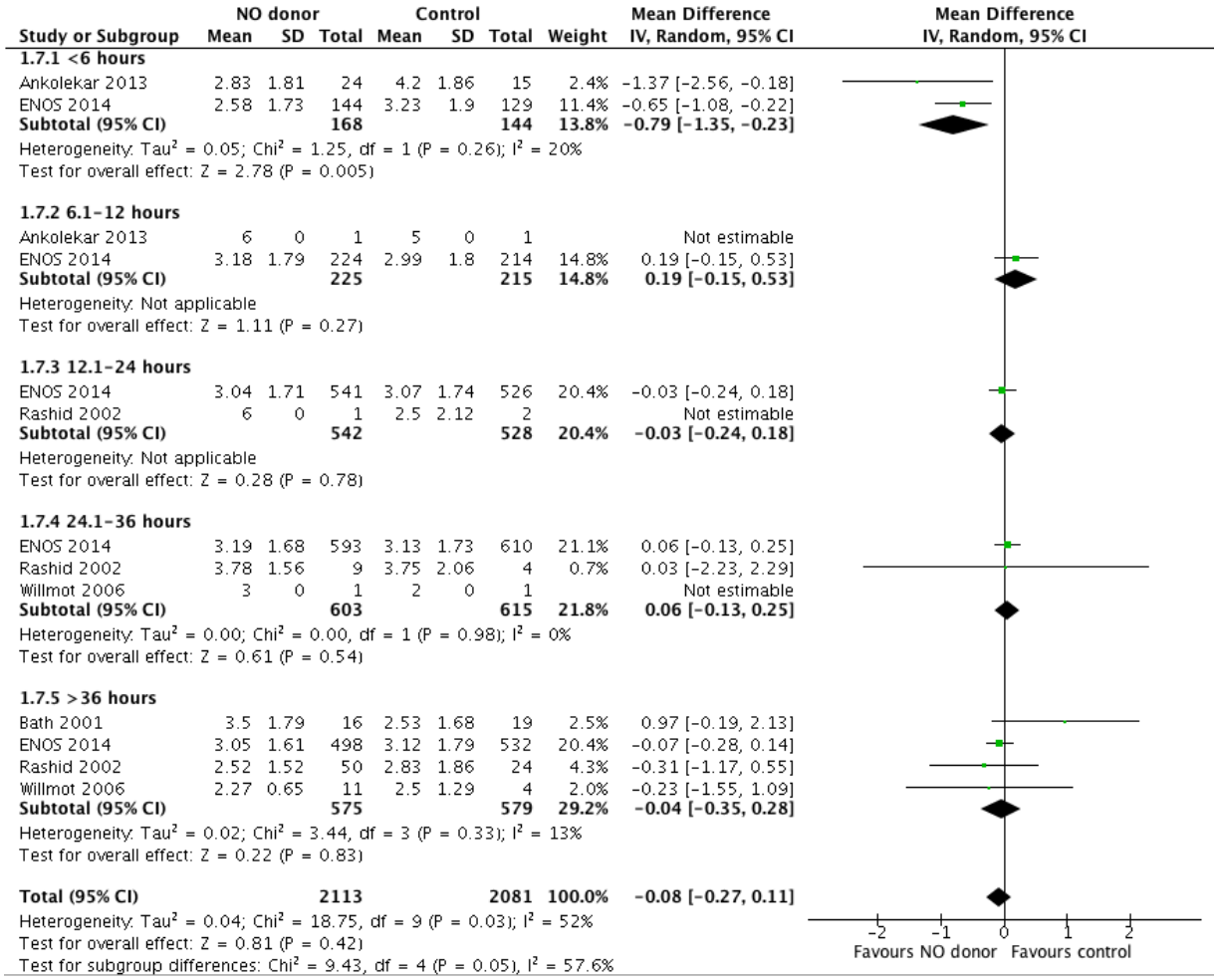


Figure 2.5: Forest plot of mean mRS end of trial GTN vs. no GTN, by time to randomisation



Secondary outcomes

Clinical outcomes are summarised in Table 2.3 with further data available in the main publication.(198) There was no significant treatment effect of GTN compared with control on death, whether measured early or end of trial (n=4197). No significant differences were noted in any subgroups for death, end of treatment, whilst death, end of trial was reduced in those randomised to GTN within 6 hours of stroke onset (OR 0.30, 95% CI 0.15 to 0.60; p=0.0006; I^2 = 0%) with significant interaction between time and death (p=0.01).

No significant difference was noted in early neurological deterioration between treatment and control in the four studies with data (n=4158). Stroke type did not change the neutral effect seen overall. Those randomised between 24 and 36 hours to GTN in one study (n=1218, two studies were not estimable due to no events) had a higher likelihood of neurological deterioration than control participants (OR 2.00, 95% CI 1.19 to 3.35; p=0.009). The other time groups revealed no difference between GTN and control. Participants randomised to GTN with a baseline SBP of <160 mmHg (n=1688) or 180-200 mmHg in two studies (n=743, two studies were not estimable due to no events) were more likely to have neurological deterioration end of treatment than controls (OR 1.62, 95% CI 1.05 to 2.50, p=0.03 and OR 1.81, 95% CI 1.05 to 3.12, p=0.03 respectively).

NIHSS was calculated using the SSS available from four trials (n=4137). There was no significant difference between treatment and control. Participants randomised to GTN within 6 hours of stroke onset had lower NIHSS scores end of treatment compared with control (MD -2.07, 95% CI -3.81 to -0.34, p=0.02), with no heterogeneity seen between the two trials and significant interaction between time and NIHSS (p=0.03).

Disability data in the form of BI was available for all five studies (n=4153) with no significant difference detected between GTN and control. Those randomised to GTN within 6 hours of stroke ictus (2 trials, n=312) had improved disability scores compared to control participants (MD 14.57, 95% CI 5.95 to 23.19, p=0.0009), with no heterogeneity seen between the studies, and significant interaction between time and BI (p=0.03).

Mood was assessed in three studies (n=3312) using the Zung depression scale. No significant difference was seen between GTN and control. Improved mood scores were seen in those randomised to GTN within 6 hours of index event compared with control participants (MD -11.12, 95% CI -17.35 to -4.90, p=0.0005) with no heterogeneity seen between the 2 studies (n=268), and significant interaction between time and ZDS (p=0007).

Two measures of quality of life were assessed: the EQ5D was transformed into a health utility status from data available in four studies (n=4088); and the EQ-VAS from four studies (n=3575). There was no significant difference between treatment and control for either

measure. Early treatment with GTN within 6 hours of onset was associated with improved quality of life at day 90 compared to those randomised to control: EQ5D MD 0.11, 95% CI 0.02 to 0.20, p=0.02, with minimal heterogeneity (I^2 = 3%); and EQ-VAS (2 trials, n=295) MD 9.96, 95% CI 2.49 to 17.43, p=0.009 with no heterogeneity. Across time to randomisation, there was a significant interaction with EQ-VAS (p=0.04), but not EQ-5D (p=0.10).

Three measures of cognition were assessed: three studies had t-MMSE data (n=2078, in two studies t-MMSE was derived from MMSE); one study also used TICS and animal naming.(84) Overall there was no significant difference between GTN and control. Improved cognitive scores were seen across all three measures in those randomised to GTN within 6 hours of onset: t-MMSE MD 3.61, 95% CI 1.68 to 5.55, p=0.0002 (2 trials, n=219, interaction p=0.003); TICS MD 5.59, 95% CI 2.63 to 8.55, p=0.0002 (1 trial, n=182, interaction p=0.002); and animal naming MD 2.94, 95% CI 0.88 to 5.00, p=0.005 (1 trial, n=192, interaction p=0.04).

Data on whether individuals received physiotherapy, occupational therapy or speech and language therapy during their admission were available from three studies (n=4042). There was no difference between treatment groups on the rates of therapy required. Rates of occupational therapy were lower, with borderline significance, in those randomised to GTN with baseline SBP 180.1-200 mmHg compared with control participants (OR 0.74, 95% CI 0.54 to 1.00, p=0.05), with no heterogeneity seen between the three studies (n=730), and

higher in those randomised to GTN with baseline SBP >200 mmHg compared with controls (OR 2.11, 95% CI 1.25 to 3.54, p=0.005), with no heterogeneity between the two studies (n=255). No other associations regarding baseline SBP were noted for rates of either physiotherapy or speech and language therapy. Neither time to randomisation nor stroke type had any effect on therapy rates between treatment and control.

Feeding route was recorded in three trials (n=4009) and assessed as non-oral feeding at day 7. Overall, there was no difference between GTN versus no GTN regarding rates of non-oral feeding at day 7. Early treatment with GTN within 6 hours of onset was associated with a non-significant tendency towards less non-oral feeding (OR 0.59, 95% CI 0.32 to 1.08, p=0.09), with no heterogeneity seen between the 2 studies (n=301). No associations of note were seen regarding other time groups from randomisation, stroke type or baseline SBP.

Headache on treatment was more common in patients randomised to GTN than to control (OR 2.37, 95% CI 1.55 to 3.62; n=4186), with no significant heterogeneity seen. GTN led to increased reporting of headache on treatment in both IS (OR 2.39, 95% CI 1.59 to 3.61, p<0.0001; n=3409; I^2 = 4%) and ICH (OR 1.91, 95% CI 1.24 to 2.93, p=0.003; n=639; I^2 = 0%) compared with control. Headache was more common in participants randomised to GTN than controls for all time periods of randomisation and all baseline SBP subgroups except >200 mmHg, which was neutral.

There was no significant difference in the rate of treatment being stopped early between those randomised to GTN or control (n=4193), although there was significant heterogeneity between trials (I^2=88%). Treatment was more likely to be stopped in those randomised to GTN after 6, and before 36, hours but these groups were dominated by ENOS.(84) Stroke type and baseline SBP did not alter the overall neutral effect seen.

Haemodynamics are the first measurements on treatment (n=4197). BP (mmHg) was significantly lowered in those randomised to GTN compared with control (systolic BP: MD -7.21, 95% CI -8.58 to -5.85; diastolic BP: MD -3.31, 95% CI -4.18 to -2.45), whilst heart rate (beats per minute) was significantly increased (MD 2.02, 95% CI 1.13 to 2.91). Of note, for heart rate there was significant heterogeneity (I^2 = 61%) between trials. Regardless of stroke type, BP was significantly lowered by GTN compared with control, and heart rate was increased. BP was significantly lowered in those randomised to GTN compared with control in all time to randomisation groups, whilst heart rate was significantly increased in those randomised to GTN after 24, and before 36, hours (MD 3.44, 95% CI 1.79 to 5.09; n=1218; participants = 1218; I^2 = 32%), with non-significant results seen in other time groups.

Table 2.3: Clinical outcomes GTN vs. no GTN, overall and in those randomised within 6 hours of onset

Clinical outcomes	GTN overall	Randomisation ≤6hours
Patients	4197	312
On treatment		
SBP (mmHg)	**-7.21 (-8.58, -5.85)**	**-9.52 (-14.28, -4.75)**
DBP (mmHg)	**-3.31 (-4.17, -2.45)**	-2.31 (-5.53, 0.90)
HR (bpm)	**2.02 (1.13, 2.91)**	1.89 (-1.62, 5.40)
Headache	**2.37 (1.55, 3.62)**	**2.22 (1.10, 4.45)**
Treatment stopped early	1.84 (0.27, 12.42)	1.03 (0.05, 22.27)
End of treatment		
Death	1.09 (0.76, 1.56)	0.82 (0.28, 2.43)
Neurological deterioration	1.24 (0.97, 1.60)	0.57 (0.28, 1.16)
NIHSS	-0.28 (-0.70, 0.14)	**-2.07 (-3.81, -0.34)**
Day 7		
Non-oral feeding	0.97 (0.82, 1.15)	0.59 (0.32, 1.08)
Hospital		
Length of stay (days)	1.29 (-6.21, 8.78)	-1.55 (-6.30, 3.21)
Physiotherapy	0.94 (0.79, 1.12)	0.90 (0.40, 2.05)
Occupational therapy	1.01 (0.72, 1.41)	1.15 (0.36, 3.68)
Speech therapy	0.95 (0.84, 1.08)	1.01 (0.44, 2.29)
End of trial		
mRS >2	0.97 (0.86, 1.10)	0.65 (0.41, 1.02)
mean mRS	-0.08 (-0.52, 0.36)	**-0.79 (-1.35, -0.23)**
Death	0.78 (0.40, 1.50)	0.30 (0.15, 0.60)
Barthel Index	-0.09 (-5.39, 5.20)	**14.57 (5.95, 23.19)**
Mood (ZDS)	-0.83 (-4.44, 2.79)	**-11.12 (-17.35, -4.90)**
Quality of life (HUS)	-0.01 (-0.17, 0.15)	**0.11 (0.02, 0.20)**
Quality of life (EQ-VAS)	1.12 (-0.91, 3.15)	**9.96 (2.49, 17.43)**
Cognition (t-MMSE)	1.13 (-1.77, 4.03)	**3.61 (1.68, 5.55)**
Cognition (TICS-M)	0.30 (-0.63, 1.23)	**5.59 (2.63, 8.55)**
Cognition (animal naming)	0.10 (-0.53, 0.73)	**2.94 (0.88, 5.00)**

Data are odds ratio (OR) or mean difference (MD) with 95% confidence intervals (CI). Significant results in bold ($p<0.05$). Bpm: beats per minute; DBP: diastolic blood pressure; EQ-VAS: EuroQoL-visual analogue scale; HR: heart rate; HUS: health utility status; mRS: modified Rankin Scale; NIHSS: National Institute of Health Stroke Scale; SBP: systolic blood pressure; t-MMSE: telephone mini-mental state examination; TICS-M: telephone interview cognition scale; ZDS: Zung depression scale

2.5 DISCUSSION

Five trials involving 4,197 participants assessed transdermal GTN in acute stroke. Overall, GTN did not significantly influence clinical outcomes including death or disability, death, neurological deterioration, severity, mood, quality of life, cognition, length of stay, therapy requirements or feeding route.

Participants randomised to GTN with baseline SBP <160 or 180-200 mmHg had increased rates of neurological deterioration compared with controls. Conversely, receiving GTN with a baseline SBP 180-200 mmHg was associated with lower rates of occupational therapy but higher rates were seen with a baseline SBP >200 mmHg. There were no consistent effects across outcomes regarding baseline SBP. Participants randomised to GTN within 6 hours of symptom onset had non-significant reductions in the rates of death or dependency (mRS>2) and non-oral feeding, and significant improvements in NIHSS at end of treatment, and death or dependency (mean mRS), death, disability, mood, quality of life, and cognition end of trial. Although participants randomised to GTN between 24 and 36 hours after stroke onset had an increased chance of neurological deterioration, no significant findings were noted in any other clinical outcomes for this time period.

In respect of haemodynamics, BP was significantly lowered by GTN as compared with control regardless of stroke type and time to randomisation, whilst heart rate was significantly increased overall,

regardless of stroke type, and in those randomised between 24 and 36 hours after stroke onset. Headache, a recognised side effect of GTN, was more commonly seen in those randomised to GTN overall, in both stroke types, at all times of randomisation and in all but those with baseline SBP >200 mmHg.

Of note, there was no significant difference in the rate of treatment being stopped early between GTN and control but there was significant heterogeneity between the trials. Those who received GTN between 6 and 36 hours of stroke onset were more likely to stop treatment early; a finding driven by ENOS.(84)

This review includes all identified trials of NO donors in people with recent stroke. All five trials studied transdermal GTN and no RCTs assessing other NO donors, L-arginine or NOS-I have been completed. The results from trials assessing GTN should not be extrapolated to other NO donors, L-arginine or NOS-I. With 4,197 participants across five trials this review has substantial external validity and is able to comment on the safety and efficacy of GTN in acute stroke. Overall, GTN was safe, lowered BP and increased heart rate, but had no beneficial effect on primary and secondary clinical outcomes. The potential benefit of GTN seen in participants randomised within six hours of onset may represent chance, but there are several explanations to suggest that the association could be real:

1. The beneficial effects seen were across multiple outcomes including death, dependency, disability, mood, NIHSS, quality of life and cognition.

2. The effects were seen in two separate trials.(83, 84)
3. The effects were seen in a population of over 300 participants. This group is equivalent in size to each of the parts of the positive NINDS trials of intravenous alteplase(199) and recent trials of mechanical thrombectomy.(19)
4. The time-dependent effect of NO donors has been demonstrated in a meta-analysis of pre-clinical studies in which early treatment within 60 minutes of ischaemia was associated with positive outcomes as compared with neutral outcomes in those studies assessing treatment up to 48 hours following induction of ischaemic stroke.(178)

Hyperacute administration of NO donors such as GTN has several potential mechanisms of action alluded to previously:

1. NO levels are low in acute stroke and replacing this deficiency may be beneficial.
2. NO donors lower BP, pulse pressure and peak systolic BP, and improve arterial compliance. Lowering BP acutely may reduce early recurrence following IS, and haematoma expansion in ICH.(130)
3. NO dilates cerebral arteries thereby increasing perilesional perfusion through the 'front door' without resultant cerebral steal.(183)
4. NO is a potent vasodilator of pial arteries leading to 'back-door' collateral reperfusion.(191)

5. NO donors are neuroprotective when administered early after preclinical IS.(178)
6. NO donors might augment the effects of established interventions such as intravenous alteplase, both through preparing patients for treatment by lowering BP, and potentially by increasing access of lytics to occluding clot.

This review has several strengths:

1. The study cohort was large, involving more than 4000 patients.
2. Individual patient data were used from all identified controlled trials of NO donors, this facilitating subgroup analyses.
3. Differing participant characteristics between trials broadened external validity.
4. The trials examined different time-windows after stroke onset including ultra-acute/pre-hospital,(83) hyper-acute (<6 hours),(84) acute (<48 hours),(84) and sub-acute (<168 hours).(110, 182, 183)
5. Safety and efficacy were assessed across a multitude of outcomes encompassing multiple clinical domains; similar results across outcome domains and trials suggest intra- and inter-trial consistency.

There are also several limitations of this review:

1. GTN was the only NO donor assessed in the included RCTs and therefore the results cannot be extrapolated to other NO donors, for which there is no RCT-based evidence.

2. ENOS accounts for 95.6% of all patients included in this review, and therefore dominates the data and subsequent analyses.
3. All these data are reported by the same research group and therefore the results need to be validated and extended by other research groups.
4. Only one of the included studies was double-blind,(110) with the remainder being single-blind(83, 84, 183) or open-label.(182) Further, headache, a common side effect of GTN, may have unblinded some participants. In addition, despite all end of trial outcome measures being performed by assessors blinded to treatment in the included studies, observer bias cannot be excluded.
5. The results relating to ultra-acute and hyper-acute treatment involve a relatively small number of patients;(83, 84) these findings should be considered provisional and require formal testing.
6. This review contains analyses involving a variety of outcomes assessed in several subgroups. Such multiple testing can lead to spurious results and it is possible that some findings simply reflect chance, e.g. increased neurological deterioration in certain BP subgroups (in the absence of a trend across BP, or a negative effect on the NIHSS), or variation in occupational therapy utilisation in BP subgroups.

A recently published systematic review and individual patient data meta-analysis assessed the same five trials of transdermal GTN under the auspices of the Blood pressure in Acute Stroke

Collaboration.(186) Both unadjusted and adjusted analyses were performed including a pre-defined subgroup analysis by time to randomisation. In this regard, the results and conclusions of both reviews are similar showing that GTN is safe but ineffective overall but may improve outcome in a time-dependent manner if given within 6 hours of stroke onset.

2.6 CONCLUSIONS

There is currently insufficient evidence to recommend the use of NO donors, L-arginine or NOS-I in acute stroke, with only RCT evidence available for the NO donor, GTN. In patients with acute stroke, transdermal GTN is safe, reduces blood pressure, increases heart rate, but does not alter outcome. When administered within 6 hours, GTN improved clinical outcomes. Therefore, we can reject the null hypotheses as follows:

- Transdermal GTN lowers BP in acute stroke.
- Transdermal GTN is safe in acute stroke.
- Transdermal GTN improves clinical outcomes when given within 6 hours of stroke onset.

Large phase III randomised trials are required to assess the administration of NO donors in ultra-acute and hyper-acute strokes:

- The safety and efficacy of NO donors other than GTN.
- The safety and efficacy of GTN administered in the ultra-acute phase/pre-hospital environment (e.g. RIGHT-2 (ISRCTN26986053)).
- The effect of GTN on long-term functional outcome and death, i.e. beyond 90 days.
- Mechanisms by which GTN might work, e.g. potentially through reducing haematoma expansion in ICH, and improving collateral supply in IS.

- The health economics of GTN (although the low cost of treatment, i.e. <£5 per patient, means that GTN will dominate if efficacy is seen for mRS).
- Whether there are particular subgroups who benefit from, or do not benefit from, GTN.
- Whether GTN acts as adjunctive therapy to mechanical thrombectomy.
- Whether GTN reduces the need for hospital-based therapies such as mechanical thrombectomy, hemicraniectomy, need for rehabilitation therapy.
- Whether low and middle income countries can utilise GTN in the hyperacute period.

CHAPTER 3:

EFFECT OF GLYCERYL TRINITRATE ON HAEMODYNAMICS IN ACUTE STROKE: DATA FROM THE EFFICACY OF NITRIC OXIDE IN STROKE (ENOS) TRIAL

Publications contributing to this chapter:

Appleton JP, Woodhouse LJ, Bereczki D, Berge E, Christensen HK, Collins R, et al. Effect of glyceryl trinitrate on haemodynamics in acute stroke: data from the Efficacy of Nitric Oxide in Stroke (ENOS) trial. Stroke 2019;50(2):405-12.

Presentations contributing to this chapter:

Effect of transdermal glyceryl trinitrate on blood pressure and other haemodynamic parameters in acute stroke: data from the Efficacy of Nitric Oxide in Stroke (ENOS) trial. British Hypertension Society Annual Scientific Meeting. Royal Marine Hotel, Dun Laoghaire, Dublin (September 2016)

Effect of transdermal glyceryl trinitrate on haemodynamic parameters in acute stroke: Data from the Efficacy of Nitric Oxide in Stroke (ENOS) trial. UK Stroke Forum, The Arena and Convention Centre, Liverpool (November 2016)

3.1 ABSTRACT

Background

Increased blood pressure (BP), heart rate and their derivatives (variability, pulse pressure, rate-pressure product [RPP]) are associated with poor clinical outcome in acute stroke. We assessed the effects of glyceryl trinitrate (GTN) on haemodynamic parameters, and these on outcome in participants in the Efficacy of Nitric Oxide in Stroke (ENOS) trial.

Methods

4011 patients with acute stroke and raised BP were randomised within 48 hours of onset to transdermal GTN or no GTN for 7 days. Peripheral haemodynamics were measured at baseline (3 measures) and daily (2 measures) during treatment. Between-visit BP variability over days 1 to 7 (as standard deviation) was assessed in quintiles. Functional outcome was assessed as modified Rankin Scale and cognition as telephone mini-mental state examination (t-MMSE) at day 90. Analyses were adjusted for baseline prognostic variables. Data are mean difference (MD) or odds ratios (OR) with 95% confidence intervals (CI).

Results

Increased baseline BP (diastolic, variability), heart rate and RPP (minimum, mean, maximum, variability) were each associated with

unfavourable functional outcome at day 90. Increased systolic BP variability over days 1 to 7 was associated with an unfavourable shift in mRS (highest quintile adjusted OR 1.65, 95% CI 1.37-1.99, trend p<0.001), worse cognitive scores (t-MMSE: highest quintile adjusted MD -2.03, 95% CI -2.84 to -1.22, trend p<0.001) and increased the odds of death at day 90 (highest quintile adjusted OR 1.57, 95% CI 1.12, 2.19, trend p<0.001). GTN lowered BP (systolic, diastolic) and RPP, and increased heart rate at day 1; and reduced systolic BP between-visit variability over days 1 to 7.

Conclusions

Increased between-visit BP variability was associated with poor functional and cognitive outcomes and increased death 90 days after acute stroke. GTN reduced BP and RPP, increased heart rate at day 1, and reduced between-visit variability of systolic BP over days 1 to 7. Agents that lower BP variability in acute stroke warrant further study.

3.2 INTRODUCTION

Elevated BP is present in 75% of patients with acute stroke (50) and is associated with increased death and poor functional outcome in all stroke types,(200, 201) recurrent stroke in ischaemic stroke (202) and haematoma expansion in ICH.(51) Increased heart rate (HR) is similarly associated with poor outcome after acute stroke.(203) Mathematical derivations of BP and HR provide useful summaries of haemodynamic parameters and include peak SBP, mean arterial pressure (MAP), pulse pressure (PP), pulse pressure index (PPI), rate-pressure product (RPP), and variability in each of them. Each parameter is associated independently with a worse functional outcome, death, recurrent stroke, and/or early neurological deterioration.(54-56, 204) Variability may be assessed within a set of measurements (within-visit) or across several sets of measurements (between-visit). Different antihypertensive drug classes have variable effects on BP variability in terms of inter-individual and intra-individual recordings in the outpatient setting.(205, 206)

Recent large trials assessing whether BP should be lowered in acute ischaemic stroke were neutral.(74, 84, 124) In contrast, lowering BP in ICH was associated with improved functional outcome in the intensive blood pressure reduction in acute cerebral haemorrhage trial-2 (INTERACT-2),(130) had a neutral effect in the antihypertensive treatment of acute cerebral haemorrhage-2 trial (ATACH-2)(133) and is recommended in clinical practice.(4) Small phase II trials of GTN (a nitric oxide donor) in acute or subacute

stroke found that it lowered peripheral and central BP, 24 hour BP, peak SBP, PP and PPI; increased heart rate; improved vascular compliance; and did not change cerebral blood flow or velocity, or increase intracranial pressure.(56, 110, 182, 183, 207) Although GTN did not modify outcome overall in the large Efficacy of Nitric Oxide in Stroke (ENOS) trial,(84) patients randomised to GTN within 6 hours of onset (ENOS-early) showed a significant improvement in functional outcome.(149) This finding was replicated in a small pilot trial of GTN given in the ambulance.(83)

We assessed the association between haemodynamic measures and outcome, and the haemodynamic effects of GTN in acute stroke using data from the ENOS trial;(84) these analyses were pre-specified in the trial's statistical analysis plan.(189)

3.3 METHODS

Hypotheses tested in this chapter

- Raised BP and its derivatives, including variability, are associated with poor clinical outcomes after acute stroke.
- Transdermal GTN lowers BP and its derivatives, including variability in acute stroke .
- Transdermal GTN increases heart rate in acute stroke.

ENOS trial

Details regarding the ENOS trial protocol, statistical analysis plan, baseline characteristics and main trial results have been published elsewhere.(84, 189, 208, 209) In brief, ENOS recruited 4011 patients within 48 hours of onset of stroke symptoms with high SBP (140-220 mmHg) to transdermal GTN (5 mg patch) or no patch for 7 days. In addition, participants taking antihypertensive medication prior to their stroke were randomised to continue or stop these drugs for 7 days. Patients or relatives/carers gave written informed consent to participate. ENOS was registered (ISRCTN99414122) and approved by ethics committees/competent authorities in all participating countries.

Haemodynamic Measurements

BP can be defined as the pressure exerted by circulating blood on the vessel wall. SBP represents the peak pressure when the ventricles are at maximal contraction within the cardiac cycle, whilst DBP is the minimum pressure when the ventricles are at rest, filling with blood.

BP is dependent on systemic vascular resistance and cardiac output, and naturally fluctuates with every heartbeat. In addition, several factors can influence the BP reading: physiological; demographics; environmental; and measurement method.

BP can be measured invasively by inserting a line with a pressure transducer into an artery. Although this provides accurate beat-to-beat BP readings, it is not without risk and therefore is only performed in high-dependency or intensive care settings. Alternatively, and more commonly, peripheral BP is ascertained from non-invasive measurement of the brachial artery in both clinical practice and trial settings. Historically this used the height of a column of mercury, hence the units millimetres of mercury (mmHg). This unit is still used today in the era of electronic, automated BP measurement. It is important (where possible) to standardise the method of BP measurement. Manual BP is subject to errors, which validated electronic devices can prevent and thus are recommended.(210)

In ENOS, peripheral BP and HR were measured using a validated automated monitor (Omron 705CP (210)) with the patient either supine or sitting using the non-hemiparetic arm, where possible at the following timepoints: three measures at baseline (pre-randomisation, day 0), and two on-treatment measures one hour post application of GTN patch (or at an equivalent time in the control group) on days 1 to 7. The following haemodynamic derivatives were calculated:

Pulse pressure (PP) = systolic BP (SBP) – diastolic BP (DBP)

Mean arterial pressure (MAP) =DBP + (PP / 3)

Pulse pressure index (PPI) = PP/MAP

Rate pressure product (RPP) = SBP x HR

Values for minimum, mean and maximum were then calculated for each haemodynamic variable.

Variation in measured and derived haemodynamic parameters was assessed as:

Standard deviation (SD)

Coefficient of variation (CoV) = (100 x SD/mean)

We chose SD as the main measure of variability due to its common use, simplicity and relevance to clinical practice.

The recording of peripheral BP and HR and interpretation of derived haemodynamic measures can be spurious in the setting of atrial fibrillation (AF). Therefore, in addition to analysing the whole population, sensitivity analyses were performed excluding those participants with AF.

The association between baseline haemodynamics and outcome was assessed by analysing each baseline measure as a continuous variable. To assess the effect of GTN on between-visit BP variability, SD and CoV over days 1 to 7 were calculated for each of systolic BP, diastolic BP and MAP. In addition to analysing these variables

continuously, they were also analysed as equal quintiles with the lowest quintile as the reference group, as done previously.(204) Correlations between mean BP and measures of variability over days 1 to 7 were calculated using Spearman's correlation coefficient.

Clinical Outcomes

The primary outcome of functional outcome at day 90 was measured using the 7-level modified Rankin Scale (mRS) scale, where 0 = independent and 6= dead. Secondary outcomes at day 90 included cognition: modified telephone interview for cognition scale (TICS-M); telephone mini-mental state examination (t-MMSE); and verbal fluency. Patients who had died by day 90 were assigned a worst score for these outcomes. Safety outcomes included all-cause mortality, early neurological deterioration (decrease of ≥5 points overall or decrease of >2 points in the consciousness domain on the Scandinavian Stroke Scale [SSS] from baseline to day 7), and recurrent stroke by day 7. Outcomes at day 90 were assessed by trained investigators, masked to treatment allocation, via telephone at national coordinating centres.

Statistical Analysis

Data were analysed by intention-to-treat in line with the ENOS trial statistical analysis plan (189) and statistical analyses adopted in the primary publication.(84) Data are number (%), median [interquartile range], or mean (SD). Baseline characteristics between groups were

assessed using X^2 for categorical variables and one-way analysis of variance (ANOVA) for continuous variables.

Comparisons between haemodynamics and outcome overall and between treatment groups were assessed using analysis of covariance (ANCOVA), binary logistic regression, ordinal logistic regression or multiple linear regression. Statistical models were adjusted for prognostic baseline covariates: age, sex, baseline mRS, history of previous stroke, history of diabetes, final diagnosis, prior nitrate use, total anterior circulation syndrome (TACS), baseline SSS, thrombolysis, feeding status, time to randomisation and baseline SBP. Analyses involving the whole population were also adjusted for treatment allocation. The resultant odds ratio (OR) or mean difference (MD) and associated 95% confidence intervals (CI) are given, with significance set at $p \leq 0.05$. No adjustment was made for multiplicity of testing. ORs were calculated for change in haemodynamic variables of 1, 10 or 100 units as appropriate for each variable in continuous analyses. Analyses were performed using SPSS version 22 (Chicago, IL).

3.4 RESULTS

The trial enrolled 4011 patients with acute stroke, with mean age 70.3 (12.2) years, male sex 2297 (57.3%), severity SSS 33.7 (13.1), and time from onset to randomisation 26 [21] hours.(84) Baseline haemodynamics for the full ENOS population (n=4011) are depicted in Table 3.1; there were no baseline imbalances between GTN and no GTN groups, with overall mean BP 167.3/89.5 mmHg, MAP 115.4 mmHg, and HR 77.5 beats per minute; 762 (19.0%) of participants had atrial fibrillation.

Table 3.1: Baseline haemodynamics GTN vs. no GTN

	Overall	GTN	No GTN
Patients (N)	4011	2000	2011
SBP min (mmHg)	160.3 (19.4)	160.4 (19.3)	160.3 (19.5)
SBP mean (mmHg)	167.3 (19.0)	167.4 (18.8)	167.1 (19.2)
SBP max (mmHg)	174.2 (20.0)	174.3 (19.7)	174.0 (20.3)
SBP SD (mmHg)	7.3 (5.6)	7.3 (5.7)	7.3 (5.4)
SBP CoV (%)	4.4 (3.5)	4.4 (3.6)	4.4 (3.3)
MAP min (mmHg)	111.1 (13.1)	111.3 (13.1)	111.0 (13.2)
MAP mean (mmHg)	115.4 (13.1)	115.6 (12.9)	115.2 (13.2)
MAP max (mmHg)	119.9 (14.0)	120.0 (13.7)	119.8 (14.3)
MAP SD (mmHg)	4.6 (3.7)	4.6 (3.8)	4.6 (3.7)
MAP CoV (%)	4.0 (3.2)	4.0 (3.3)	4.0 (3.2)
DBP min (mmHg)	84.7 (13.1)	84.8 (13.1)	84.6 (13.2)
DBP mean (mmHg)	89.5 (13.1)	89.7 (13.0)	89.4 (13.3)
DBP max (mmHg)	94.6 (14.6)	94.8 (14.3)	94.4 (14.8)
DBP SD (mmHg)	5.3 (4.5)	5.3 (4.6)	5.2 (4.5)
DBP CoV (%)	5.9 (5.0)	5.9 (5.1)	5.8 (5.0)
HR min (bpm)	74.5 (14.5)	74.7 (14.6)	74.3 (14.4)
HR mean (bpm)	77.5 (14.7)	77.7 (14.8)	77.3 (14.6)
HR max (bpm)	80.6 (15.7)	80.8 (15.8)	80.4 (15.7)
HR SD (bpm)	3.3 (3.7)	3.3 (3.7)	3.2 (3.6)
HR CoV (%)	4.2 (4.5)	4.2 (4.6)	4.2 (4.4)
PP (mmHg)	77.7 (16.9)	77.7 (16.9)	77.8 (17.0)
PPI	0.68 (0.15)	0.68 (0.15)	0.68 (0.15)
RPP min (mmHg.bpm)	12163.1 (2787.6)	12183.5 (2758.1)	12142.9 (2817.1)
RPP mean (mmHg.bpm)	12968.1 (2884.8)	12998.6 (2859.2)	12937.8 (2910.5)
RPP max (mmHg.bpm)	13782.8 (3153.7)	13817.0 (3123.4)	13748.7 (3183.8)
RPP SD (mmHg.bpm)	853.9 (767.1)	861.4 (778.2)	846.5 (756.1)
RPP CoV (%)	6.6 (5.5)	6.6 (5.6)	6.5 (5.4)

Data are number (%) or mean (standard deviation [SD]). BP: blood pressure; CoV: coefficient of variation; DBP: diastolic BP; HR: heart rate; MAP: mean arterial pressure; PP: pulse pressure; PPI: pulse pressure index; RPP: rate pressure product; SBP: systolic BP.

Baseline haemodynamics and functional outcome

Higher baseline values of the following haemodynamic variables were associated in adjusted analyses with an unfavourable shift in the mRS at day 90 (Table 3.2): maximum and within-visit variability of DBP; maximum and within-visit variability of MAP; minimum, mean, maximum and within-visit variability of HR; minimum, mean, maximum and within-visit variability of RPP. However, a sensitivity analysis excluding participants with AF found that only increasing HR, RPP and their variability were associated with unfavourable shifts in functional outcome (Table 3.2).

Increasing baseline values of minimum, mean, maximum and within-visit variability of SBP and PP, and mean PPI were associated with an unfavourable shift in functional outcome in unadjusted analyses, but no associations were maintained after adjustment for baseline prognostic covariates. Although increasing baseline values of minimum MAP, minimum and mean DBP were associated with a favourable shift in mRS in unadjusted analyses, these associations were not maintained after adjustment.

Table 3.2: Baseline haemodynamics vs. functional outcome (mRS) at day 90, overall and excluding those with AF

	n	Overall Unadjusted OR (95% CI)	p	Overall Adjusted OR (95% CI)	p	n	Excluding AF: Adjusted OR (95% CI)	p
SBP min (mmHg)*	3995	1.04 (1.01, 1.07)	**0.014**	1.02 (0.99, 1.05)	0.30	3234	1.02 (0.99, 1.05)	0.31
SBP mean (mmHg)*	3995	1.05 (1.02, 1.08)	**0.001**	1.02 (0.99, 1.05)	0.16	3234	1.02 (0.99, 1.06)	0.19
SBP max (mmHg)*	3995	1.05 (1.02, 1.08)	**<0.001**	1.02 (1.00, 1.05)	0.11	3234	1.02 (0.99, 1.05)	0.16
SBP SD (mmHg)	3990	1.02 (1.01, 1.03)	**0.004**	1.01 (1.00, 1.02)	0.13	3228	1.01 (1.00, 1.02)	0.23
SBP CV (%)	3990	1.02 (1.00, 1.04)	**0.014**	1.01 (1.00, 1.03)	0.15	3228	1.01 (0.99, 1.03)	0.24
MAP min (mmHg)*	3995	0.95 (0.91, 0.99)	**0.012**	1.03 (0.98, 1.07)	0.24	3234	1.02 (0.97, 1.07)	0.50
MAP mean (mmHg)*	3995	0.98 (0.94, 1.02)	0.27	1.04 (1.00, 1.09)	0.07	3234	1.03 (0.98, 1.08)	0.32
MAP max (mmHg)*	3995	1.01 (0.97, 1.05)	0.65	1.05 (1.01, 1.09)	**0.017**	3234	1.03 (0.99, 1.08)	0.17
MAP SD (mmHg)	3990	1.04 (1.03, 1.06)	**<0.001**	1.02 (1.01, 1.04)	**0.009**	3229	1.01 (1.00, 1.03)	0.13
MAP CV (%)	3990	1.05 (1.04, 1.07)	**<0.001**	1.02 (1.01, 1.04)	**0.012**	3229	1.02 (1.00, 1.04)	0.13
DBP min (mmHg)*	3995	0.88 (0.84, 0.91)	**<0.001**	1.02 (0.97, 1.07)	0.40	3234	1.00 (0.95, 1.05)	0.95
DBP mean (mmHg)*	3995	0.92 (0.88, 0.96)	**<0.001**	1.04 (1.00, 1.09)	0.08	3234	1.01 (0.96, 1.07)	0.59
DBP max (mmHg)*	3995	0.97 (0.94, 1.01)	0.14	1.05 (1.01, 1.09)	**0.017**	3234	1.02 (0.98, 1.07)	0.32
DBP SD (mmHg)	3990	1.04 (1.03, 1.06)	**<0.001**	1.02 (1.01, 1.03)	**0.006**	3229	1.01 (1.00, 1.03)	0.12
DBP CV (%)	3990	1.04 (1.03, 1.06)	**<0.001**	1.02 (1.00, 1.03)	**0.007**	3229	1.01 (1.00, 1.02)	0.08
HR min (bpm)*	3991	1.13 (1.09, 1.18)	**<0.001**	1.10 (1.06, 1.14)	**<0.001**	3231	1.11 (1.06, 1.16)	**<0.001**
HR mean (bpm)*	3991	1.16 (1.12, 1.21)	**<0.001**	1.11 (1.07, 1.16)	**<0.001**	3231	1.12 (1.07, 1.17)	**<0.001**
HR max (bpm)*	3991	1.17 (1.13, 1.21)	**<0.001**	1.11 (1.07, 1.15)	**<0.001**	3231	1.12 (1.08, 1.17)	**<0.001**
HR SD (bpm)	3986	1.05 (1.03, 1.07)	**<0.001**	1.03 (1.01, 1.04)	**0.001**	3226	1.02 (1.00, 1.04)	**0.020**
HR CV (%)	3986	1.03 (1.02, 1.05)	**<0.001**	1.02 (1.01, 1.03)	**0.005**	3226	1.01 (1.00, 1.03)	0.10
PP min (mmHg)*	3995	1.07 (1.04, 1.10)	**<0.001**	0.99 (0.96, 1.02)	0.47	3234	1.01 (0.97, 1.04)	0.78
PP mean (mmHg)*	3995	1.12 (1.09, 1.16)	**<0.001**	1.01 (0.97, 1.04)	0.79	3234	1.02 (0.98, 1.06)	0.25
PP max (mmHg)*	3995	1.13 (1.10, 1.16)	**<0.001**	1.01 (0.98, 1.04)	0.62	3234	1.02 (0.99, 1.06)	0.24

PP SD (mmHg)	3990	1.02 (1.02, 1.03)	**<0.001**	1.01 (1.00, 1.02)	0.06	3229	1.01 (1.00, 1.02)	0.20
PP CV (%)	3990	1.01 (1.01, 1.02)	**<0.001**	1.01 (1.00, 1.01)	0.13	3229	1.00 (1.00, 1.01)	0.48
PPI mean	3995	4.02 (2.80, 5.77)	**<0.001**	0.88 (0.59, 1.30)	0.51	3234	1.16 (0.73, 1.83)	0.53
RPP min (mmHg.bpm)§	3991	1.01 (1.01, 1.01)	**<0.001**	1.01 (1.00, 1.01)	**<0.001**	3231	1.01 (1.00, 1.01)	**<0.001**
RPP mean (mmHg.bpm)§	3991	1.01 (1.01, 1.01)	**<0.001**	1.01 (1.00, 1.01)	**<0.001**	3231	1.01 (1.00, 1.01)	**<0.001**
RPP max (mmHg.bpm)§	3991	1.01 (1.01, 1.01)	**<0.001**	1.01 (1.00, 1.01)	**<0.001**	3231	1.01 (1.00, 1.01)	**<0.001**
RPP SD (mmHg.bpm)§	3986	1.03 (1.02, 1.03)	**<0.001**	1.02 (1.01, 1.02)	**<0.001**	3226	1.01 (1.00, 1.02)	**0.006**
RPP CV (%)	3986	1.03 (1.02, 1.04)	**<0.001**	1.02 (1.01, 1.03)	**0.001**	3226	1.01 (1.00, 1.02)	0.08

Effect of baseline (day 0) haemodynamic variables on functional outcome (mRS) at day 90. Ordinal logistic regression with adjustment for age, sex, baseline mRS, history of previous stroke, history of diabetes, final diagnosis, prior nitrate use, baseline SSS, total anterior circulation stroke, thrombolysis, feeding status, time to randomisation, baseline SBP, GTN vs no GTN, continue/stop. * OR per 10 units change; § OR per 100 units change. AF: atrial fibrillation; BP: blood pressure; CoV: coefficient of variation; DBP: diastolic BP; HR: heart rate; MAP: mean arterial pressure; mRS: modified Rankin Scale; PP: pulse pressure; PPI: pulse pressure index; RPP: rate pressure product; SBP: systolic BP; SD: standard deviation

Between-visit BP variability over days 1 to 7 and outcome

The highest quintile of between-visit variability of SBP (SD) over days 1 to 7 was associated with an unfavourable shift in mRS at day 90 (OR 1.65, 95% CI 1.37-1.99, p<0.001, trend p<0.001, Figure 3.1). This was seen across both those randomised, and not randomised, to GTN and when analysed as a continuous variable. These associations were maintained when participants with AF were excluded.

Similarly, the highest quintile of between-visit variability of SBP (SD) over days 1 to 7 was associated with worse cognitive scores at day 90 compared with the lowest quintile: t-MMSE MD -2.03, 95% CI -2.84 to -1.22, p<0.001, trend p<0.001; TICS-M MD -2.68, 95% CI -3.84 to -1.51, p<0.001, trend p<0.001; verbal fluency MD -1.87, 95% CI -2.73 to -1.00, p<0.001, trend p<0.001, Figure 3.2. These associations were also seen in those randomised, and not randomised, to GTN and maintained in a sensitivity analysis excluding participants with AF.

Further, similar associations with day 90 outcomes were seen across all measures (SD and CoV) of between-visit BP variability for SBP (Figure 3.3), DBP (Figures 3.4 and 3.5) and MAP (Figures 3.6 and 3.7).

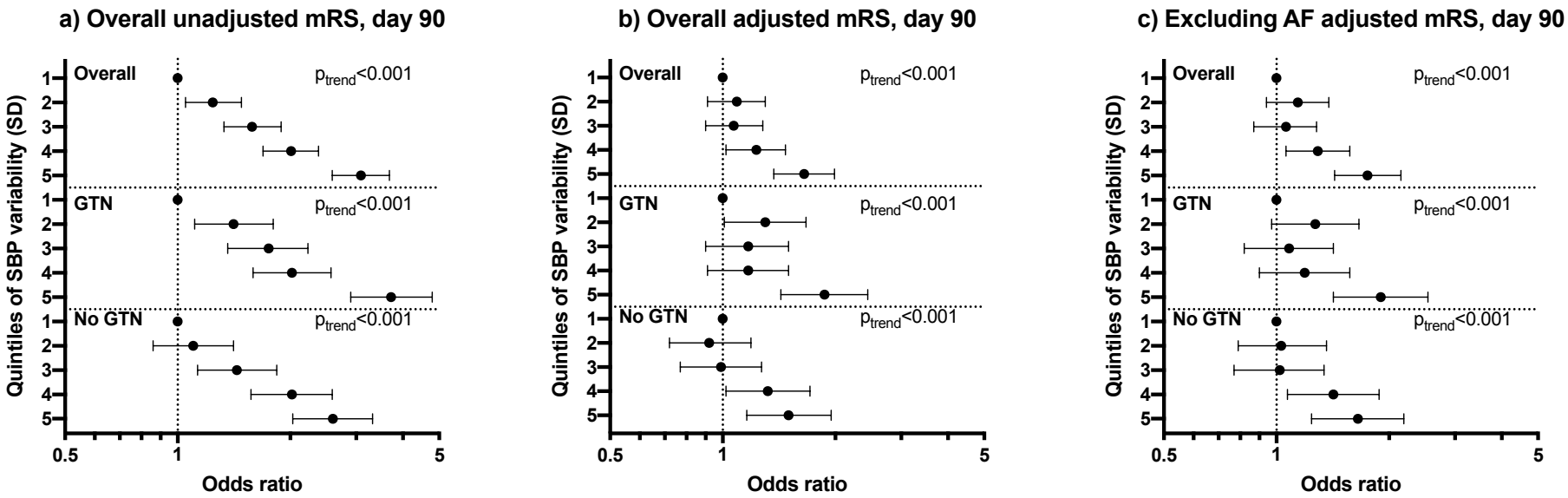


Figure 3.1: Effect of SBP variability over days 1 to 7 (SD) on mRS at day 90

Quintiles of SBP variability over days 1 to 7 (SD) vs. mRS at day 90 overall and in GTN and no GTN groups, with the first quintile as reference, (a) overall unadjusted (n=3978), (b) overall adjusted (n=3978), (c) excluding AF participants adjusted (n=3221). Ordinal logistic regression with adjustment for age, sex, baseline mRS, history of previous stroke, history of diabetes, final diagnosis, prior nitrate use, baseline SSS, total anterior circulation stroke, thrombolysis, feeding status, time to randomisation, baseline SBP, GTN vs no GTN, continue/stop. Data are odds ratios with 95% confidence intervals.

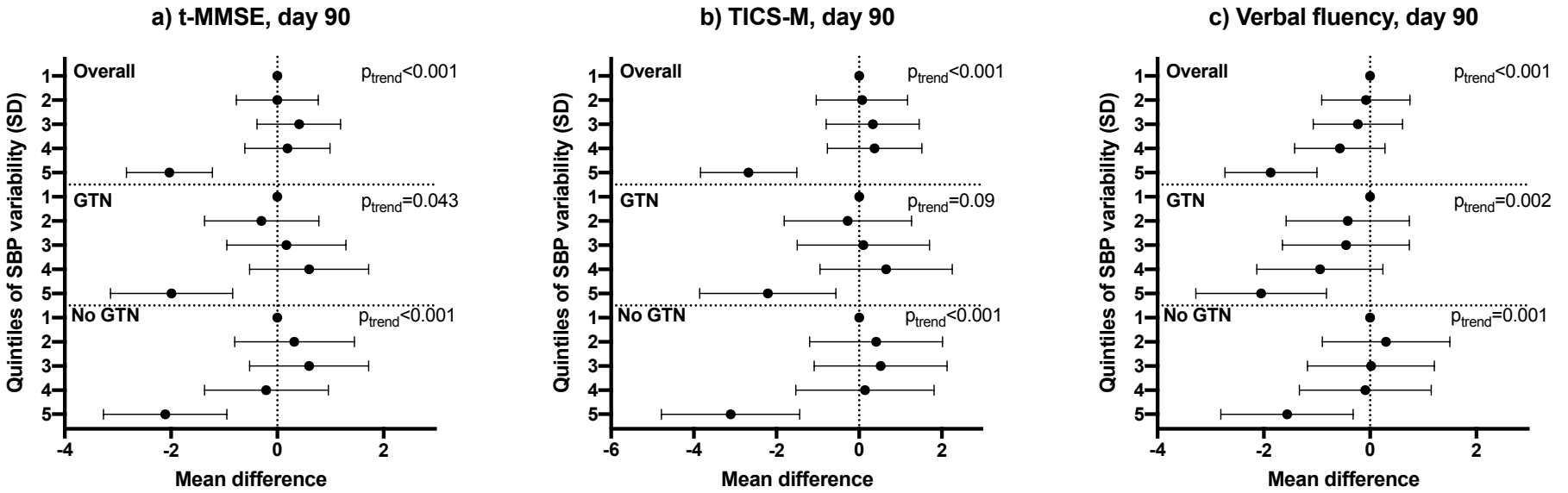


Figure 3.2: Effect of SBP variability over days 1 to 7 (SD) on cognition scores at day 90

Quintiles of SBP variability over days 1 to 7 (SD) vs. cognitive outcomes at day 90 overall and in GTN and no GTN groups, with the first quintile as reference, (a) t-MMSE (n=2019), (b) TICS-M (n=2001), (c) verbal fluency (n=2352). Multiple linear regression with adjustment for age, sex, baseline mRS, history of previous stroke, history of diabetes, final diagnosis, prior nitrate use, baseline SSS, total anterior circulation stroke, thrombolysis, feeding status, time to randomisation, baseline SBP, GTN vs no GTN, continue/stop. Data are mean difference with 95% confidence intervals.

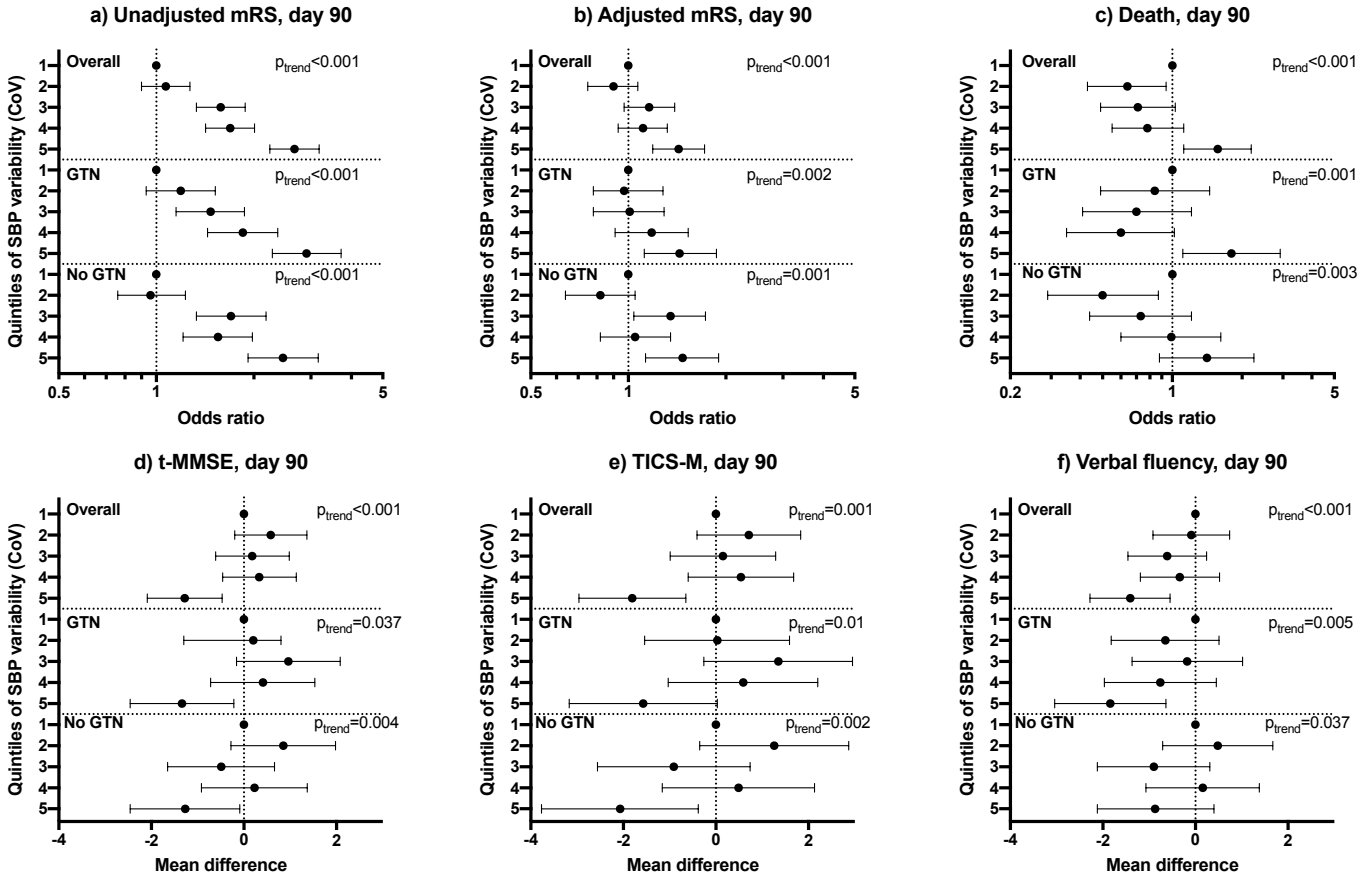


Figure 3.3: Effect of SBP variability over days 1 to 7 (CoV) on day 90 outcomes

a-b: Quintiles of SBP variability over days 1 to 7 (CoV) vs. mRS at day 90 overall and in GTN and no GTN groups, with the first quintile as reference, (a) overall unadjusted (n=3978), (b) overall adjusted (n=3978).
c: Quintiles of SBP variability over days 1 to 7 (CoV) vs. death at day 90 overall (n=3982) and in GTN and no GTN groups, with the first quintile as reference.
d-f: Quintiles of SBP variability over days 1 to 7 (CoV) vs. cognitive outcomes at day 90 overall and in GTN and no GTN groups, with the first quintile as reference, (d) t-MMSE (n=2019), (e) TICS-M (n=2001), (f) verbal fluency (n=2352).
Ordinal (a-b) or binary (c) logistic or multiple linear (d-f) regression with adjustment for age, sex, baseline mRS, history of previous stroke, history of diabetes, final diagnosis, prior nitrate use, baseline SSS, total anterior circulation stroke, thrombolysis, feeding status, time to randomisation, baseline SBP, GTN vs no GTN, continue/stop. Data are odds ratios or mean difference with 95% confidence intervals.

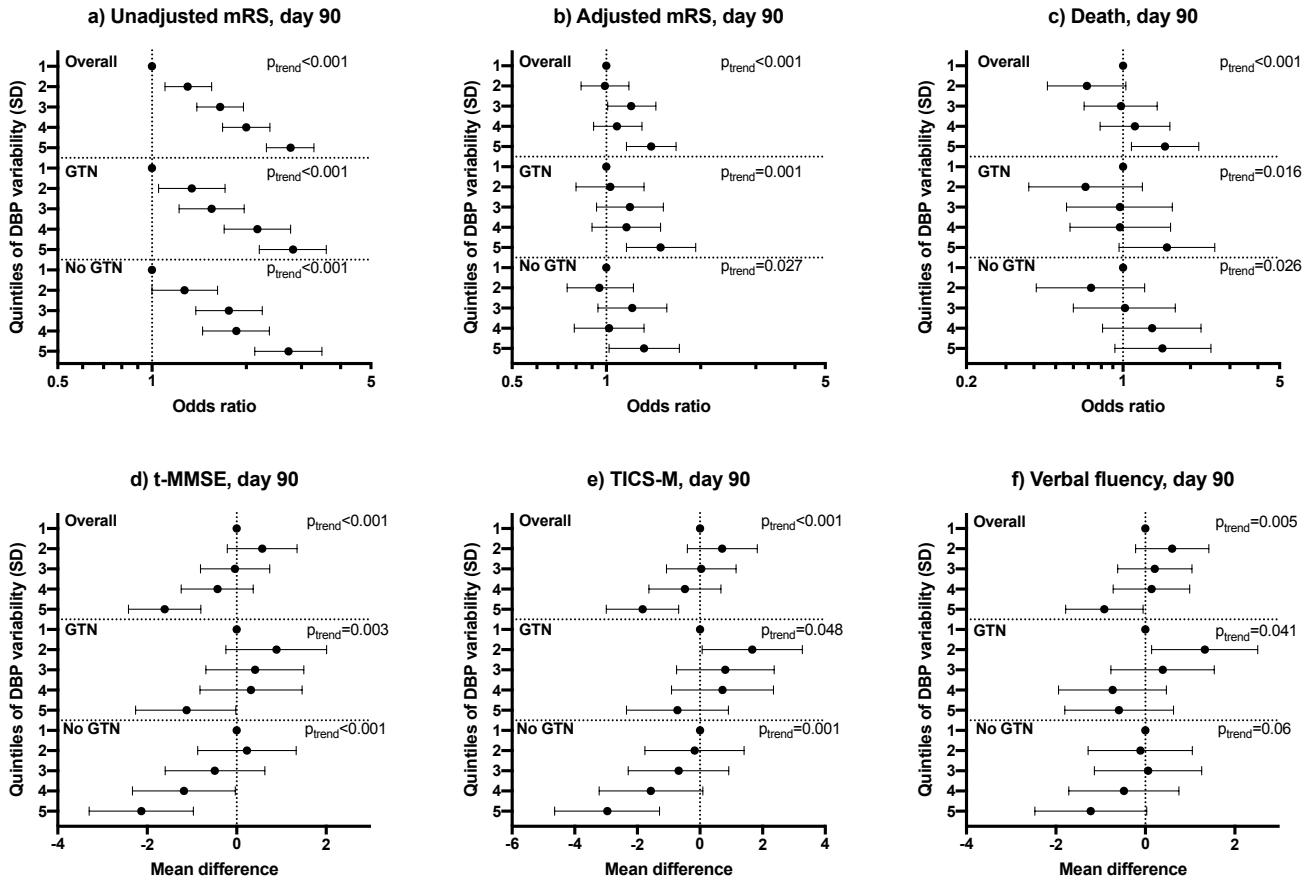


Figure 3.4: Effect of DBP variability over days 1 to 7 (SD) on day 90 outcomes

a-b: Quintiles of DBP variability over days 1 to 7 (SD) vs. mRS at day 90 overall and in GTN and no GTN groups, with the first quintile as reference, (a) overall unadjusted (n=3978), (b) overall adjusted (n=3978).
c: Quintiles of DBP variability over days 1 to 7 (SD) vs. death at day 90 overall (n=3982) and in GTN and no GTN groups, with the first quintile as reference.
d-f: Quintiles of DBP variability over days 1 to 7 (SD) vs. cognitive outcomes at day 90 overall and in GTN and no GTN groups, with the first quintile as reference, (d) t-MMSE (n=2019), (e) TICS-M (n=2001), (f) verbal fluency (n=2352).
Ordinal (a-b) or binary (c) logistic or multiple linear (d-f) regression with adjustment for age, sex, baseline mRS, history of previous stroke, history of diabetes, final diagnosis, prior nitrate use, baseline SSS, total anterior circulation stroke, thrombolysis, feeding status, time to randomisation, baseline SBP, GTN vs no GTN, continue/stop. Data are odds ratios or mean difference with 95% confidence intervals.

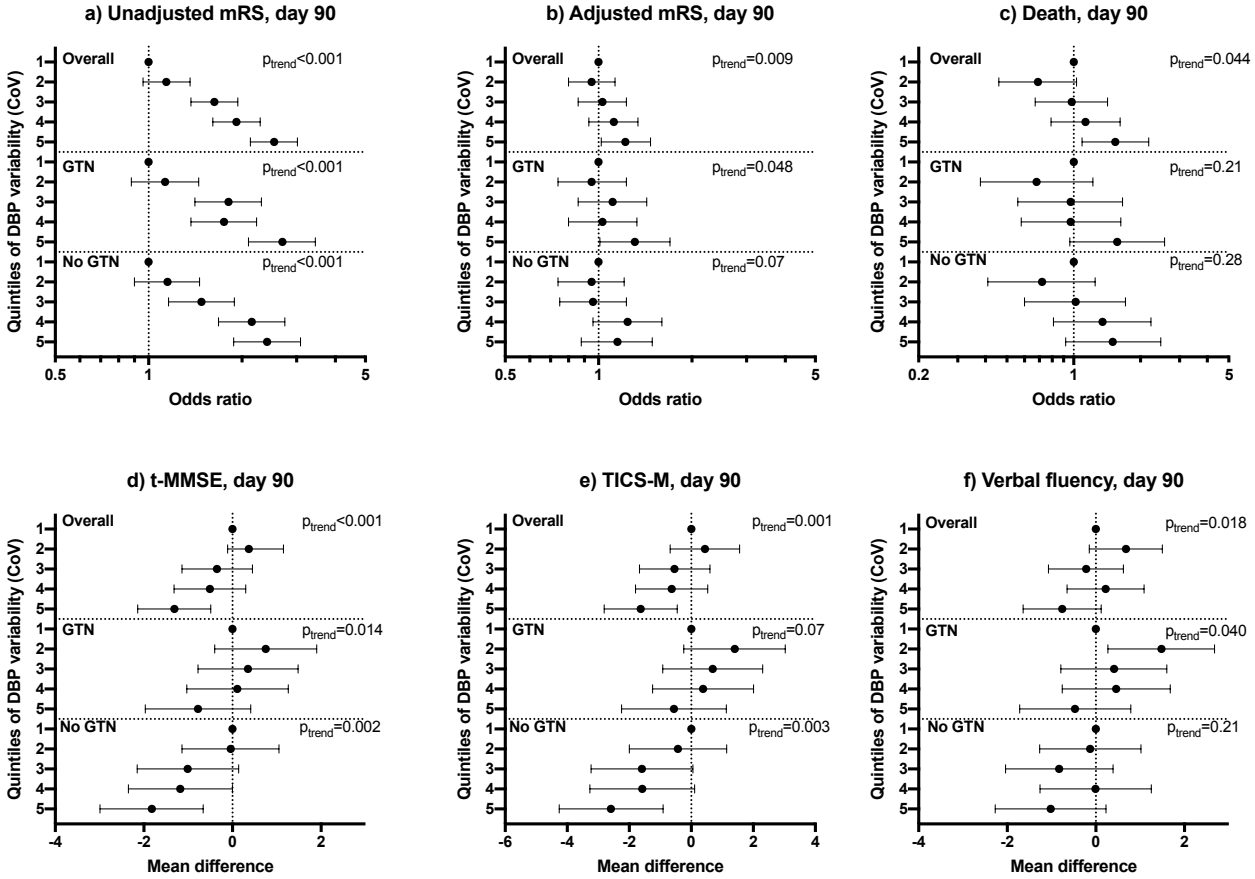


Figure 3.5: Effect of DBP variability over days 1 to 7 (CoV) on day 90 outcomes

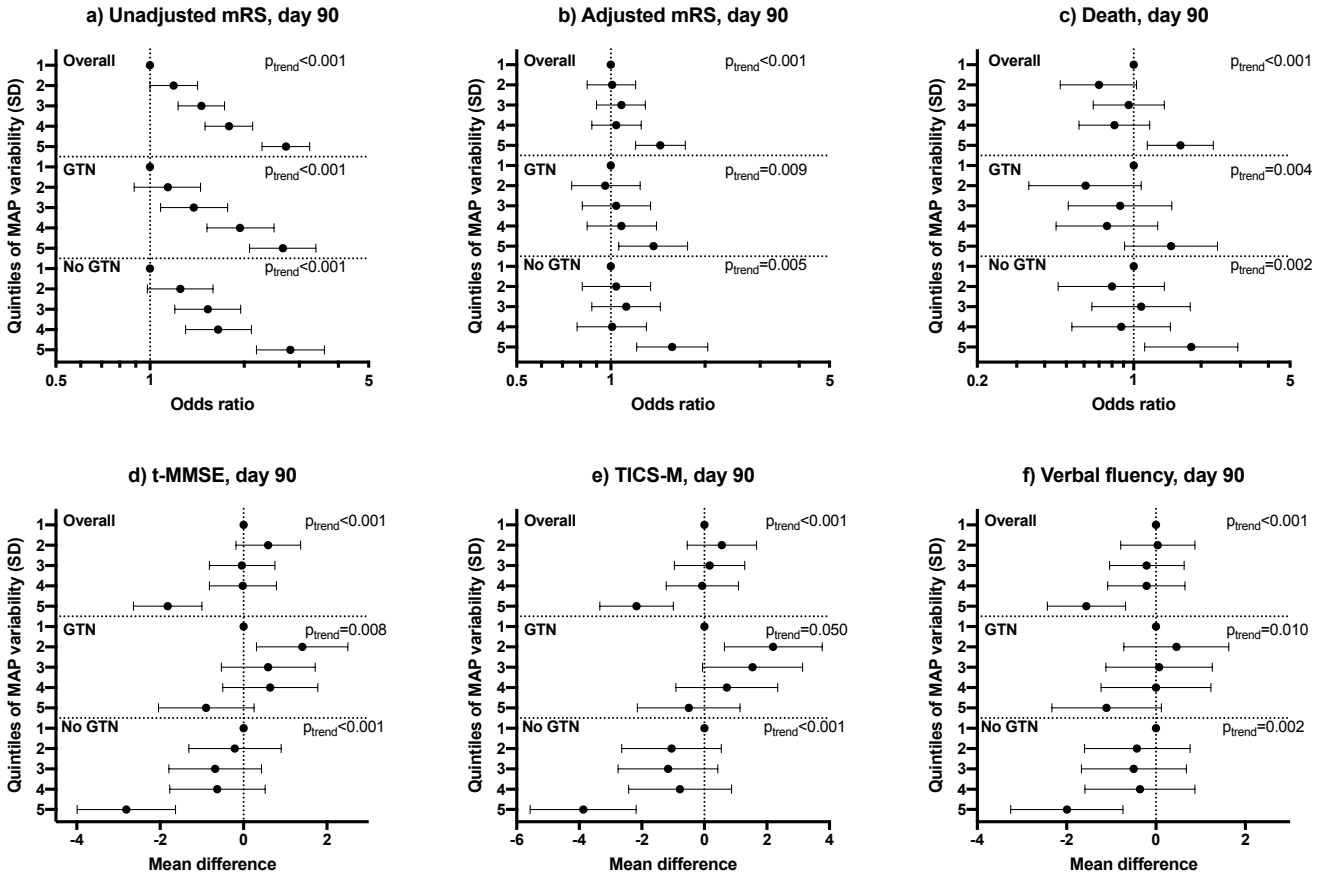


Figure 3.6: Effect of MAP variability over days 1 to 7 (SD) on day 90 outcomes

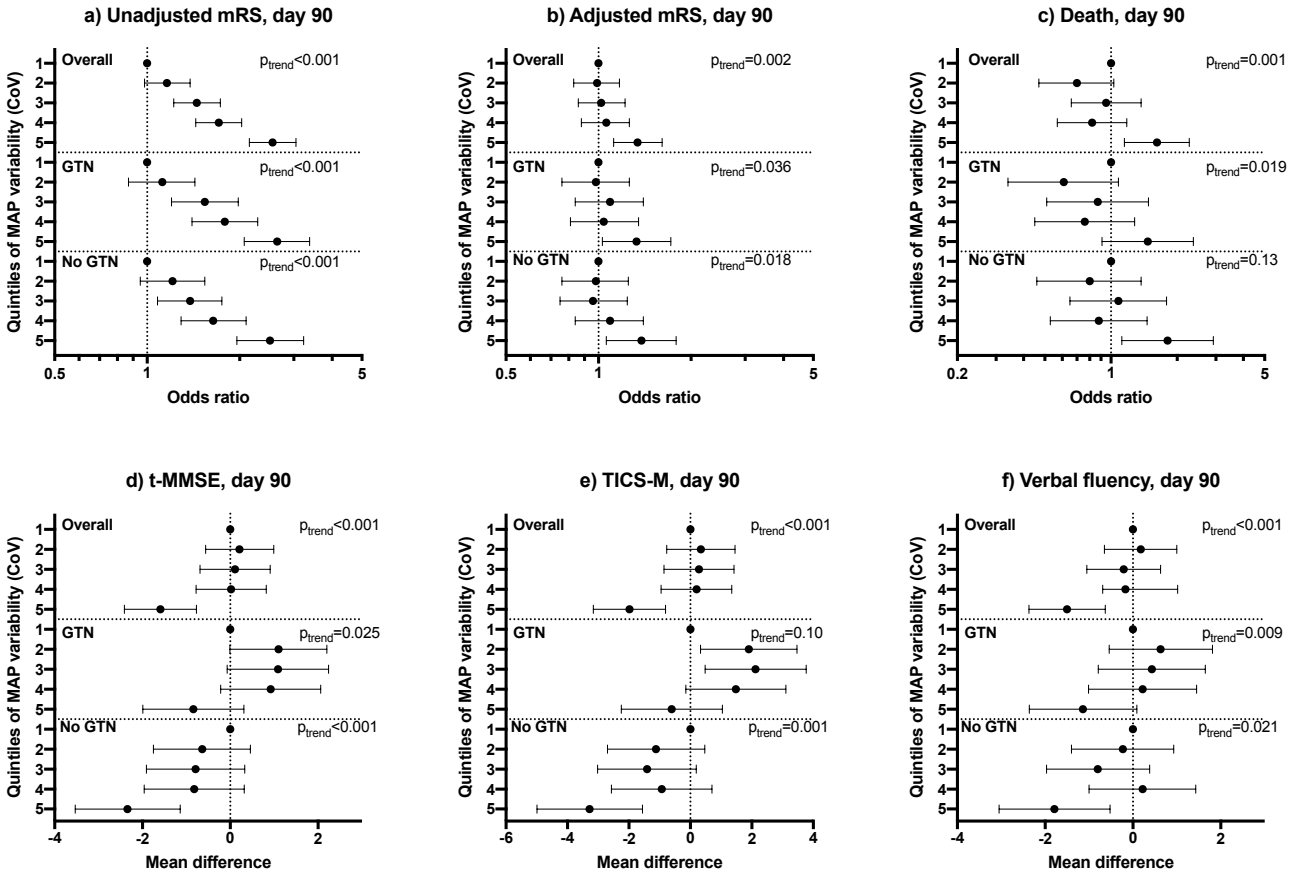


Figure 3.7: Effect of MAP variability over days 1 to 7 (CoV) on day 90 outcomes

The middle 3 quintiles of between-visit variability of SBP (SD) were associated with reduced death at day 7 compared with the lowest quintile, whilst the highest quintile was associated with an increased risk of death at day 90 (OR 1.57, 95% CI 1.12, 2.19, p=0.009, trend p<0.001, Figure 3.8). A similar effect on death at day 90 was noted in those with increased between-visit variability of SBP randomised to GTN, which was not seen in those who did not receive GTN. No significant association was noted regarding stroke recurrence at day 7. Overall, the trend p for early neurological deterioration was significant (p=0.042) with a suggestion of a U-shaped trend across the quintiles, but no significant associations within the group were seen (Figure 3.9). The differing associations between SBP variability and early and late clinical outcomes were also seen across all measures of between-visit variability assessed: SBP CoV (Figures 3.3 and 4.10; DBP SD and CoV (Figures 3.4, 3.5, 3.11, 3.12); MAP SD and CoV (Figures 3.6, 3.7, 3.13, 3.14).

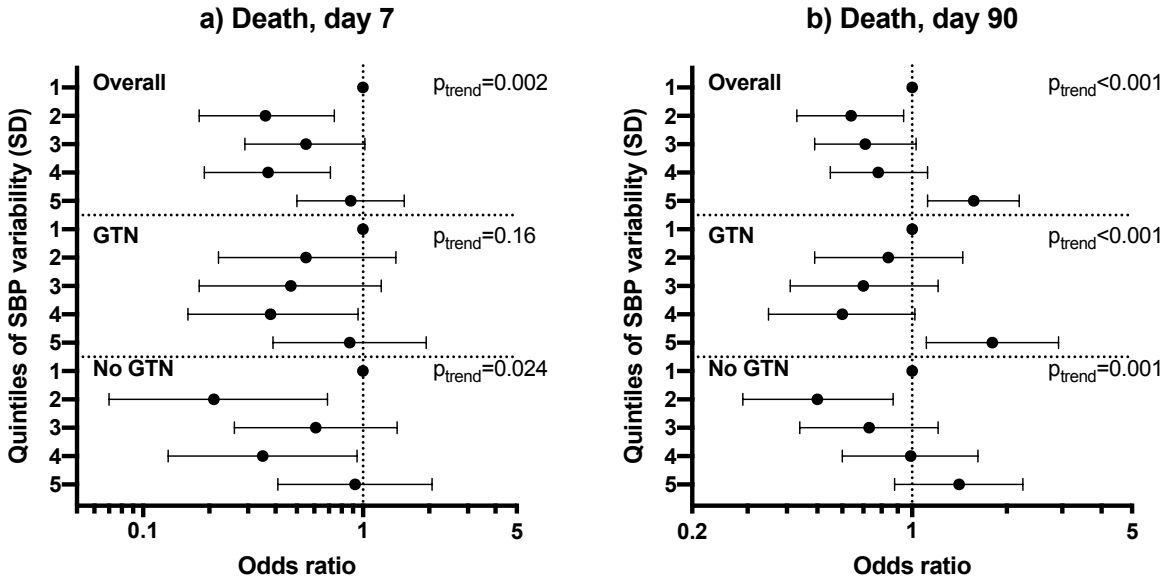


Figure 3.8: Effect of SBP variability over days 1 to 7 (SD) on death at day 7 and 90

Quintiles of SBP variability over days 1 to 7 (SD) vs. death overall (n=3982) and in GTN and no GTN groups, with the first quintile as reference: (a) death at day 7; (b) death at day 90. Binary logistic regression with adjustment for age, sex, baseline mRS, history of previous stroke, history of diabetes, final diagnosis, prior nitrate use, baseline SSS, total anterior circulation stroke, thrombolysis, feeding status, time to randomisation, baseline SBP, GTN vs no GTN, continue/stop. Data are odds ratios with 95% confidence intervals. GTN: glyceryl trinitrate; mRS: modified Rankin Scale; SBP: systolic blood pressure; SD: standard deviation; SSS: Scandinavian Stroke Scale

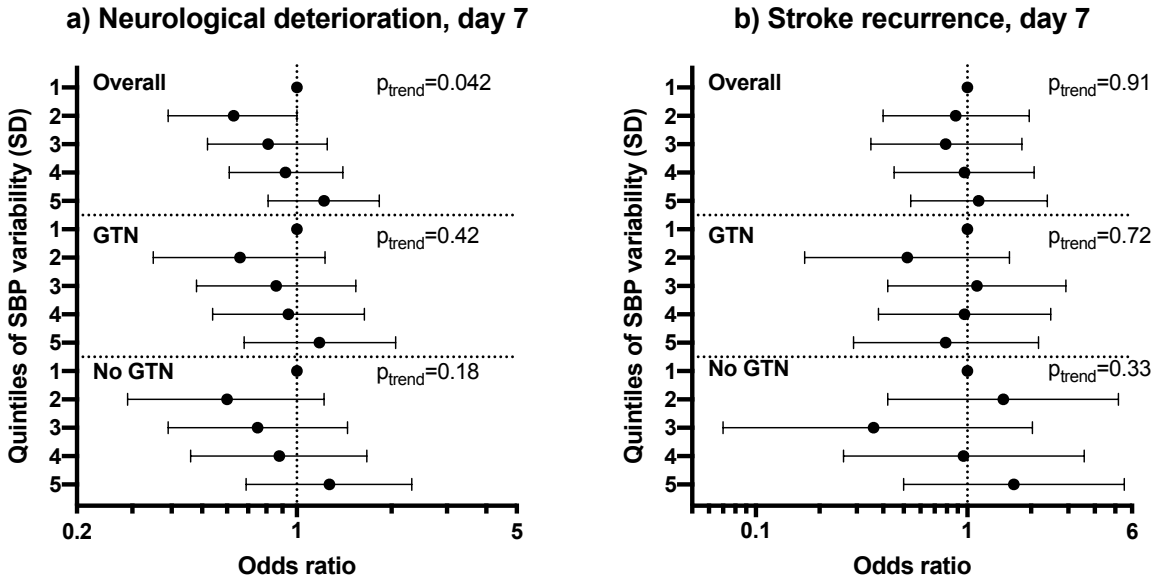


Figure 3.9: Effect of SBP variability over days 1 to 7 (SD) on neurological deterioration and stroke recurrence at day 7

Quintiles of SBP variability over days 1 to 7 (SD) vs. (a) neurological deterioration and (b) stroke recurrence at day 7, overall (n=3982) and in GTN and no GTN groups, with the first quintile as reference. Binary logistic regression with adjustment for age, sex, baseline mRS, history of previous stroke, history of diabetes, final diagnosis, prior nitrate use, baseline SSS, total anterior circulation stroke, thrombolysis, feeding status, time to randomisation, baseline SBP, GTN vs no GTN, continue/stop. Data are odds ratios or mean difference with 95% confidence intervals. GTN: glyceryl trinitrate; mRS: modified Rankin Scale; SBP: systolic blood pressure; SD: standard deviation; SSS: Scandinavian Stroke Scale.

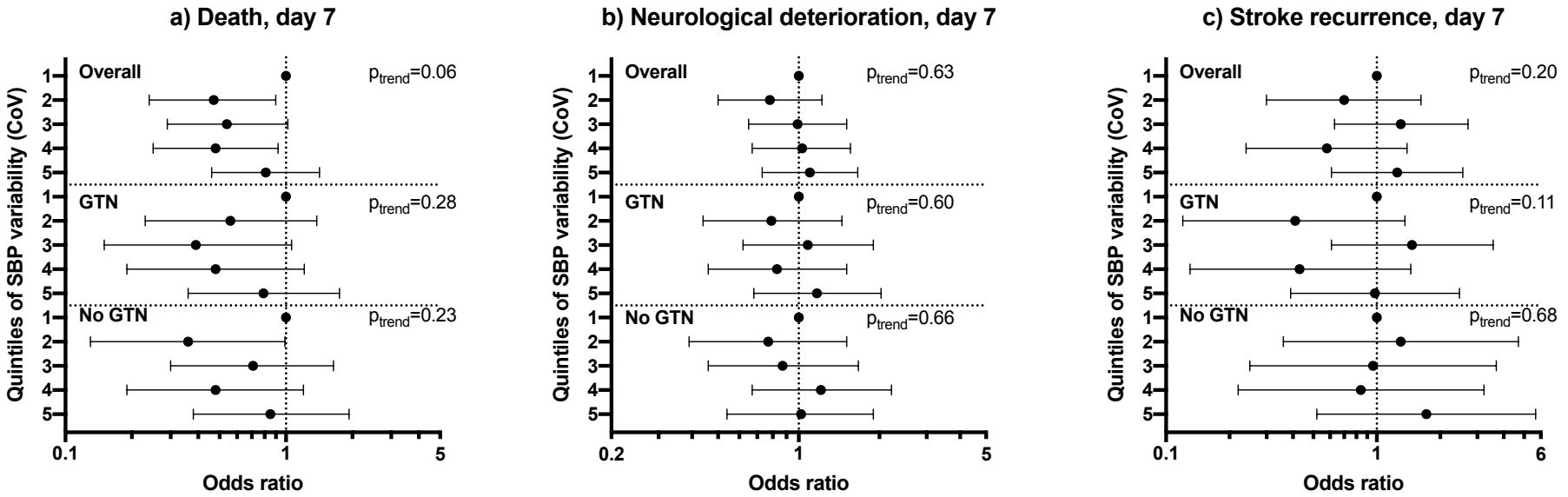


Figure 3.10: Effect of SBP variability over days 1 to 7 (CoV) on day 7 outcomes

Quintiles of SBP variability over days 1 to 7 (CoV) vs. (a) death, (b) neurological deterioration, and (c) stroke recurrence at day 7, overall (n=3982) and in GTN and no GTN groups, with the first quintile as reference.
Binary logistic regression with adjustment for age, sex, baseline mRS, history of previous stroke, history of diabetes, final diagnosis, prior nitrate use, baseline SSS, total anterior circulation stroke, thrombolysis, feeding status, time to randomisation, baseline SBP, GTN vs no GTN, continue/stop. Data are odds ratios or mean difference with 95% confidence intervals. CoV: coefficient of variation; GTN: glyceryl trinitrate; mRS: modified Rankin Scale; SBP: systolic blood pressure; SSS: Scandinavian Stroke Scale.

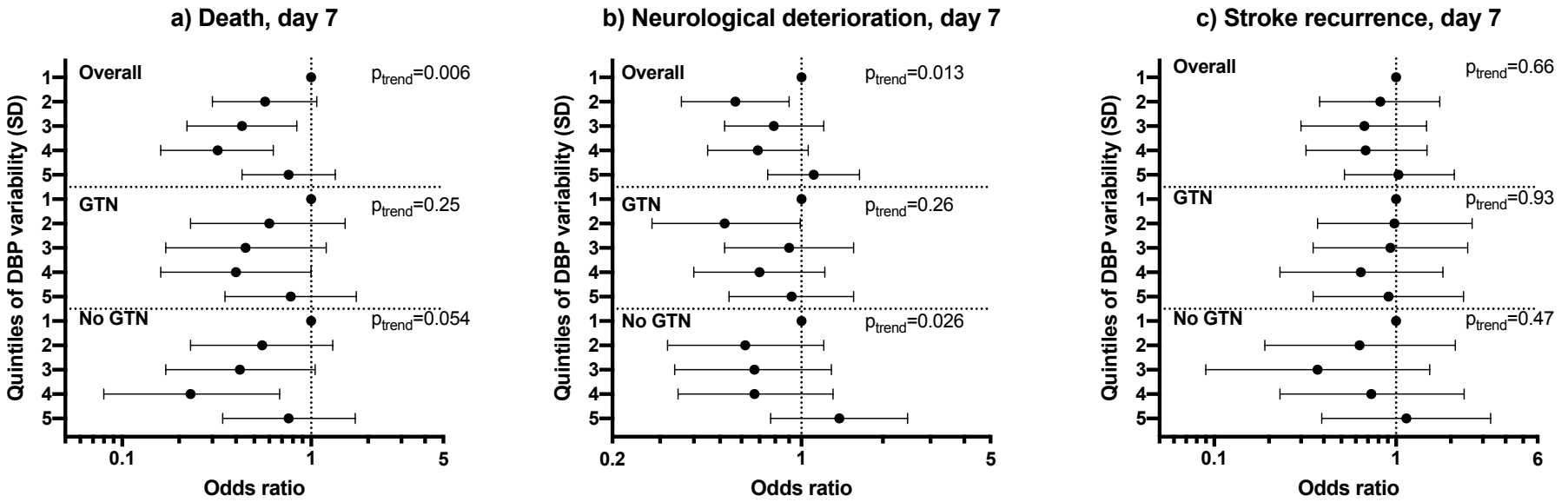


Figure 3.11: Effect of DBP variability over days 1 to 7 (SD) on day 7 outcomes

Quintiles of DBP variability over days 1 to 7 (SD) vs. (a) death, (b) neurological deterioration, and (c) stroke recurrence at day 7, overall (n=3982) and in GTN and no GTN groups, with the first quintile as reference. Binary logistic regression with adjustment for age, sex, baseline mRS, history of previous stroke, history of diabetes, final diagnosis, prior nitrate use, baseline SSS, total anterior circulation stroke, thrombolysis, feeding status, time to randomisation, baseline SBP, GTN vs no GTN, continue/stop. Data are odds ratios or mean difference with 95% confidence intervals. DBP: diastolic blood pressure; GTN: glyceryl trinitrate; mRS: modified Rankin Scale; SD: standard deviation; SSS: Scandinavian Stroke Scale.

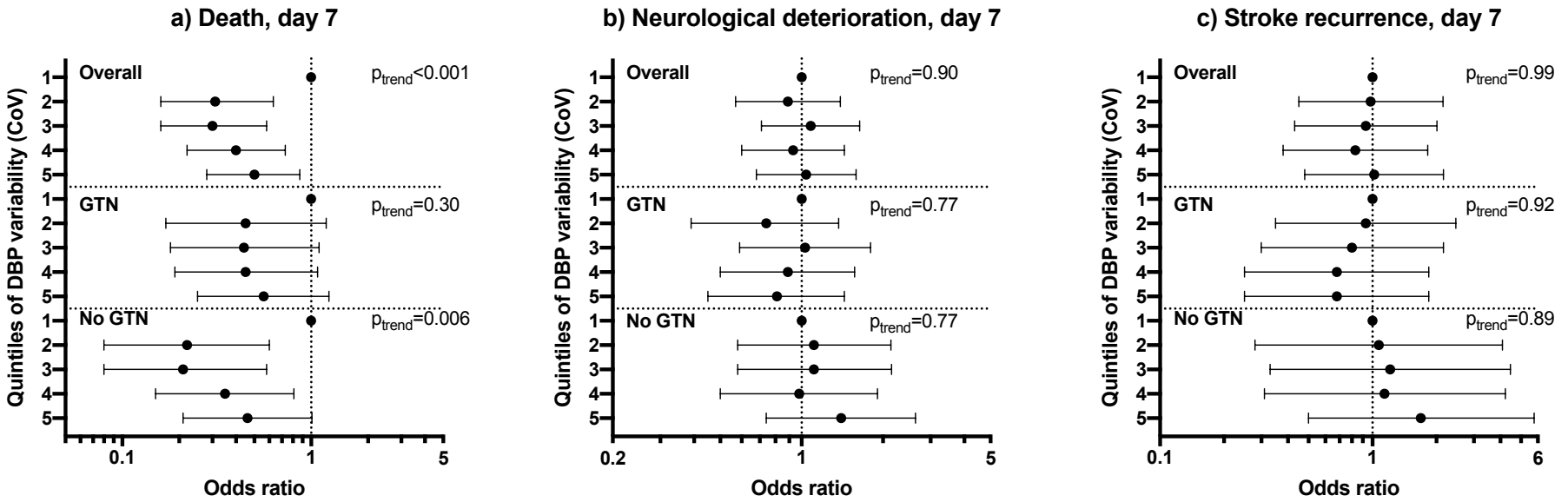


Figure 3.12: Effect of DBP variability over days 1 to 7 (CoV) on day 7 outcomes

Quintiles of DBP variability over days 1 to 7 (CoV) vs. (a) death, (b) neurological deterioration, and (c) stroke recurrence at day 7, overall (n=3982) and in GTN and no GTN groups, with the first quintile as reference. Binary logistic regression with adjustment for age, sex, baseline mRS, history of previous stroke, history of diabetes, final diagnosis, prior nitrate use, baseline SSS, total anterior circulation stroke, thrombolysis, feeding status, time to randomisation, baseline SBP, GTN vs no GTN, continue/stop. Data are odds ratios with 95% confidence intervals. CoV: coefficient of variation; DBP: diastolic blood pressure; GTN: glyceryl trinitrate; mRS: modified Rankin Scale; SSS: Scandinavian Stroke Scale.

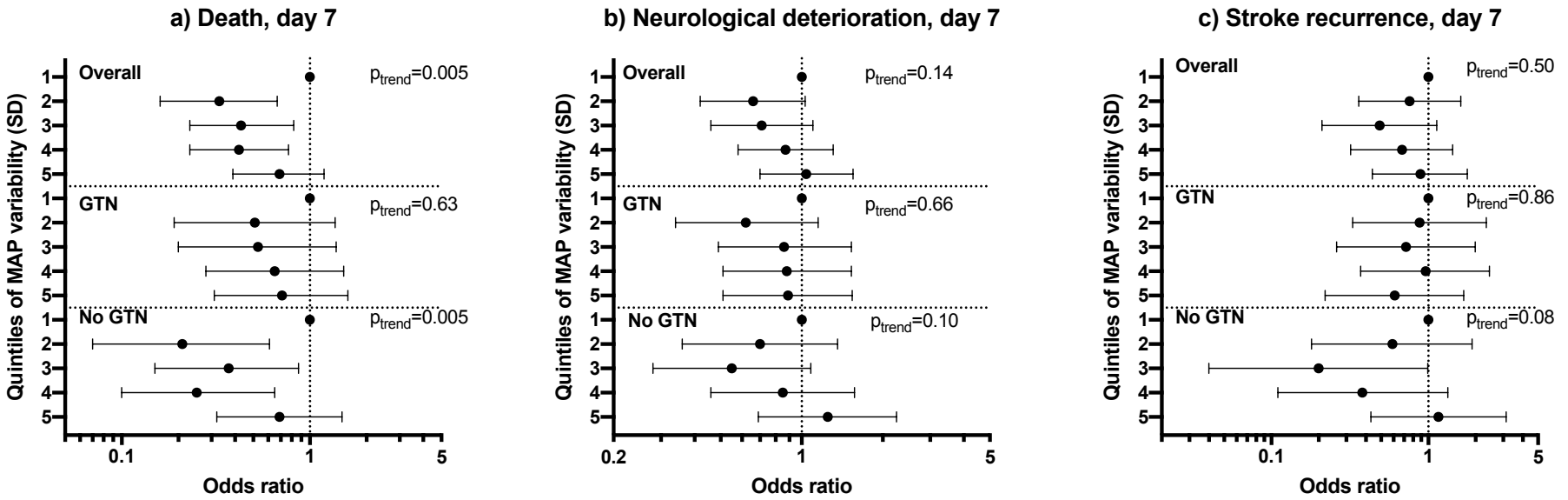


Figure 3.13: Effect of MAP variability over days 1 to 7 (SD) on day 7 outcomes

Quintiles of MAP variability over days 1 to 7 (SD) vs. (a) death, (b) neurological deterioration, and (c) stroke recurrence at day 7, overall (n=3982) and in GTN and no GTN groups, with the first quintile as reference. Binary logistic regression with adjustment for age, sex, baseline mRS, history of previous stroke, history of diabetes, final diagnosis, prior nitrate use, baseline SSS, total anterior circulation stroke, thrombolysis, feeding status, time to randomisation, baseline SBP, GTN vs no GTN, continue/stop. Data are odds ratios or mean difference with 95% confidence intervals. GTN: glyceryl trinitrate; MAP: mean arterial pressure; mRS: modified Rankin Scale; SD: standard deviation; SSS: Scandinavian Stroke Scale.

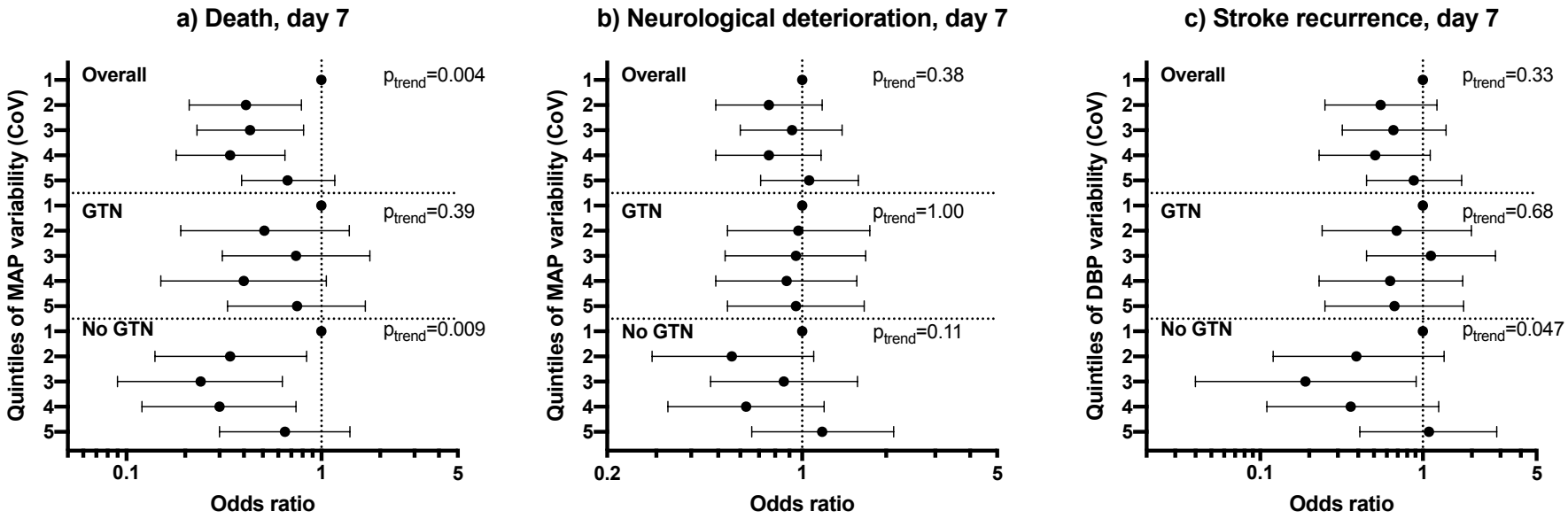


Figure 3.14: Effect of MAP variability over days 1 to 7 (CoV) on day 7 outcomes

Quintiles of MAP variability over days 1 to 7 (CoV) vs. (a) death, (b) neurological deterioration, and (c) stroke recurrence at day 7, overall (n=3982) and in GTN and no GTN groups, with the first quintile as reference. Binary logistic regression with adjustment for age, sex, baseline mRS, history of previous stroke, history of diabetes, final diagnosis, prior nitrate use, baseline SSS, total anterior circulation stroke, thrombolysis, feeding status, time to randomisation, baseline SBP, GTN vs no GTN, continue/stop. Data are odds ratios with 95% confidence intervals. CoV: coefficient of variation; GTN: glyceryl trinitrate; MAP: mean arterial pressure; mRS: modified Rankin Scale; SSS: Scandinavian Stroke Scale.

Mean SBP over days 1 to 7 was weakly correlated with measures of between-visit SBP variability (Spearman's correlation coefficient: SD 0.137; CoV -0.155). Similar weak correlations between mean DBP and MAP and their corresponding measures of variability were seen. In addition, there was no correlation between within-individual BP trend over the treatment period and mRS at day 90, highlighting that the associations between variability and outcome seen were not mediated by trend in mean BP over time.

Haemodynamic effects of GTN vs. no GTN

Table 3.3 shows the effect of GTN vs. no GTN on haemodynamic parameters at day 1 i.e. on treatment (n=3851, 96%). GTN lowered minimum, mean and maximum SBP by 7 mmHg, DBP by 3.6 mmHg and MAP by 4.7 mmHg. Mean PP was reduced by 3.4 mmHg. GTN increased minimum, mean and maximum HR by 1.4 bpm, but reduced minimum, mean and maximum RPP (the product of SBP and HR), i.e. GTN's effect on HR was negated by its BP-lowering effect. GTN had no effect on within-visit variability of any haemodynamic variable at day 1. GTN had the same effects on haemodynamics overall as in those without AF (Table 3.4).

Over 7 days of treatment, GTN lowered mean BP by 2.9 / 2.1 mmHg, MAP by 2.3 mmHg, PP by 0.8 mmHg, and increased HR by 1 bpm on average (Table 3.5). These results are dampened in comparison to the day 1 data (Table 3.3), highlighting tachyphylaxis seen with GTN over longer treatment periods. Although GTN did not influence within-

visit variability on day 1, over days 1 to 7 GTN lowered between-visit variability of SBP (SD) by 0.4 mmHg. In contrast, GTN did not change SBP CoV or between-visit variability of DBP over days 1 to 7. These effects were maintained when analysed in those without AF (Table 3.6).

Table 3.3: Effect of GTN vs. no GTN on haemodynamic variables at day 1

	GTN	No GTN	Unadjusted Mean Difference (95% CI)	p	Adjusted Mean Difference (95% CI)	p
Patients (N)	1914	1937				
SBP min	152.9 (22.7)	159.9 (22.8)	-7.03 (-8.47, -5.59)	**<0.001**	-6.92 (-8.11, -5.72)	**<0.001**
SBP mean	156.6 (22.6)	163.6 (22.5)	-7.03 (-8.45, -5.61)	**<0.001**	-6.98 (-8.12, -5.84)	**<0.001**
SBP max	160.4 (23.2)	167.4 (22.9)	-7.03 (-8.49, -5.57)	**<0.001**	-6.98 (-8.17, -5.80)	**<0.001**
SBP SD	5.9 (5.9)	5.9 (6.4)	0.01 (-0.40, 0.42)	0.96	-0.03 (-0.41, 0.40)	0.99
SBP CV (%)	3.8 (3.9)	3.6 (4.1)	0.15 (-0.12, 0.41)	0.28	0.14 (-0.13, 0.40)	0.31
MAP min	106.1 (15.2)	110.6 (15.3)	-4.57 (-5.53, -3.61)	**<0.001**	-4.59 (-5.38, -3.8)	**<0.001**
MAP mean	108.4 (15.2)	113.1 (15.1)	-4.65 (-5.61, -3.69)	**<0.001**	-4.72 (-5.49, -3.95)	**<0.001**
MAP max	110.8 (15.7)	115.5 (15.6)	-4.73 (-5.72, -3.75)	**<0.001**	-4.79 (-5.62, -3.97)	**<0.001**
MAP SD	3.7 (4.0)	3.8 (4.2)	-0.12 (-0.4, 0.15)	0.38	-0.13 (-0.40, 0.14)	0.35
MAP CV (%)	3.4 (3.7)	3.4 (3.9)	0.03 (-0.23, 0.28)	0.84	0.02 (-0.24, 0.27)	0.91
DBP min	81.7 (14.1)	85.1 (14.6)	-3.39 (-4.30, -2.49)	**<0.001**	-3.39 (-4.11, -2.67)	**<0.001**
DBP mean	84.3 (14.1)	87.8 (14.5)	-3.46 (-4.37, -2.56)	**<0.001**	-3.59 (-4.3, -2.88)	**<0.001**
DBP max	87.0 (14.9)	90.5 (15.3)	-3.53 (-4.48, -2.58)	**<0.001**	-3.71 (-4.51, -2.90)	**<0.001**
DBP SD	4.2 (4.8)	4.3 (4.9)	-0.10 (-0.42, 0.22)	0.53	-0.12 (-0.44, 0.20)	0.47
DBP CV (%)	4.9 (5.5)	4.9 (5.5)	0.07 (-0.30, 0.43)	0.71	0.04 (-0.32, 0.41)	0.81
HR min	77.2 (15.0)	75.6 (14.8)	1.61 (0.67, 2.55)	**0.001**	1.31 (0.68, 1.94)	**<0.001**
HR mean	79.0 (15.1)	77.3 (14.9)	1.68 (0.73, 2.63)	**0.001**	1.35 (0.73, 1.96)	**<0.001**
HR max	80.7 (15.6)	79.0 (15.5)	1.74 (0.76, 2.73)	**0.001**	1.42 (0.75, 2.09)	**<0.001**
HR SD	2.8 (4.0)	2.7 (3.8)	0.10 (-0.16, 0.36)	0.44	0.10 (-0.16, 0.35)	0.45
HR CV (%)	3.5 (4.9)	3.5 (4.8)	0.07 (-0.25, 0.39)	0.68	0.06 (-0.26, 0.38)	0.71
PP	72.3 (17.9)	75.9 (19.0)	-3.57 (-4.74, -2.40)	**<0.001**	-3.38 (-4.26, -2.51)	**<0.001**
PPI	0.67 (0.16)	0.68 (0.16)	-0.01 (-0.02, 0.01)	0.33	0.00 (-0.01, 0.01)	0.48
RPP min	11945.6 (3001.3)	12241.8 (3067.4)	-296.21 (-488.22, -104.2)	**0.003**	-323.14 (-464.77, -181.51)	**<0.001**
RPP mean	12386.5 (3054.9)	12664.0 (3104.4)	-277.50 (-472.37, -82.63)	**0.005**	-318.67 (-458.12, -179.22)	**<0.001**
RPP max	12827.4 (3210.0)	13086.2 (3238.8)	-258.79 (-462.8, -54.77)	**0.013**	-300.61 (-452.78, -148.44)	**<0.001**

RPP SD	689.7 (818.7)	661.4 (805.3)	28.29 (-82.34, 25.77)	0.31	25.42 (-27.86, 78.70)	0.35
RPP CV (%)	5.6 (6.3)	5.3 (6.4)	0.28 (-0.14, 0.71)	0.19	0.27 (-0.15, 0.69)	0.21

Analysis of covariance (ANCOVA) with adjustment for baseline variable. BP: blood pressure; CoV: coefficient of variation; DBP: diastolic BP; HR: heart rate; MAP: mean arterial pressure; PP: pulse pressure; PPI: pulse pressure index; RPP: rate pressure product; SBP: systolic BP; SD: standard deviation.

Table 3.4: Effect of GTN vs. no GTN on haemodynamic variables at day 1 excluding those with AF

	GTN	No GTN	Unadjusted Mean Difference (95% CI)	p	Adjusted Mean Difference (95% CI)	p
Patients (N)	1534	1582				
SBP min	153.1 (22.9)	160.4 (23.2)	-7.34 (-8.96, -5.72)	**<0.001**	-7.10 (-8.43, -5.77)	**<0.001**
SBP mean	156.7 (22.9)	164.1 (22.9)	-7.37 (-8.97, -5.76)	**<0.001**	-7.14 (-8.42, -5.86)	**<0.001**
SBP max	160.3 (23.4)	167.7 (23.4)	-7.39 (-9.03, -5.75)	**<0.001**	-7.14 (-8.47, -5.80)	**<0.001**
SBP SD	5.6 (5.6)	5.7 (6.3)	-0.10 (-0.54, 0.34)	0.66	-0.11 (-0.55, 0.33)	0.62
SBP CV (%)	3.6 (3.6)	3.5 (4.1)	0.07 (-0.21, 0.36)	0.61	0.07 (-0.22, 0.35)	0.65
MAP min	105.9 (15.5)	110.7 (15.4)	-4.82 (-5.91, -3.74)	**<0.001**	-4.77 (-5.65, -3.89)	**<0.001**
MAP mean	108.1 (15.4)	113.0 (15.4)	-4.88 (-5.96, -3.80)	**<0.001**	-4.84 (-5.71, -3.99)	**<0.001**
MAP max	110.4 (15.9)	115.3 (15.8)	-4.93 (-6.05, -3.82)	**<0.001**	-4.85 (-5.77, -3.92)	**<0.001**
MAP SD	3.5 (3.9)	3.6 (4.2)	-0.12 (-0.42, 0.18)	0.42	-0.13 (-0.42, 0.17)	0.41
MAP CV (%)	3.3 (3.6)	3.2 (3.7)	0.04 (-0.23, 0.31)	0.77	0.03 (-0.24, 0.30)	0.81
DBP min	81.3 (14.2)	85.0 (14.6)	-3.64 (-4.66, -2.63)	**<0.001**	-3.64 (-4.44, -2.84)	**<0.001**
DBP mean	83.9 (14.2)	87.5 (14.6)	-3.64 (-4.65, -2.62)	**<0.001**	-3.69 (-4.48, -2.90)	**<0.001**
DBP max	86.4 (14.9)	90.0 (15.3)	-3.63 (-4.69, -2.56)	**<0.001**	-3.70 (-4.59, -2.81)	**<0.001**
DBP SD	3.9 (4.8)	4.0 (4.6)	-0.03 (-0.37, 0.32)	0.88	-0.12 (-0.44, 0.20)	0.47
DBP CV (%)	4.7 (5.5)	4.5 (5.2)	0.18 (-0.21, 0.57)	0.37	0.16 (-0.23, 0.55)	0.41
HR min	76.2 (14.6)	74.8 (14.5)	1.44 (0.41, 2.46)	**0.006**	1.35 (0.68, 2.02)	**<0.001**
HR mean	77.8 (14.6)	76.3 (14.6)	1.48 (0.45, 2.51)	**0.005**	1.38 (0.73, 2.04)	**<0.001**
HR max	79.3 (14.9)	77.8 (15.1)	1.53 (0.47, 2.58)	**0.005**	1.40 (0.69, 2.11)	**<0.001**
HR SD	2.4 (3.2)	2.4 (3.6)	0.04 (-0.21, 0.29)	0.76	0.04 (-0.21, 0.29)	0.76
HR CV (%)	3.1 (4.2)	3.1 (4.5)	0.03 (-0.29, 0.36)	0.85	0.02 (-0.30, 0.34)	0.90
PP	72.8 (17.7)	76.6 (19.1)	-3.73 (-5.02, -2.44)	**<0.001**	-3.44 (-4.42, -2.47)	**<0.001**
PPI	0.68 (0.15)	0.68 (0.16)	-0.01 (-0.02, 0.01)	0.41	0.00 (-0.01, 0.01)	0.61
RPP min	11811.1 (2990.1)	12143.8 (3057.3)	-232.57 (-545.37, -119.77)	**0.002**	-314.19 (-467.97, -160.41)	**<0.001**
RPP mean	12219.5 (3031.9)	12536.7 (3097.8)	-317.26 (-532.96, -101.56)	**0.004**	-305.64 (-456.26, -155.01)	**<0.001**
RPP max	12627.7 (3150.6)	12929.7 (3232.2)	-301.95 (-526.57, -77.34)	**0.008**	-290.21 (-453.97, -126.46)	**0.001**

RPP SD	632.4 (701.9)	617.1 (793.4)	15.34 (-40.04, 70.72)	0.59	13.43 (-41.48, 68.33)	0.63
RPP CV (%)	5.2 (5.8)	5.0 (6.3)	0.24 (-0.21, 0.69)	0.30	0.22 (-0.23, 0.66)	0.33

Analysis of covariance (ANCOVA) with adjustment for baseline variable. AF: atrial fibrillation; BP: blood pressure; CoV: coefficient of variation; DBP: diastolic BP; HR: heart rate; MAP: mean arterial pressure; PP: pulse pressure; PPI: pulse pressure index; RPP: rate pressure product; SBP: systolic BP; SD: standard deviation.

Table 3.5: Effect of GTN vs. no GTN on haemodynamic variables over days 1 to 7 overall

	GTN	No GTN	Unadjusted Mean Difference (95% CI)	p	Adjusted Mean Difference (95% CI)	p
Patients (N)	1996	2001				
SBP min	129.2 (20.1)	131.3 (20.2)	-2.14 (-3.39, -0.89)	**0.001**	-2.25 (-3.38, -1.11)	**<0.001**
SBP mean	153.3 (19.0)	156.0 (18.8)	-2.71 (-3.88, -1.54)	**<0.001**	-2.87 (-3.81, -1.91)	**<0.001**
SBP max	178.3 (22.9)	181.6 (22.5)	-3.31 (-4.71, -1.90)	**<0.001**	-3.48 (-4.64, -2.32)	**<0.001**
SBP SD	15.5 (6.2)	15.9 (6.4)	-0.38 (-0.77, 0.01)	0.056	-0.40 (-0.78, -0.02)	**0.041**
SBP CV (%)	10.2 (4.1)	10.3 (4.2)	-0.08 (-0.34, 0.18)	0.53	-0.08 (-0.34, 0.17)	0.52
MAP min	91.0 (13.6)	93.1 (13.7)	-2.05 (-2.90, -1.20)	**<0.001**	-2.21 (-2.95, -1.46)	**<0.001**
MAP mean	106.9 (12.6)	109.1 (12.7)	-2.15 (-2.93, -1.37)	**<0.001**	-2.33 (-2.96, -1.71)	**<0.001**
MAP max	123.8 (15.0)	126.0 (15.2)	-2.28 (-3.22, -1.35)	**<0.001**	-2.48 (-3.27, -1.70)	**<0.001**
MAP SD	10.4 (4.1)	10.4 (4.2)	-0.02 (-0.28, 0.24)	0.89	-0.03 (-0.29, 0.22)	0.81
MAP CV (%)	9.8 (3.9)	9.6 (3.9)	0.17 (-0.07, 0.42)	0.17	0.17 (-0.07, 0.42)	0.16
DBP min	69.0 (11.9)	70.9 (12.3)	-1.95 (-2.70, -1.20)	**<0.001**	-2.12 (-2.75, -1.48)	**<0.001**
DBP mean	83.8 (11.2)	85.6 (11.8)	-1.87 (-2.59, -1.16)	**<0.001**	-2.07 (-2.61, -1.52)	**<0.001**
DBP max	100.3 (14.5)	102.2 (14.6)	-1.97 (-2.87, -1.07)	**<0.001**	-2.16 (-2.94, -1.39)	**<0.001**
DBP SD	9.7 (3.9)	9.7 (3.9)	0.01 (-0.24, 0.25)	0.97	0.00 (-0.25, 0.24)	0.97
DBP CV (%)	11.7 (4.7)	11.5 (4.6)	0.24 (-0.05, 0.53)	0.10	0.26 (-0.03, 0.54)	0.08
HR min	66.1 (12.0)	65.2 (11.4)	0.97 (0.24, 1.69)	**0.009**	0.82 (0.23, 1.41)	**0.006**
HR mean	78.2 (12.4)	77.1 (12.0)	1.12 (0.36, 1.88)	**0.004**	0.93 (0.38, 1.49)	**0.001**
HR max	92.2 (16.7)	90.9 (16.9)	1.22 (0.18, 2.26)	**0.020**	1.01 (0.15, 1.87)	**0.020**
HR SD	8.4 (4.7)	8.3 (4.6)	0.07 (-0.22, 0.36)	0.63	0.06 (-0.23, 0.34)	0.70
HR CV (%)	10.7 (5.6)	10.7 (5.5)	-0.01 (-0.36, 0.33)	0.95	-0.01 (-0.36, 0.33)	0.94
PP	69.5 (14.6)	70.4 (14.8)	-0.84 (-1.75, 0.08)	0.07	-0.79 (-1.48, -0.10)	**0.025**
PPI	0.65 (0.12)	0.65 (0.13)	0.00 (0.00, 0.01)	0.28	0.01 (0.00, 0.01)	**0.034**
RPP min	9361.5 (2225.5)	9368.4 (2164.7)	-7.00 (-143.16, 129.19)	0.92	-29.78 (-145.93, 86.37)	0.62
RPP mean	11984.6 (2417.4)	12028.3 (2411.9)	-43.73 (-193.5, 106.05)	0.57	-78.39 (-193.08, 36.30)	0.18
RPP max	15090.2 (3398.3)	15195.5 (3502.7)	-105.31 (-319.37, 108.75)	0.34	-152.13 (-325.96, 21.71)	0.09

RPP SD	1805.8 (874.1)	1841.8 (908.1)	-36.01 (-91.33, 19.30)	0.20	-42.45 (-94.99, 10.10)	0.11
RPP CV (%)	15.1 (6.7)	15.3 (6.7)	-0.16 (-0.58, 0.25)	0.44	-0.18 (-0.59, 0.23)	0.39

Data mean (SD) or mean difference (95% confidence intervals). Analysis of covariance (ANCOVA) with adjustment for baseline variable. BP: blood pressure; CoV: coefficient of variation; DBP: diastolic BP; HR: heart rate; MAP: mean arterial pressure; PP: pulse pressure; PPI: pulse pressure index; RPP: rate pressure product; SBP: systolic BP; SD: standard deviation.

Table 3.6: Effect of GTN vs. no GTN on haemodynamic variables over days 1 to 7 excluding those with AF

	GTN	No GTN	Unadjusted Mean Difference (95% CI)	p	Adjusted Mean Difference (95% CI)	p
Patients (N)	1602	1636				
SBP min	130.7 (20.1)	132.4 (20.3)	-1.74 (-3.13, -0.34)	**0.015**	-1.75 (-3.00, -0.50)	**0.006**
SBP mean	154.2 (19.3)	156.6 (19.3)	-2.40 (-3.73, -1.07)	**<0.001**	-2.43 (-3.50, -1.37)	**<0.001**
SBP max	178.9 (23.3)	181.8 (23.1)	-2.92 (-4.52, -1.32)	**<0.001**	-2.92 (-4.24, -1.61)	**<0.001**
SBP SD	15.2 (6.2)	15.6 (6.3)	-0.43 (-0.86, 0.05)	0.053	-0.43 (-0.85, -0.01)	**0.046**
SBP CV (%)	9.9 (4.0)	10.1 (4.0)	-0.14 (-0.41, 0.14)	0.34	-0.13 (-0.41, 0.15)	0.35
MAP min	91.8 (13.6)	93.6 (13.8)	-1.78 (-2.73, -1.84)	**<0.001**	-1.87 (-2.69, -1.04)	**<0.001**
MAP mean	107.2 (12.8)	109.1 (12.9)	-1.94 (-2.83, -1.05)	**<0.001**	-2.05 (-2.74, -1.35)	**<0.001**
MAP max	123.8 (15.3)	125.7 (15.4)	-1.96 (-3.02, -0.90)	**<0.001**	-2.01 (-2.89, -1.12)	**<0.001**
MAP SD	10.1 (4.1)	10.2 (4.1)	-0.05 (-0.33, 0.23)	0.89	-0.04 (-0.32, 0.24)	0.77
MAP CV (%)	9.5 (3.8)	9.4 (3.8)	0.11 (-0.15, 0.38)	0.41	0.11 (-0.15, 0.38)	0.40
DBP min	69.4 (12.0)	71.2 (12.4)	-1.77 (-2.61, -0.93)	**<0.001**	-1.86 (-2.56, -1.17)	**<0.001**
DBP mean	83.7 (11.4)	85.4 (11.8)	-1.71 (-2.51, -0.91)	**<0.001**	-1.85 (-2.45, -1.25)	**<0.001**
DBP max	99.8 (14.7)	101.6 (14.7)	-1.82 (-2.83, -0.81)	**<0.001**	-1.94 (-2.81, -1.06)	**<0.001**
DBP SD	9.4 (3.9)	9.4 (3.8)	-0.04 (-0.31, 0.22)	0.75	-0.04 (-0.31, 0.22)	0.76
DBP CV (%)	11.3 (4.7)	11.2 (4.6)	0.15 (-0.17, 0.47)	0.35	0.15 (-0.17, 0.46)	0.36
HR min	65.7 (11.5)	64.9 (11.1)	0.82 (0.04, 1.60)	**0.039**	0.80 (0.20, 1.41)	**0.010**
HR mean	77.0 (11.8)	76.1 (11.7)	0.97 (0.16, 1.78)	**0.019**	0.94 (0.36, 1.53)	**0.002**
HR max	90.3 (15.5)	89.3 (16.0)	0.99 (-0.10, 2.07)	0.07	0.95 (0.04, 1.85)	**0.040**
HR SD	7.9 (4.3)	7.9 (4.2)	0.03 (-0.27, 0.32)	0.86	0.03 (-0.27, 0.32)	0.85
HR CV (%)	10.3 (5.2)	10.3 (5.2)	-0.05 (-0.41, 0.31)	0.80	-0.05 (-0.41, 0.30)	0.77
PP	70.4 (14.5)	71.1 (15.0)	-0.69 (-1.71, 0.33)	0.18	-0.59 (-1.35, -0.18)	0.13
PPI	0.66 (0.12)	0.65 (0.13)	0.01 (0.00, 0.01)	0.28	0.01 (0.00, 0.01)	**0.033**
RPP min	9393.1 (2195.1)	9381.8 (2121.9)	11.31 (-137.44, 160.07)	0.88	16.95 (-107.39, 141.28)	0.79
RPP mean	11884.0 (2380.8)	11916.9 (2394.0)	-32.89 (-197.42, 131.65)	0.70	-33.27 (-155.89, 89.34)	0.60
RPP max	14854.3 (3296.5)	14954.8 (3454.4)	-100.47 (-333.22, 132.28)	0.40	-102.87 (-291.00, 85.26)	0.28

RPP SD	1717.6 (817.1)	1761.9 (855.8)	-44.29 (-101.98, 13.40)	0.13	-44.55 (-101.76, 12.65)	0.13
RPP CV (%)	14.5 (6.2)	14.7 (6.3)	-0.24 (-0.68, 0.19)	0.27	-0.25 (-0.68, 0.18)	0.26

Data mean (SD) or mean difference (95% confidence intervals). Analysis of covariance (ANCOVA) with adjustment for baseline variable. AF: atrial fibrillation; BP: blood pressure; CoV: coefficient of variation; DBP: diastolic BP; HR: heart rate; MAP: mean arterial pressure; PP: pulse pressure; PPI: pulse pressure index; RPP: rate pressure product; SBP: systolic BP; SD: standard deviation.

3.5 DISCUSSION

In this pre-specified secondary analysis of ENOS trial data, baseline blood pressure and heart rate haemodynamic parameters were associated with an unfavourable shift in functional outcome at day 90. Increased between-visit BP variability over days 1 to 7 was associated with an unfavourable shift in mRS, worse cognitive scores and increased odds of death at day 90. GTN lowered BP and RPP, whilst increasing HR at day 1, and reduced between-visit variability of SBP over days 1 to 7. Therefore, we can reject the null hypotheses and state that:

- Raised BP and its derivatives, including variability, are associated with poor clinical outcomes after acute stroke.
- Transdermal GTN lowers BP and its derivatives, including variability in acute stroke .
- Transdermal GTN increases heart rate in acute stroke.

Data from the International Stroke Trial (IST) demonstrated a U-shaped curve between SBP 24 hours after ischaemic stroke and outcome.(200) Two other trials found that high baseline SBP within 8 hours of ischaemic stroke onset was associated with neurological impairment at day 7 and symptomatic ICH but not functional outcome at 3 and 6 months.(55, 211) Although we found no association between baseline SBP and functional outcome at 90 days, increased maximum DBP and MAP were both independently associated with an unfavourable shift in mRS at day 90 in the whole population. One

cohort found that baseline DBP and MAP were associated with increased neurological impairment at day 7, whilst MAP – and not DBP – was associated with death or dependency at 90 days.(55) In contrast, others have reported no effect of DBP on early outcomes at day 10.(56)

All derived measures of higher baseline HR and RPP were associated with an unfavourable shift in mRS at day 90 in both the overall population and in those without AF. High baseline HR has been associated with increased death, heart failure and dependency at 90 days in both acute ischaemic and haemorrhagic stroke.(203, 212, 213) As well as being a surrogate for clinical frailty and comorbidity burden,(214) high baseline HR may also represent underlying dehydration, anaemia and stroke severity, which are all independently associated with poor prognosis after stroke.(215, 216) Although there are fewer data pertaining to RPP in acute stroke, increased baseline RPP has been associated with death or dependency at day 90.(55) In addition to confirming this finding, we demonstrated that increased within-visit variability of RPP at baseline was associated with an unfavourable shift in mRS, a novel finding in acute stroke.

In addition to RPP, within-visit variability of DBP, MAP and HR at baseline were associated with unfavourable shifts in mRS at day 90. There are conflicting data with some authors reporting no association between baseline within-visit BP or HR variability and 90-day functional outcome in ischaemic stroke,(212) whilst others have

demonstrated associations between increased SBP variability at baseline, and the first 24 hours of admission, and poor functional outcome.(55, 211) Similarly, increased baseline within-visit SBP variability has been associated with worse early clinical outcomes in some analyses,(56, 211) but not others.(55) In our analysis, baseline readings were taken prior to randomisation and therefore over a much shorter period than the above studies.

Increased between-visit variability of SBP over days 1 to 7 was associated with an unfavourable shift in mRS, worse cognitive scores and increased odds of death at day 90 independent of trend in mean BP over time. This suggests that fluctuations in BP in the days after stroke may have a greater influence on 90-day outcome than absolute mean BP; in line with a recently reported post-hoc analysis in ICH patients.(217) A meta-analysis of seven studies assessing BP variability in acute stroke (ICH and ischaemic stroke) demonstrated a significant association between SBP variability and poor functional outcome.(204) INTERACT-2 also reported that increased variability of DBP and MAP were associated with an unfavourable shift in mRS,(54) in line with the results of the present analysis. Our finding of SBP variability being associated with less death in the middle quintiles may be inherent to the outcome being studied; participants who died within 7 days were included in the analysis. Such patients had fewer BP measurements and therefore less potentially measurable variability, skewing the data towards the lowest quintile.

This is the first trial-based analysis to assess the association between between-visit BP variability after acute stroke and cognitive scores at 90 days. A Chinese observational study (n=796) found that increased SBP variability over the first 7 days after acute ischaemic stroke was associated with post-stroke cognitive impairment at 90 days, defined as a Montreal cognitive assessment (MoCA) score <26.(218) Here, we have demonstrated – with a much larger population including ICH – that increased between-visit variability of SBP, DBP and MAP over days 1 to 7 were all independently associated with worse cognitive scores at 90 days across three cognitive domains.

Transdermal GTN significantly lowered SBP, DBP, MAP, mean BP, PP and PPI, and increased HR in a pooled analysis of three phase II studies of acute and subacute stroke.(207) Using a much larger dataset, we have confirmed GTN's aforementioned effects on haemodynamics. The significant reduction in RPP at day 1, implies that GTN's ability to increase HR is negated by its BP-lowering effect. GTN significantly lowered between-visit variability of SBP over days 1 to 7, mirroring a similar finding in a pooled analysis of four GTN pilot studies.(219)

The timing of haemodynamic measurements in relation to stroke onset is important. The maintenance of cerebral blood flow through autoregulation is impaired in acute stroke with cerebral perfusion pressure becoming dependent on systemic BP.(62) Potentially viable brain tissue may therefore be sensitive to greater variability in BP

with peaks increasing the risk of haemorrhagic transformation in ischaemic stroke or haematoma expansion in ICH, and cerebral oedema in both stroke types, whilst troughs cause further ischaemic injury.(51, 204) If so, then medications that lower BP variability may be best assessed as early as possible after stroke onset, before these effects manifest. Although this potential time-dependent mechanism is speculative, it may be one way in which GTN may exert its apparent beneficial impact on clinical outcomes.(219) The efficacy of transdermal GTN given within four hours of onset is currently being assessed in the large Rapid Intervention with Glyceryl trinitrate in Hypertensive stroke Trial 2 (RIGHT-2).(188)

The strengths of the present study include: its large sample size using data from one of the largest BP trials in acute stroke; the number of haemodynamic measures and use of a validated BP monitor; the use of pre-specified analyses and ordinal logistic regression to increase power compared with binary analysis of mRS; and generalisability with analyses including the vast majority of recruited participants from a variety of countries, stroke services and patient populations.

This study has several limitations. First, some comparisons were observational and the results should be interpreted with caution due to the inherent risk of confounding. Despite adjusting for baseline prognostic factors, we cannot exclude reverse causality, for example if patients with larger, more severe strokes also had increased BP variability. Second, the inclusion criterion of SBP 140-220 mmHg

systematically excluded patients and thus reduces generalisability, although this is likely to have attenuated rather than enhanced the strength of reported associations. Third, although a validated automated monitor was used for measurements, beat-to-beat data were not available, limiting the ability to detect within-clinic variability. Fourth, a minority of patients had AF which can reduce the accuracy of BP and HR measurements; however, sensitivity analyses where patients with AF were excluded did not materially alter the results. Fifth, cognitive outcome data were only available for around 50% of participants and their analyses may have lacked power, weakening the observed associations but given the severity of the stroke population recruited this is inevitable. Sixth, we do not have outcome data beyond three months and it is unclear whether the associations found with mRS persist over the longer term. Last, participants were recruited a median of 26 hours following stroke onset, which is longer than previous studies that have detected associations between haemodynamic measures and functional outcome.(204)

Smooth lowering of elevated BP with avoidance of peaks and troughs over the first days after stroke should be considered by clinicians in both acute ICH and ischaemic stroke. Whether smooth and sustained BP control is beneficial has yet to be tested directly in randomised controlled trials. Therefore, future studies assessing whether it is feasible to reduce BP variability in acute stroke and, if so, whether it is beneficial are warranted. If BP variability is a modifiable target in

acute stroke, then agents that lower it, including GTN, may be of benefit.

CHAPTER 4:

IMPACT OF HYDRATION STATUS ON HAEMODYNAMICS, EFFECTS OF ACUTE BLOOD PRESSURE LOWERING TREATMENT, AND PROGNOSIS AFTER STROKE

Publications contributing to this chapter:

*Billington CK, *Appleton JP, Berge E, Sprigg N, Glover M, Bath PM. Impact of hydration status on haemodynamics, effects of acute blood pressure lowering treatment, and prognosis after stroke. British Journal of Clinical Pharmacology. Epub 08 Sept 2018, DOI: 10.1111/bcp.13761

*both authors contributed equally

Presentations contributing to this chapter:

Effect of transdermal glyceryl trinitrate on blood pressure: Relationship with hydration status. European Stroke Organisation Conference. Prague, Czech Republic (May 2017)

Transdermal glyceryl trinitrate does not cause precipitous changes in blood pressure in dehydrated acute stroke patients. British and Irish Hypertension Society Annual Scientific Meeting. The Technology and Innovation Centre, University of Strathclyde, Glasgow (September 2017)

4.1 ABSTRACT

Background

High blood pressure (BP) is common in acute stroke and associated with poor outcome, but the Efficacy of Nitric Oxide in Stroke (ENOS) trial showed no beneficial effect of antihypertensive treatment in this situation. Antihypertensive agents have accentuated effects in dehydrated patients. We assessed the association of dehydration with haemodynamics, effects of antihypertensive treatment and prognosis in the ENOS trial.

Methods

ENOS randomised 4011 patients with acute stroke and raised systolic BP to glyceryl trinitrate (GTN) patch or no GTN, and to continue or to stop existing antihypertensive treatment within 48 hours of onset. The primary outcome was functional outcome (modified Rankin Scale, mRS) at day 90. Blood markers of dehydration at baseline were collected at two sites (n=310) and their relation to haemodynamics and outcome was assessed.

Results

There were no significant associations between dehydration markers and fall in blood pressure from baseline to day 1, and no significant interaction with allocated treatment. Overall, increasing urea was associated with an unfavourable shift in mRS (OR 3.43, 95% CI 1.42

to 8.32, p=0.006) and increased risk of death at day 90 (HR 4.55, 95% CI 1.51 to 13.66, p=0.007).

Conclusions

Blood pressure lowering treatment was safe in dehydrated patients, with no precipitous changes in BP, this supporting its use in acute stroke prior to blood markers of dehydration becoming available. Increased baseline urea was associated with poor prognosis after stroke.

4.2 INTRODUCTION

High blood pressure (BP) is common in acute stroke and associated independently with a poor outcome in both ischaemic stroke and intracerebral haemorrhage.(200) Lowering elevated BP is recommended in acute intracerebral haemorrhage,(144) and is safe in ischaemic stroke.(84, 124) Most drug classes that might be useful for lowering BP (including angiotensin converting enzyme-inhibitors, angiotensin receptor antagonists, nitrates) have accentuated vasodepressant effects when patients are dehydrated or hypovolaemic.(220) Reduced circulating volume is also common in stroke, especially if admission to hospital is delayed thereby allowing dehydration to develop. Hypovolaemia may reduce cerebral perfusion and increase the infarct core in ischaemic stroke(63) and the perihaematomal ischaemia in intracerebral haemorrhage.(64) It may also lead to renal impairment and is associated with venous thromboembolism.(136, 221) As a consequence, dehydration has been associated with poor clinical outcomes following acute stroke,(215, 222, 223) and adequate hydration after stroke is recommended in clinical guidelines.(136)

The Efficacy of Nitric Oxide in Stroke (ENOS) trial assessed the effect of transdermal glyceryl trinitrate (GTN) on outcome in 4011 patients.(84) Overall, GTN did not alter clinical outcomes despite lowering BP by 7/3.5 mmHg at day 1,(84) but when administered within 6 hours of stroke onset, GTN improved multiple clinical outcomes at day 90.(149) The aim of the present planned substudy

was to assess the impact of dehydration on haemodynamic changes, effect of blood pressure reduction on clinical outcomes, and prognosis after stroke.

4.3 METHODS

Hypothesis

Transdermal GTN is safe in the setting of acute stroke patients with blood markers of dehydration.

ENOS trial

The ENOS trial protocol, statistical analysis plan, baseline characteristics and main trial results have been published elsewhere.(84, 189, 208, 209) In brief, ENOS recruited 4011 people with acute stroke within 48 hours of onset and high systolic BP (140-220 mmHg) and randomised them to GTN 5 mg patch or no patch for 7 days. In addition, participants taking antihypertensive medication prior to the index event were randomised to continue or stop these drugs for 7 days. Patients or relatives/carers gave written consent to participate. ENOS was registered (ISRCTN99414122) and approved by ethics committees/competent authorities in all participating countries.

Biomarkers of Dehydration

Biochemical biomarkers were recorded at two sites (Nottingham City Hospital and Queen's Medical Centre, Nottingham UK) including full blood count (haemoglobin, red cell count, haematocrit/packed cell volume) and biochemistry (sodium, potassium, urea, creatinine, glucose). Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Diseases (MDRD) equation: 186 x serum creatinine (μmol/L)$^{-1.154}$ x age (years)$^{-0.203}$ x (0.742 if

female) x (1.210 if Black)). Since ethnicity was not recorded in ENOS, participants of Black ethnicity will have an under-estimated eGFR.

In addition, we calculated the following markers of dehydration, which are all elevated in dehydrated patients:

i) Urea:Creatinine (mg/dL),
ii) 2 Na + Glucose + Urea (mmol/L) (224),
iii) 2 Na + 2 K + Glucose + Urea (mmol/L) (225)

The latter two are also formulae for osmolarity. Ethanol may be added to the calculations to refine the estimate of osmolarity but was not measured in ENOS. Furthermore, ENOS did not routinely collect information on clinical markers of dehydration such as thirst or skin turgor.

Dehydration was defined as: Na>145 mmol/l, urea>7.5 mmol/l, urea:creatinine ratio>20, calculated osmolarity >297 mmol/l, eGFR <30 ml/min/1.73m^2, haematocrit >0.54 L/L for men and >0.47 L/L for women, and red cell count >6.5 cells/μL for men and >5.8 cells/μL for women.

Haemodynamic Outcomes

Peripheral haemodynamics (BP and HR) were measured at baseline (three measurements) and on days 1 to 7 (two measurements), using a validated automated monitor (Omron 705CP).(210).

Clinical Outcomes

The primary outcome (functional outcome) was measured using the modified Rankin Scale (mRS, a 7-level ordered categorical scale where 0 = independent and 6 = dead) at day 90. Clinical safety outcomes included all-cause death at day 90, and headache, hypotension, hypertension at day 7, and change in the Scandinavian Stroke Scale (SSS, a marker of neurological improvement) from baseline to day 7. Multiple secondary outcomes were also assessed at day 90. All day 90 outcomes were assessed centrally by telephone by trained investigators based at the International Coordinating Centre in Nottingham; assessors were masked to treatment allocation.

Statistical Analysis

Data were analysed by intention-to-treat in line with the ENOS trial statistical analysis plan and statistical analyses adopted in the primary publication.(189) Data are shown as number (%), median [interquartile range, IQR], or mean (standard deviation, SD). Baseline characteristics between groups were assessed using X^2 for categorical variables and one-way analysis of variance (ANOVA) for continuous variables.

Associations between dehydration markers and haemodynamic changes from baseline to day 1 were assessed using multiple linear regression, after adjustment for age, sex and allocated treatment, with resultant standardised regression coefficients (β) given.

Interaction p-values were calculated by adding an interaction term for treatment and dehydration marker to the models.

Associations between dehydration markers and clinical outcomes were assessed using Cox proportional hazard regression, binary logistic regression, multiple linear regression or ordinal logistic regression. The impact of dehydration on the effect of treatment was assessed by introducing an interaction term for treatment and dehydration status to the analyses. Statistical models were adjusted for prognostic baseline covariates including age, sex, systolic BP, SSS and time to randomisation. Analyses involving the whole population were also adjusted for treatment allocation. The resultant hazard ratio (HR), mean difference (MD) or odds ratio (OR) and associated 95% confidence intervals (CI) are given, with significance set at $p \leq 0.05$. Analyses were performed using SPSS version 22 (Chicago, IL).

4.4 RESULTS

This sub-study included 310 participants (GTN 158, no GTN 152; continue pre-stroke antihypertensives 74, temporarily stop 76) from two trial sites in Nottingham, UK. Of these, 294 had data on one or more laboratory measure of dehydration. Baseline characteristics and biochemical markers of dehydration are shown in Table 4.1. Clinical characteristics were well balanced, and blood markers of dehydration did not differ between randomised groups (GTN vs. no GTN, or Continue vs. Stop pre-stroke antihypertensives), except for raised haematocrit (Table 4.1).

Table 4.1: Baseline characteristics of patients enrolled into this ENOS sub-study.

	All	GTN		No GTN		p	Continue		Stop		p
Participants	310		n		n			n		n	
Age (yr)	73.2 (11.7)	73.1 (11.7)	158	73.3 (11.7)	152	-	77.0 (9.2)	74	76.1 (8.9)	76	-
Sex, male (%)	169 (54.5)	84 (53.2)	158	85 (55.9)	152	-	36 (48.6)	74	33 (43.4)	76	-
Drugs pre-stroke (%)											
ACE-I	57 (18.4)	26 (16.5)	158	31 (20.4)	152	-	30 (40.5)	74	26 (34.2)	76	-
ARB	18 (5.8)	12 (7.6)	158	6 (3.9)	152	-	7 (9.5)	74	10 (13.2)	76	-
ß-receptor antagonist	68 (21.9)	39 (24.7)	158	29 (19.1)	152	-	28 (37.8)	74	36 (47.4)	76	-
Calcium channel blocker	56 (18.1)	34 (21.5)	158	22 (14.5)	152	-	25 (33.8)	74	25 (32.9)	76	-
Diuretic	62 (20)	38 (24.1)	158	24 (15.8)	152	-	24 (32.4)	74	31 (40.8)	76	-
Others	14 (4.5)	11 (7)	158	3 (2)	152	-	8 (10.8)	74	5 (6.6)	76	-
Stroke type, ischaemic (%)	264 (85.2)	131 (82.9)	158	133 (87.5)	152	-	66 (89.2)	74	66 (86.8)	76	-
Stroke severity (SSS, /58)	32.5 (13.9)	32.4 (13.9)	158	32.6 (14)	152	-	33.0 (13.7)	74	32.2 (13.1)	76	-
Stroke syndrome, TACS (%)	124 (40)	70 (44.3)	158	54 (35.5)	152	-	33 (44.6)	74	30 (39.5)	76	-

Systolic blood pressure (mmHg)	166.6 (20.1)	167.7 (19.1)	158	165.4 (21.1)	152	-	166.9 (19.9)	74	165.0 (18.0)	76	-
Diastolic blood pressure (mmHg)	89.1 (14.3)	89.7 (15.5)	158	88.4 (12.9)	152	-	87.1 (14.3)	74	85.2 (12.6)	76	-
Heart rate (bpm)	75.4 (14.4)	74.9 (13.8)	158	76 (14.9)	152	-	77.3 (16.4)	74	73.6 (14.1)	76	-
Time, onset to randomisation (hr)	27.6 (12.1)	27.5 (12.2)	158	27.8 (12.0)	152	-	25.4 (11.7)	74	25.6 (12.0)	76	-
Blood analyses											
Haematocrit (L/L)	0.42 (0.04)	0.42 (0.04)	152	0.41 (0.04)	142	0.33	0.41 (0.04)	69	0.41 (0.04)	70	0.61
Haemoglobin (g/L)	139 (16.3)	140 (16.5)	152	138 (16.1)	142	0.30	136 (14.2)	69	139 (15.0)	70	0.31
Red cell count (10E12/L)	4.6 (0.5)	4.6 (0.5)	152	4.6 (0.5)	142	0.54	4.5 (0.5)	69	4.6 (0.5)	70	0.56
Sodium (mmol/L)	138.3 (3.6)	138.3 (3.9)	154	138.4 (3.4)	143	0.69	138.1 (4.0)	70	138.5 (3.8)	72	0.57
Potassium (mmol/L)	4.1 (0.5)	4.1 (0.5)	149	4.2 (0.4)	141	0.16	4.0 (0.5)	67	4.0 (0.5)	71	0.92
Urea (mmol/L)	6.5 (2.6)	6.4 (2.6)	154	6.6 (2.6)	143	0.45	7.1 (2.7)	70	7.0 (2.2)	72	0.87
Creatinine (µmol/L)	94.4 (32)	93.1 (25.3)	154	95.8 (37.9)	143	0.47	98.6 (43.4)	70	97.7 (25.4)	72	0.88
eGFR (ml/min/1.73m^2)	69.7 (20.8)	69.5 (19.7)	154	69.9 (22.1)	143	0.85	65.4 (19.3)	70	63.2 (18.3)	72	0.51
Glucose (mmol/L)	6.7 (1.9)	6.7 (1.9)	126	6.7 (1.9)	114	0.93	6.8 (1.9)	59	6.9 (1.7)	52	0.87

Urea:Creatinine (mg/dL)	17.4 (5.3)	17 .0 (4.6)	154	17.8 (5.9)	143	0.21	18.4 (4.9)	70	18.0 (4.6)	72	0.67
Osmolarity A (mmol/l)	289.6 (7.7)	288.9 (8.5)	126	290.3 (6.7)	112	0.17	289.0 (8.9)	59	291.5 (7.7)	51	0.13
Osmolarity B (mmol/l)	297.8 (7.8)	297.2 (8.7)	122	298.6 (6.7)	111	0.17	296.8 (9.3)	56	299.3 (7.8)	50	0.14
Dehydration markers											
Na>145 mmol/l (%)	3 (1)	3 (1.9)	154	0 (0)	143	0.09	1 (1.4)	70	1 (1.4)	72	0.98
Urea >7.5 mmol/L (%)	69 (23.2)	35 (22.7)	155	34 (23.8)	143	0.83	20 (28.6)	70	25 (34.7)	72	0.43
eGFR <30 ml/min/1.73m^2 (%)	4 (1.3)	1 (0.6)	154	3 (2.1)	143	0.28	2 (2.9)	70	0 (0)	72	0.15
Urea:Creatinine >20 (%)	75 (25.3)	36 (23.4)	154	39 (27.3)	143	0.44	26 (37.1)	70	19 (26.4)	72	0.17
Osmolarity A >297 mmol/L (%)	31 (13)	17 (13.5)	126	14 (12.5)	112	0.82	10 (16.9)	59	13 (25.5)	51	0.27
Osmolarity B >297 mmol/L (%)	129 (55.6)	62 (51.2)	121	67 (60.4)	111	0.16	30 (53.6)	56	31 (62.0)	50	0.38
Haematocrit >0.54 L/L (male), >0.47 L/L (female) (%)	6 (2.0)	6 (3.9)	152	0 (0)	142	0.017	1 (1.4)	69	1 (1.4)	70	0.99
Haemoglobin >180 g/L (male), >165 g/L (female) (%)	3 (1)	3 (2)	152	0 (0)	142	0.09	1 (1.4)	69	1 (1.4)	70	0.99
Red cell count >6.5 (male), >5.8 (female) (%)	0 (0)	0 (0)	152	0 (0)	142	-	0 (0)	69	0 (0)	70	-

Data are number (%) or mean (standard deviation); comparison by Chi-square test or one-way analysis of variance (ANOVA). ACE-I: Angiotensin-converting enzyme inhibitor; ARB: Angiotensin receptor blocker; bpm: beats per minute;

eGFR: estimated glomerular filtration rate; K: potassium; Na: sodium; Osmolarity A: 2 Na+Glucose+Urea; Osmolarity B: 2 Na+2 K+Glucose+ Urea (mmol/L); SSS: Scandinavian Stroke Scale; TACS: total anterior circulation syndrome.

The relationship between blood biomarkers of dehydration and change in haemodynamic parameters from baseline to day 1 is shown split by randomisation to GTN or no GTN and Stop or Continue pre-stroke antihypertensives (Table 4.2). There were no significant interactions between treatment with GTN vs. no GTN and dehydration markers on the change in BP and heart rate from baseline to day 1. GTN lowered BP and increased heart rate, but dehydration markers did not significantly influence these findings. Similarly, there were no significant interactions between continuing vs. stopping pre-stroke antihypertensives and dehydration markers on haemodynamic changes from baseline to day 1.

We compared effects of blood pressure lowering treatment (GTN vs. no-GTN, and Continue vs. Stop) on neurological impairment and clinical safety events during the first 7 days and outcome at 3 months, by level of urea (Table 4.3) and level of urea:creatinine ratio (Table 4.4). There were no differences in change in neurological impairment or in rates of reported hypotension, hypertension or headache by day 7 in those randomised to GTN compared to no GTN, or those randomised to Stop vs. Continue their pre-stroke antihypertensives. In those with a raised urea (>7.5 mmol/L) there was a tendency towards an unfavourable shift in mRS and increased death at 90 days when randomised to GTN vs. no GTN (p for interaction=0.047 and 0.050, respectively); a finding not seen with raised urea:creatinine ratio. No significant interactions were noted in regard to Stop vs. Continue.

Table 4.2: Relationship between blood measures of hydration status and allocated treatment and % change in SBP, DBP and HR from day 0 to day 1, by treatment allocation

Allocated treatment (Rx)	Measure of hydration status (MHS)	ΔSBP					ΔDBP					ΔHR				
		β for Rx	p	β for MHS	p	p interaction	β for Rx	p	β for MHS	p	p interaction	β for Rx	p	β for MHS	p	p interaction
GTN/No GTN	Sodium	-0.264	<0.001	0.175	0.35	0.64	-0.143	0.015	0.014	0.81	0.54	0.158	0.008	-0.029	0.62	0.08
	Urea	-0.263	<0.001	0.019	0.76	0.50	-0.135	0.021	0.138	0.027	0.34	0.156	0.009	-0.032	0.61	0.64
	eGFR	-0.264	<0.001	-0.002	0.97	0.57	-0.143	0.015	-0.045	0.49	0.67	0.158	0.008	0.054	0.41	0.24
	Urea: Creatinine	-0.261	<0.001	0.045	0.47	0.37	-0.135	0.021	0.096	0.13	0.54	0.161	0.007	0.036	0.58	0.54
	Osmolarity A	-0.257	<0.001	0.007	0.92	0.54	-0.198	0.003	-0.085	0.19	0.73	0.190	0.004	-0.082	0.21	0.12
	Osmolarity B	-0.256	<0.001	0.007	0.91	0.59	-0.205	0.002	-0.073	0.26	0.71	0.201	0.003	-0.084	0.21	0.15
	Haematocrit	-0.258	<0.001	0.003	0.96	0.34	-0.134	0.024	-0.039	0.54	0.26	0.158	0.008	0.011	0.86	0.83
	Haemoglobin	-0.257	<0.001	-0.007	0.91	0.45	-0.132	0.026	-0.057	0.37	0.23	0.156	0.009	0.028	0.66	0.89
	Red cell count	-0.259	<0.001	0.045	0.46	0.78	-0.134	0.023	-0.053	0.39	0.08	0.159	0.007	-0.021	0.74	0.36
Cont / Stop	Sodium	-0.112	0.19	0.013	0.88	0.51	0.024	0.78	-0.027	0.76	0.98	-0.043	0.62	-0.080	0.35	0.88
	Urea	-0.112	0.20	-0.038	0.67	0.80	0.024	0.78	0.050	0.57	0.27	-0.042	0.63	0.041	0.64	0.98
	eGFR	-0.111	0.20	-0.021	0.82	0.89	0.025	0.77	-0.001	0.99	0.59	-0.044	0.61	0.039	0.66	0.85
	Urea: Creatinine	-0.114	0.19	0.024	0.80	0.35	0.018	0.83	0.108	0.24	0.90	-0.045	0.60	0.065	0.47	0.68
	Osmolarity A	-0.111	0.27	-0.029	0.77	0.74	-0.061	0.54	-0.154	0.12	0.84	-0.075	0.45	-0.131	0.18	0.63
	Osmolarity B	-0.100	0.33	-0.036	0.72	0.62	-0.040	0.70	-0.137	0.17	0.63	-0.062	0.54	-0.129	0.20	0.71
	Haematocrit	-0.097	0.27	-0.063	0.48	0.83	0.036	0.68	-0.069	0.44	0.14	-0.040	0.65	-0.057	0.52	0.16
	Haemoglobin	-0.102	0.25	-0.081	0.37	0.57	0.030	0.73	-0.102	0.264	0.09	-0.041	0.64	-0.040	0.66	0.28
	Red cell count	-0.095	0.28	0.010	0.12	0.56	0.034	0.70	-0.085	0.35	0.70	-0.040	0.65	-0.027	0.76	0.14

Multiple linear regression with adjustment for age and sex, allocated treatment and hydration status. Data are standardised regression coefficient (β) with associated p-values. β= standardised regression coefficient; interaction p = allocated treatment*blood measure of hydration status; MHS: measure of hydration status; Rx: allocated treatment

Table 4.3: Effects of GTN vs. non-GTN, and effects of Continue v. Stop antihypertensive treatment on change in neurological status and clinical events during the first 7 days and outcome at 3 months, by level of urea

	Urea	All	GTN	No GTN	OR/MD/HR (95% CI)	p	p interaction	Cont.	Stop	OR/MD/HR (95% CI)	p	p interaction
Day 7, n		310	158	152				74	76			
ΔSSS 0-7	>7.5	2.1 (10.5)	0.7 (12.6)	3.6 (7.7)	-3.02 (-8.33, 2.29)	0.26	0.25	-1.1 (10.3)	3.0 (12.8)	-4.98 (-12.59, 2.63)	0.19	0.16
	<7.5	5.6 (10.0)	5.8 (10.0)	5.5 (10.1)	0.06 (-2.63, 2.75)	0.97	-	4.9 (11.1)	3.5 (10.3)	1.85 (-2.70, 6.39)	0.42	-
Hypotension (%)	>7.5	4 (5.8)	4 (11.4)	0 (0)	-	-	-	3 (15.0)	1 (4.0)	8.59 (0.42, 177.24)	0.16	0.07
	<7.5	5 (2.2)	4 (3.4)	1 (0.9)	3.77 (0.41, 34.84)	0.24	-	1 (2.0)	3 (6.4)	0.99 (0.87, 1.13)	0.86	-
Hypertension (%)	>7.5	3 (4.3)	1 (2.9)	2 (5.9)	0.31 (0.02, 4.47)	0.39	0.31	0 (0)	2 (8.0)	-	-	-
	<7.5	10 (4.4)	7 (5.9)	3 (2.8)	2.42 (0.57, 10.29)	0.23	-	2 (4.0)	1 (2.1)	2.32 (0.15, 34.96)	0.54	-
Headache (%)	>7.5	11 (15.9)	6 (17.1)	5 (14.7)	1.28 (0.32, 5.11)	0.72	0.31	4 (20.0)	2 (8.0)	2.24 (0.34, 14.70)	0.40	0.22
	<7.5	41 (18.0)	29 (24.4)	12 (11.0)	2.76 (1.30, 5.87)	0.008	-	3 (6.0)	5 (10.6)	0.65 (0.14, 3.02)	0.58	-
Day 90												
Functional outcome (mRS) *	>7.5	3.5 (1.9)	3.9 (1.9)	3.1 (1.7)	2.19 (0.92, 5.26)	0.08	0.047	3.2 (1.8)	3.6 (1.9)	0.57 (0.19, 1.67)	0.30	0.26
	<7.5	3.0 (1.6)	3.0 (1.6)	3.0 (1.6)	0.96 (0.61, 1.52)	0.87	-	3.4 (1.7)	3.3 (1.5)	1.14 (0.56, 2.34)	0.72	-
Death (%)	>7.5	15 (21.7)	11 (31.4)	4 (11.8)	4.42 (1.26, 15.44)	0.020	0.050	3 (15.0)	6 (24.0)	0.53 (0.12, 2.31)	0.40	0.51
	<7.5	19 (8.4)	10 (8.5)	9 (8.3)	1.08 (0.43, 2.75)	0.87	-	6 (12.0)	5 (10.6)	1.47 (0.37, 5.87)	0.59	-

Multiple linear regression, binary logistic regression, ordinal logistic regression or Cox proportional hazards regression with adjustment for age, sex and time to randomisation. Data are n (%), mean (SD), mean difference (MD), odds ratio (OR) or hazard ratio (HR) with 95 confidence intervals (CI). *ordinal logistic regression. BP: blood pressure; CI: confidence interval; GTN: glyceryl trinitrate; mRS: modified Rankin Scale; OR: odds ratio; SD: standard deviation; SSS: Scandinavian Stroke Scale

Table 4.4: Effects of GTN vs. non-GTN, and effects of Continue v. Stop antihypertensive treatment on change in neurological status and clinical events during the first 7 days and outcome at 3 months by urea:creatinine ratio

	Ur:Cr	All	GTN	No GTN	OR/MD/HR (95% CI)	p	p interaction	Cont.	Stop	HR/OR/MD/HR (95% CI)	p	p interaction
Day 7, n		297	154	143				n=70	n=72			
ΔSSS 0-7	>20	3.3 (10.9)	2.8 (11.7)	3.8 (10.3)	-0.77 (-6.50, 4.96)	0.79	0.95	3.7 (10.3)	2.4 (12.9)	1.30 (-7.84, 10.44)	0.78	0.54
	<20	5.3 (10.0)	5.2 (10.6)	5.5 (9.3)	-0.87 (-3.67, 1.94)	0.54	-	2.8 (11.8)	3.7 (10.5)	-0.80 (-5.51, 3.91)	0.74	-
Hypotension (%)	>20	3 (4.0)	2 (5.6)	1 (2.6)	2.37 (0.18, 30.99)	0.51	-	2 (7.7)	1 (5.3)	1.46 (0.10, 20.81)	0.78	0.65
	<20	6 (2.7)	6 (5.1)	0 (0)	-	-	-	2 (4.5)	3 (5.7)	1.07 (0.15, 7.70)	0.95	-
Hypertension (%)	>20	3 (4.0)	2 (5.6)	1 (2.6)	2.92 (0.23, 37.98)	0.41	0.63	0 (0)	1 (5.3)	-	-	-
	<20	10 (4.5)	6 (5.1)	4 (3.8)	1.40 (0.37, 5.30)	0.62	-	2 (4.5)	2 (3.8)	1.38 (0.16, 11.58)	0.77	-
Headache (%)	>20	10 (13.3)	8 (22.2)	2 (5.1)	7.38 (1.28, 42.67)	0.026	0.16	3 (11.5)	2 (10.5)	0.92 (0.11, 7.85)	0.94	0.84
	<20	42 (18.9)	27 (22.9)	15 (14.4)	1.74 (0.86, 3.52)	0.13	-	4 (9.1)	5 (9.4)	1.07 (0.26, 1.38)	0.93	-
Day 90												
Functional outcome (mRS)*	>20	3.6 (1.7)	3.7 (1.8)	3.5 (1.5)	1.03 (0.46, 2.33)	0.94	0.84	3.5 (1.6)	3.6 (1.8)	0.73 (0.25, 2.19)	0.58	0.99
	<20	3.0 (1.7)	3.0 (1.7)	2.9 (1.7)	1.23 (0.77, 1.97)	0.38	-	3.2 (1.8)	3.3 (1.6)	0.88 (0.43, 1.77)	0.71	-
Death (%)	>20	13 (17.3)	8 (22.2)	5 (12.8)	2.66 (0.85, 8.34)	0.09	0.53	3 (11.5)	4 (21.1)	0.57 (0.12, 2.68)	0.48	0.44
	<20	21 (9.5)	13 (11.1)	8 (7.7)	1.52 (0.62, 3.68)	0.36	-	6 (13.6)	7 (13.2)	1.15 (0.36, 3.63)	0.81	-

Multiple linear regression, binary logistic regression, ordinal logistic regression or Cox proportional hazards regression with adjustment for age, sex and time to randomisation. Data are n (%), mean (SD), mean difference (MD), odds ratio (OR) or hazard ratio (HR) with 95 confidence intervals (CI).* ordinal logistic regression. BP: blood pressure; CI: confidence interval; GTN: glyceryl trinitrate; mRS: modified Rankin Scale; OR: odds ratio; SD: standard deviation; SSS: Scandinavian Stroke Scale.
Ur:Cr= Urea: creatinine ratio high >20, normal <20

The associations between markers of dehydration and mRS and death at day 90 were assessed across the total available population (Table 4.5). High urea at baseline was associated with an unfavourable shift in mRS at day 90 and increased death at day 90. Although there was no significant association between baseline creatinine and mRS at day 90, increasing creatinine was associated with an increased risk of death at day 90. In contrast, a high sodium at baseline was associated with a favourable shift in mRS at day 90. We did not find any significant relationships in adjusted analyses between other markers of dehydration and clinical outcomes at day 90 (Table 4.5).

The rate of venous thromboembolism by day 7 in the population studied was low (n=2), and therefore further analysis to establish any association with markers of dehydration was not deemed appropriate.

Table 4.5: Relationships between baseline markers of dehydration and modified Rankin Scale (mRS) or death at 3 months.

	mRS				Death			
	Unadjusted	**p**	**Adjusted**	**p**	**Unadjusted**	**p**	**Adjusted**	**p**
Sodium*	**0.46 (0.26, 0.81)**	**0.007**	**0.49 (0.28, 0.87)**	**0.015**	0.71 (0.30, 1.68)	0.43	0.81 (0.34, 1.93)	0.64
Sodium >145	1.21 (0.16, 8.94)	0.85	0.60 (0.08, 4.68)	0.63	-	-	-	-
Potassium	0.87 (0.55, 1.36)	0.54	1.34 (0.84, 2.17)	0.22	1.18 (0.55, 2.55)	0.67	1.45 (0.70, 2.99)	0.32
Urea*	**5.31 (2.36, 11.95)**	**<0.001**	**3.43 (1.42, 8.32)**	**0.006**	**3.30 (1.30, 8.38)**	**0.012**	**4.55 (1.51, 13.66)**	**0.007**
Urea >7.5	**1.71 (1.06, 2.76)**	**0.028**	1.47 (0.88, 2.44)	0.14	1.67 (0.85, 3.29)	0.14	1.95 (0.94, 4.03)	0.07
Urea tertiles								
<5.4	1.00	-	1.00	-	1.00	-	1.00	-
5.4-6.9	1.48 (0.90, 2.42)	0.12	1.11 (0.67, 1.86)	0.84	1.15 (0.42, 3.17)	0.79	1.42 (0.49, 4.11)	0.52
>6.9	**2.04 (1.25, 3.34)**	**0.004**	1.69 (0.99, 2.89)	0.056	1.62 (0.64, 4.07)	0.31	1.76 (0.64, 4.81)	0.27
Creatinine*	1.04 (0.98, 1.11)	0.18	1.06 (0.98, 1.13)	0.13	1.08 (0.99, 1.17)	0.07	**1.11 (1.02, 1.20)**	**0.019**
eGFR*	**0.89 (0.81, 0.98)**	**0.021**	0.93 (0.83, 1.04)	0.23	0.90 (0.75, 1.08)	0.27	0.84 (0.68, 1.04)	0.11
eGFR <30	2.71 (0.47, 15.55)	0.26	6.54 (0.97, 44.04)	0.053	1.97 (0.27, 14.40)	0.51	2.44 (0.30, 20.02)	0.41
eGFR								
<60.3	1.48 (0.91, 2.43)	0.12	1.19 (0.67, 2.10)	0.55	1.13 (0.50, 2.53)	0.77	1.49 (0.56, 3.97)	0.43
60.3-77.8	1.53	0.09	1.20	0.49	0.69	0.45	0.60	0.60

	(0.94, 2.51)		(0.71, 2.01)		(0.27, 1.80)		(0.22, 1.64)	
>77.8	1.00	-	1.00	-	1.00		1.00	
Glucose*	**4.27 (1.27, 14.32)**	**0.019**	1.61 (0.46, 5.60)	0.45	3.74 (0.74, 18.90)	0.11	1.58 (0.22, 11.15)	0.65
Urea: Creatinine	**1.07 (1.02, 1.11)**	**0.002**	1.03 (0.99, 1.08)	0.13	1.02 (0.97, 1.08)	0.45	1.01 (0.95, 1.08)	0.67
Osmolarity A*	0.90 (0.67, 1.20)	0.46	0.80 (0.60, 1.09)	0.16	1.21 (0.69, 2.12)	0.51	1.11 (0.65, 1.88)	0.70
Osmolarity B*	0.88 (0.66, 1.17)	0.38	0.80 (0.60, 1.08)	0.15	1.17 (0.66, 2.08)	0.59	1.10 (0.64, 1.88)	0.74
Haematocrit^{+}	0.65 (0.41, 1.05)	0.08	0.74 (0.44, 1.25)	0.26	1.64 (0.79, 3.38)	0.18	1.42 (0.62, 3.26)	0.41
Haematocrit, increased	1.45 (0.35, 6.04)	0.61	0.74 (0.17, 3.23)	0.69	1.17 (0.16, 8.54)	0.88	0.82 (0.10, 6.39)	0.85
Haematocrit								
<0.4	1.00	-	1.00	-	1.00	-	1.00	-
0.4-0.44	0.80 (0.49, 1.31)	0.38	0.97 (0.58, 1.6)	0.90	0.89 (0.38, 2.12)	0.80	1.10 (0.44, 2.75)	0.83
>0.44	0.67 (0.41, 1.1)	0.11	0.76 (0.45, 1.31)	0.33	2.15 (0.98, 4.71)	0.057	2.13 (0.87, 5.21)	0.10
Haemoglobin*	0.89 (0.79, 1.01)	0.08	0.92 (0.80, 1.05)	0.21	1.13 (0.93, 1.37)	0.24	1.06 (0.85, 1.34)	0.60
Red cell count	0.94 (0.62, 1.44)	0.79	0.91 (0.58, 1.45)	0.70	1.79 (0.95, 3.36)	0.07	1.60 (0.81, 3.15)	0.18

Analysis by ordinal logistic regression or Cox proportional hazards regression; with adjustment for age, sex, systolic blood pressure, stroke severity (Scandinavian Stroke Scale), time from onset to randomisation, continue/stop and GTN/no GTN. Results are odds ratio (OR) or hazard ratio (HR) and 95% confidence intervals (CI). Significant ($p<0.05$) results in bold. *OR per 10 units change, $^{+}$OR per 0.1 L/L change. eGFR: estimated glomerular filtration rate; GTN: glyceryl trinitrate.

4.5 DISCUSSION

In this pre-planned substudy of patients with acute stroke, measures of dehydration ranged between 0% and 55.6%. There was no difference in BP change in dehydrated patients between those randomised to GTN vs. no GTN, and between those who continued vs. stopped their pre-stroke antihypertensives. Further, no differences in neurological status or clinical safety events at day 7 in dehydrated patients were seen across randomised groups. In a multivariable analysis we found that increased urea was associated with an unfavourable shift in mRS and more death at day 90 overall. No consistent findings were noted for other markers of dehydration.

Conventional medical teaching suggests that giving antihypertensive medication, including nitrates, in the context of dehydration may lead to precipitous drops in BP. However, in this cohort of acute stroke patients, GTN did not have this effect. This incongruity may stem from the dose used (5 mg) and route of administration (transdermal), which is supported by the modest BP lowering effect seen across the trial in those randomised to GTN (7/3.5 mmHg lower after the first dose than those randomised to no GTN).(84) In addition, continuing pre-stroke antihypertensives did not cause large falls in BP in the context of dehydration, compared to stopping antihypertensives. The borderline interactions seen in relation to GTN and mRS and death at day 90 in those with raised urea are not supported by the neutral effects seen on haemodynamics across multiple dehydration markers.

Such effects were not seen in those randomised to GTN with raised urea:creatinine and may, therefore, represent chance. More data are needed to confirm or refute this finding.

Several biochemical markers of dehydration were assessed using this dataset; only increasing serum urea was associated with 90 day clinical outcome after acute stroke. In an earlier observational study of 2042 patients within 48 hours of stroke, raised urea at baseline was associated with an increased risk of death up to 7 years later.(226) In addition, raised creatinine and urea:creatinine ratio, and reduced creatinine clearance, at baseline were associated with higher mortality risk.(226) Similarly, elevated urea on admission was associated with a higher mortality rate during initial hospitalisation in a cohort of 388 stroke patients.(227)

Other groups have noted associations between several markers of dehydration, other than urea, and outcome in acute stroke. First, elevated blood urea nitrogen: creatinine ratio has been associated with poor neurological outcome and worse functional outcome in 2570 patients with acute ischaemic stroke.(222) Similarly, in a UK cohort of 2591 acute stroke patients raised urea:creatinine ratio was associated with an increased likelihood of being dead or dependent at hospital discharge.(215) Second, elevated plasma osmolality on admission has been associated with increased mortality after stroke.(223) Third, reduced baseline eGFR has been associated with poor functional outcome after both ischaemic stroke(228) and intracerebral

haemorrhage,(229) and long term mortality and new cardiovascular morbidity over a 10 year period in acute stroke overall.(230) Last, urine specific gravity has been identified as a predictor of early neurological deterioration within 3 days of acute ischaemic stroke.(231) In summary, although a number of dehydration markers have been associated with outcome after stroke, there are no consistent findings for any particular marker, perhaps reflecting that there is no gold standard measure of dehydration.

The strengths of this study include the variety of blood dehydration parameters assessed, and the pre-specified nature of this analysis within the context of a large randomised, controlled trial with almost complete follow-up. Nevertheless, there are certain limitations. First, the data are limited to two sites and therefore may not be extrapolated to larger datasets. Extrapolation to more severely dehydrated stroke patients may not be appropriate as patients with overt clinical dehydration may have been less likely to be recruited into ENOS. Further, the relatively small population studied meant the analyses of clinical outcomes, although exploratory, were underpowered and therefore the findings may represent chance. Second, although prognostic factors were adjusted for in analyses, we cannot exclude that the associations seen are due to chance or confounded by other factors such as infection, vomiting, medication and co-morbidities. We did not collect information on fluid supplementation prior to or following blood being taken, which may have influenced the participant's fluid status, dehydration status and

clinical outcomes measured. Further, diuretic use pre-stroke has been associated with dehydration in patients presenting with acute stroke,(215) which may have confounded our results involving the assessment of GTN vs. no GTN. However, we did not see any significant associations between continuing pre-stroke antihypertensives and outcome in dehydrated stroke patients in this cohort. Third, no adjustment was made for multiplicity of testing and therefore some findings may represent chance, highlighted by the opposite directions of association with day 90 outcomes seen for sodium and urea, and neutral findings in relation to creatinine and urea:creatinine ratio. Last, whether the negative effects of dehydration in acute stroke patients are seen in the longer-term is not answered by this analysis of outcomes assessed at a relatively early stage post-stroke.

In summary, transdermal GTN or continuation of pre-existing antihypertensive treatment did not cause precipitous drops in BP in dehydrated acute stroke patients nor were there any significant effects on safety outcomes. These data reject the null hypothesis and demonstrate that transdermal GTN is safe in the setting of acute stroke patients with blood markers of dehydration. This is of reassurance and supports the use of GTN in acute stroke prior to blood markers of dehydration being available. The ongoing Rapid Intervention with Glyceryl trinitrate in Hypertensive stroke Trial-2 (RIGHT-2) is assessing the safety and efficacy of GTN in the ambulance within four hours of symptom onset and will add to this

data in the ultra-acute setting.(188) Dehydration, when measured as urea, was associated with poor clinical outcomes after acute stroke. Whether rehydration of dehydrated acute stroke patients has the potential to improve clinical outcomes requires further assessment in randomised controlled trials.

CHAPTER 5:

BLOOD PRESSURE LOWERING USING TRANSDERMAL GLYCERYL TRINITRATE IN ACUTE STROKE PATIENTS WITH CAROTID STENOSIS IS SAFE

Publications (accepted) contributing to this chapter:

Appleton JP, Woodhouse LJ, Belcher A, Bereczki D, Berge E, Caso V, et al. Blood pressure lowering using transdermal glyceryl trinitrate in acute ischaemic stroke patients with carotid stenosis is safe. Stroke and Vascular Neurology 2019

Presentations contributing to this chapter:

Effect of transdermal glyceryl trinitrate in acute stroke patients with carotid stenosis. Association of British Neurologists Annual meeting, International Conference Centre, Birmingham (May 2018)

The effect of transdermal glyceryl trinitrate in acute stroke patients with carotid stenosis: data from the efficacy of nitric oxide in stroke trial. European Stroke Organisation Conference, Gothenburg, Sweden (May 2018)

5.1 ABSTRACT

Background

There is concern that blood pressure lowering in acute stroke may compromise cerebral perfusion and worsen outcome in the presence of carotid stenosis. We assessed the effect of glyceryl trinitrate (GTN) in patients with carotid stenosis using data from the Efficacy of Nitric Oxide in Stroke (ENOS) trial.

Methods

ENOS randomised 4011 patients with acute stroke and raised systolic blood pressure (140-220 mmHg) to transdermal GTN or no GTN within 48 hours of onset. Those on pre-stroke antihypertensives were also randomised to stop or continue their medication for 7 days. The primary outcome was the modified Rankin Scale (mRS) at day 90. Ipsilateral carotid stenosis was split: <30%; 30-<50%; 50-<70%; ≥70%. Data are odds ratios (OR) with 95% confidence intervals (CI) adjusted for baseline prognostic factors.

Results

2023 (60.5%) ischaemic stroke participants had carotid imaging. As compared with <30%, ≥70% ipsilateral stenosis was associated with an unfavourable shift in mRS (worse outcome) at 90 days (OR 1.88, 95% CI 1.44-2.44, p<0.001). Those with ≥70% stenosis who received GTN vs. no GTN had a favourable shift in mRS (OR 0.56, 95% CI

0.34-0.93, p=0.024). In those with 50-<70% stenosis, continuing vs. stopping pre-stroke antihypertensives was associated with worse disability, mood, quality of life and cognition at 90 days. Clinical outcomes did not differ across bilateral stenosis groups.

Conclusions

Following ischaemic stroke, severe ipsilateral carotid stenosis is associated with worse functional outcome at 90 days. GTN appears safe in ipsilateral or bilateral carotid stenosis, and might improve outcome in severe ipsilateral carotid stenosis.

5.2 INTRODUCTION

Blood pressure (BP) is elevated in 75% of patients presenting with acute ischaemic stroke (50) and is associated independently with poor clinical outcomes.(200, 201) Lowering elevated BP appears safe in acute ischaemic stroke, but has failed to show clinical benefit.(68) There is a specific concern regarding BP lowering in the 15% of patients with significant carotid stenosis in whom cerebral perfusion may be compromised and where reducing BP might extend the ischaemic core and potentially worsen outcome.(51) Data on BP reduction in severe ipsi- or bi-lateral carotid stenosis are limited, although a meta-analysis found that lower BP was associated with an increased rate of stroke recurrence in bilateral carotid stenosis.(152) In the ultra-acute prehospital and acute hospital situation, information on carotid stenosis is often not available and it is unclear whether BP lowering is safe in this group of stroke patients.

The Efficacy of Nitric Oxide in Stroke (ENOS) trial assessed the safety and efficacy of transdermal glyceryl trinitrate (GTN), and of continuing pre-stroke antihypertensives, in 4011 acute stroke patients.(84) Although GTN lowered BP by 7/3.5 mmHg at day 1, GTN did not influence functional outcome at 90 days.(84) However, when administered within 6 hours of stroke onset GTN improved several clinical outcomes.(149) The aim of the current pre-planned substudy (232) was to assess the safety and efficacy of BP lowering on clinical outcomes in patients with acute ischaemic stroke and carotid stenosis.

5.3 METHODS

Hypothesis

BP lowering with transdermal GTN is safe in acute stroke patients with ipsilateral carotid stenosis.

ENOS trial

Details pertaining to the ENOS trial protocol, statistical analysis plan, baseline characteristics and main trial results have been published.(149, 208, 209, 233) In summary, ENOS recruited 4011 acute stroke patients within 48 hours of onset with high systolic BP (140-220 mmHg) and randomised them to GTN 5 mg patch or no patch for 7 days. Those participants taking antihypertensive medication prior to their index event were also randomised to continue or stop these drugs for 7 days. Known carotid stenosis was not an exclusion criterion. Written consent to participate was given by patients or relatives/carers in those who lacked capacity. ENOS was approved by ethics committees/competent authorities in participating countries and registered (ISRCTN99414122).

Carotid stenosis

Clinical information on carotid stenosis was collected by investigators during the participant's index event admission. Clinical imaging using either carotid Doppler, magnetic resonance (MR) angiography or computed tomography (CT) angiography was performed as per local protocol. Investigators entered the % of stenosis of both left and right internal carotid arteries using North American Symptomatic Carotid

Endarterectomy Trial (NASCET) criteria where available (Appendix 10.2).(9) Data was checked and validated but no central adjudication of carotid imaging was performed.

Participants who had a final diagnosis of ischaemic stroke and who had carotid data available were included in this substudy. Grades of carotid stenosis were defined as follows:
Unilateral carotid stenosis ipsilateral to the symptomatic hemisphere:(9) <30%, 30-<50%, 50-<70%, ≥70%
Bilateral carotid stenosis (% for both carotid arteries): <30%, 30-<50%, ≥50%. Therefore, patients with grossly asymmetrical stenosis were excluded from these analyses.

Haemodynamic Measures

BP and heart rate were measured peripherally at baseline (three measurements) and on days 1 to 7 (two measurements/day), using validated automated equipment (Omron 705 CP).(210)

Clinical Outcomes

The primary outcome in ENOS was functional outcome measured using the modified Rankin Scale(234) (mRS, a 7-level categorical scale where 0 = independent and 6= dead) at 90 days. Day 90 secondary outcomes included disability (Barthel Index)(235), mood (Zung depression scale)(195), quality of life (Health utility status calculated using European Quality of Life 5-dimensions 3-level, and visual analogue scale)(236) and cognition (telephone mini-mental

state examination [MMSE],(237) modified telephone interview for cognition scale [TICS-M],(238) and verbal fluency). Patients who had died by day 90 were assigned a worst score for the outcomes. Safety outcome data was collected on all-cause mortality at day 90, early neurological deterioration (a minimum 5 point reduction overall or >2 point reduction in the consciousness domain from baseline to day 7 on the Scandinavian Stroke Scale [SSS]), symptomatic hypotension, hypertension or headache by day 7. The National Institute of Health Stroke Scale (NIHSS) was calculated from the SSS.(194) Day 90 outcomes were recorded by trained blinded assessors via telephone at national coordinating centres.

Statistical Analysis

In line with the ENOS trial statistical analysis plan and statistical analyses performed in the primary publication, data were analysed by intention-to-treat.(233) Data are number (%), median [interquartile range, IQR], or mean (standard deviation, SD). Baseline characteristics between grades of carotid stenosis were assessed using X^2 for categorical variables and one-way analysis of variance (ANOVA) for continuous variables.

Associations between carotid stenosis grades and outcomes were assessed using multiple linear regression, ordinal logistic regression or binary logistic regression with adjustment for baseline prognostic covariates including age, sex, baseline mRS score, history of previous stroke, history of diabetes mellitus, total anterior circulation stroke,

nitrate use, baseline SSS, thrombolysis, feeding status, time to randomisation, and baseline systolic BP. Analyses involving the whole population were also adjusted for treatment allocation. Associations between BP change from baseline to day 1 and outcome across degrees of carotid stenosis were assessed per 10 mmHg reduction in BP. Interaction p-values were obtained by adding an interaction term to statistical models. Data are mean difference (MD) or odds ratio (OR) and associated 95% confidence intervals (CI), with significance defined as $p \leq 0.05$. Analyses were performed using SPSS version 24 (Chicago, IL USA).

5.4 RESULTS

Of 4011 participants, 2023 (50.4%) had a final diagnosis of ischaemic stroke and carotid imaging data (GTN 1002 vs. no GTN 1021, Table 5.1). 1319 (32.9%) patients with ischaemic stroke did not have carotid imaging, typically in those with more severe stroke (carotid imaging: SSS 36.6 (12.4), no imaging: SSS 30.6 (13.7), p<0.001); there was no relationship between country of enrolment and whether carotid imaging was performed (data not shown). Of 2023 participants with carotid data, 148 (7.3%) had 50-<70% ipsilateral stenosis, 213 (10.5%) had ≥70% ipsilateral stenosis and 97 (4.8%) had ≥50% bilateral stenosis. Age and rates of treated hypertension, diabetes mellitus, atrial fibrillation, history of transient ischaemic attack, ischaemic heart disease, and peripheral arterial disease differed across grades of ipsilateral carotid stenosis. Those with higher degrees of ipsilateral carotid stenosis were more likely to be male, current smokers, have more severe strokes with higher NIHSS and lower Glasgow coma scale scores, fewer cardioembolic and small vessel disease-related strokes, and more received thrombolysis treatment (Table 5.1). As compared to patients with carotid imaging data, those without had a worse functional outcome at day 90: mRS 4 [3] vs. 3 [3], OR 1.76, 95% CI 1.54-2.01, p<0.001.

Table 5.1: Baseline characteristics of all ischaemic stroke patients with carotid data and by ipsilateral carotid stenosis

	All IS	GTN	No GTN	Continue	Stop	Stenosis <30%	Stenosis 30-<50%	Stenosis 50-<70%	Stenosis ≥70%	p
Number of patients	2023	1002	1021	534	525	1431	224	148	213	
Age (years)	69.1 (11.4)	68.8 (11.3)	69.4 (11.5)	71.6 (10.5)	70.9 (10.5)	68.3 (11.6)	71.2 (10.7)	73.3 (9.9)	68.9 (10.8)	<0.001
Sex, male (%)	1193 (59.0)	599 (59.8)	594 (58.2)	286 (53.6)	283 (53.9)	817 (57.1)	141 (62.9)	91 (61.5)	141 (66.2)	0.036
Premorbid mRS >1 (%)	209 (10.3)	95 (9.5)	114 (11.2)	71 (13.3)	64 (12.2)	135 (9.4)	27 (12.1)	20 (13.5)	24 (11.3)	0.29
Medical history (%)										
Hypertension	1307 (64.6)	624 (47.7)	683 (66.9)	512 (95.9)	503 (95.8)	903 (63.1)	146 (65.2)	108 (73.0)	144 (67.6)	0.078
Treated hypertension	1072 (53.0)	516 (51.5)	556 (54.5)	533 (99.8)	522 (99.4)	720 (50.3)	134 (59.8)	98 (66.2)	115 (54.0)	<0.001
Diabetes mellitus	353 (17.4)	164 (16.4)	189 (18.5)	125 (23.4)	121 (23.0)	245 (17.1)	38 (17.0)	38 (25.7)	28 (13.1)	0.020
Atrial fibrillation	333 (16.5)	169 (16.9)	164 (16.1)	135 (25.3)	116 (22.1)	224 (15.7)	43 (19.2)	36 (24.3)	30 (14.1)	0.024
Stroke	295 (14.6)	150 (15.0)	145 (14.2)	113 (21.2)	97 (18.5)	207 (14.5)	37 (16.5)	25 (16.9)	22 (10.3)	0.22
TIA	286 (14.1)	147 (14.7)	139 (13.6)	91 (17.0)	96 (18.3)	179 (12.5)	43 (19.2)	24 (16.2)	39 (18.3)	0.010

IHD	380 (18.8)	191 (19.1)	189 (18.5)	136 (25.5)	153 (29.1)	248 (17.3)	59 (26.3)	38 (25.7)	34 (16.0)	0.001
PAD	65 (3.2)	29 (2.9)	36 (3.5)	23 (4.3)	22 (4.2)	35 (2.4)	10 (4.5)	8 (5.4)	12 (5.6)	0.018
Hyper-lipidaemia	587 (29.0)	293 (29.2)	294 (28.8)	204 (38.2)	216 (41.1)	412 (28.8)	60 (26.8)	52 (35.1)	57 (26.8)	0.29
Smoking, current	573 (28.3)	278 (27.7)	295 (28.9)	111 (20.8)	109 (20.8)	380 (26.6)	71 (31.7)	37 (25.0)	83 (39.0)	0.010
Alcohol >21 units per week	176 (8.7)	92 (9.2)	84 (8.2)	38 (7.1)	29 (5.5)	116 (8.1)	19 (10.9)	11 (7.4)	28 (13.1)	0.10
Side of lesion, right (%)	1047 (51.8)	509 (50.8)	538 (52.7)	278 (52.4)	264 (50.4)	722 (50.5)	109 (48.7)	89 (60.1)	127 (59.6)	0.010
NIHSS (/42), calculated	9.9 (5.3)	9.8 (5.3)	10.0 (5.4)	10.3 (5.5)	10.2 (5.4)	9.7 (5.2)	10.1 (5.4)	9.6 (5.0)	11.6 (5.6)	<0.001
GCS <15 (%)	460 (22.7)	222 (22.2)	238 (23.3)	134 (25.1)	145 (27.6)	300 (21.0)	61 (27.2)	32 (21.6)	65 (30.5)	0.006
TOAST classification*										
Cardioembolic	358 (17.7)	181 (18.1)	177 (17.3)	133 (24.9)	117 (22.3)	271 (18.9)	41 (18.3)	24 (16.2)	22 (10.3)	0.021
Large vessel	527 (26.1)	254 (25.3)	273 (26.7)	143 (49.5)	146 (27.8)	200 (14.0)	53 (23.7)	90 (60.8)	180 (84.5)	<0.001
Small Vessel	808 (39.9)	402 (40.1)	406 (39.8)	188 (35.2)	199 (37.9)	649 (45.4)	105 (46.9)	34 (23.0)	16 (7.5)	<0.001
Other	394 (19.5)	202 (20.2)	192 (18.8)	93 (17.4)	87 (16.6)	333 (23.3)	32 (14.3)	17 (11.5)	12 (5.6)	<0.001
Haemodynamics										
BP, Systolic (mmHg)	166.6 (18.5)	167.1 (18.3)	166.1 (18.7)	165.4 (18.9)	167.7 (17.8)	166.6 (18.6)	166.6 (18.7)	165.1 (17.2)	167.5 (18.3)	0.70

BP, Diastolic (mmHg)	89.2 (13.0)	89.9 (13.1)	88.5 (12.8)	87.5 (13.3)	88.2 (12.6)	90.1 (13.0)	88.2 (13.3)	87.7 (12.9)	86.7 (11.8)	<0.001
Heart rate (bpm)	76.8 (14.4)	77.1 (14.5)	76.5 (14.2)	75.8 (14.5)	76.1 (14.5)	76.5 (14.6)	78.4 (13.1)	78.7 (15.4)	75.3 (13.1)	0.039
Time to randomisation [hours]	25.6 [21.2]	24.9 [21.5]	26.0 [21.1]	25.2 [18.8]	23.9 [22.1]	26.0 [21.6]	24.0 [20.1]	23.9 [17.8]	24.8 [19.0]	0.08
Thrombolysis (%)	239 (11.8)	107 (10.7)	132 (12.9)	71 (13.3)	63 (12.0)	156 (10.9)	24 (10.7)	20 (13.5)	39 (18.3)	0.015

*total may exceed 100% due to mixed causality. X^2 for categorical variables or one-way analysis of variance (ANOVA) for continuous variables across grades of carotid stenosis.

Relationship between carotid stenosis and outcome

Across all patients and as compared to participants with <30% ipsilateral stenosis, those with ≥70% stenosis had an unfavourable shift in mRS (worse outcome) at day 90 (OR 1.88, 95% CI 1.44-2.44, p<0.001, Table 5.2, Figure 5.1); significant associations with worse disability and quality of life, more depression and poorer cognitive scores were also seen. In addition, those with ≥70% stenosis had an increased rate of recurrent ischaemic stroke, clinical deterioration, neurological deterioration, and higher NIHSS scores at day 7 (Table 5.2).

Effects of GTN vs. no GTN

Those with ≥70% ipsilateral stenosis who were randomised to GTN had a significant shift in mRS to less death or dependency at 90 days: OR 0.56, 95%CI 0.34-0.93, p=0.024 (Table 5.3, Figure 5.2). However, GTN did not influence mRS across the other carotid stenosis groups, although there were higher cognitive scores at day 90 in those with 50-<70% stenosis but not in other stenosis groups. Headache, a recognised side-effect of GTN, was more common in those with ≥70% stenosis who were randomised to GTN; non-significant increases in headache with GTN were also reported in the other carotid stenosis groups. Safety outcomes did not differ between randomised groups (Table 5.3).

Table 5.2: Clinical outcomes by degree of ipsilateral carotid stenosis

	Stenosis <30%	Stenosis 30-<50%			Stenosis 50-<70%			Stenosis ≥70%		
		n (%) / mean (SD)	OR/MD (95% CI)	p	n (%) / mean (SD)	OR/MD (95% CI)	p	n (%) / mean (SD)	OR/MD (95% CI)	p
Number of Participants	1431	224	-	-	148	-	-	213	-	-
Primary outcome	1426	224	-	-	148	-	-	212	-	-
mRS (/6)*	2 [3]	2[2]	1.03 (0.80, 1.33)	0.83	3 [2]	1.21 (0.89, 1.64)	0.23	3 [2]	1.88 (1.44, 2.44)	<0.001
Day 7 (or Discharge)										
Death, by cause (%)	9 (0.6)	0 (0)	-	-	1 (0.7)	1.27 (0.15, 11.03)	0.83	5 (2.3)	3.51 (1.04, 11.86)	0.043
Symptomatic recurrent stroke (%)	14 (1.0)	4 (1.8)	1.60 (0.50, 5.09)	0.43	1 (0.7)	0.40 (0.05, 3.29)	0.40	6 (2.8)	2.73 (0.99, 7.52)	0.052
Ischaemic	12 (0.8)	3 (1.3)	1.32 (0.36, 4.90)	0.68	1 (0.7)	0.49 (0.06, 3.92)	0.48	6 (2.8)	3.29 (1.17, 9.26)	0.024
NIH Stroke Scale (/42), calculated	6.8 (5.5)	7.5 (6.1)	0.17 (-0.32, 0.65)	0.50	6.6 (5.4)	-0.17 (-0.77, 0.42)	0.56	9.1 (6.0)	0.86 (0.36, 1.37)	0.001
Clinical deterioration (%)	62 (4.3)	15 (6.7)	1.53 (0.84, 2.77)	0.16	7 (4.8)	0.92 (0.40, 2.10)	0.83	23 (10.8)	2.61 (1.55, 4.39)	<0.001
Neurological deterioration (%)	59 (4.1)	9 (4.0)	0.97 (0.47, 2.02)	0.94	4 (2.7)	0.57 (0.20, 1.63)	0.30	18 (8.5)	2.12 (1.21, 3.74)	0.009
Headache (%)	192 (13.4)	20 (8.9)	0.80 (0.48, 1.32)	0.38	23 (15.5)	1.53 (0.93, 2.51)	0.09	30 (14.1)	1.31 (0.84, 2.03)	0.23
Hypotension (%)	21 (1.5)	3 (1.3)	0.89 (0.25, 3.10)	0.85	3 (2.0)	1.10 (0.31, 3.87)	0.88	2 (0.9)	0.65 (0.15, 2.86)	0.57

Hypertension (%)	78 (5.5)	14 (6.3)	1.22 (0.66, 2.23)	0.53	7 (4.7)	0.97 (0.43, 2.20)	0.94	12 (5.6)	1.06 (0.56, 2.02)	0.86
Death or discharge to institution (%)	356 (24.9)	50 (22.3)	0.81 (0.57, 1.15)	0.24	30 (20.3)	0.61 (0.39, 0.95)	0.029	72 (33.8)	1.34 (0.96, 1.87)	0.08
Day 90										
Death (%)	67 (4.7)	21 (9.4)	1.85 (1.06, 3.22)	0.030	11 (7.4)	1.43 (0.71, 2.90)	0.32	25 (11.8)	2.52 (1.48, 4.27)	0.001
Barthel Index	77.0 (31.3)	72.8 (35.4)	-1.43 (-5.29, 2.43)	0.47	72.4 (33.6)	-2.44 (-7.10, 2.22)	0.30	61.3 (38.0)	-10.62 (-14.57, -6.67)	<0.001
Zung Depression Scale (ZDS, /100)	52.3 (20.5)	57.2 (22.8)	3.57 (0.55, 6.58)	0.021	53.8 (22.0)	0.65 (-3.06, 4.37)	0.73	61.1 (22.5)	6.53 (3.41, 9.64)	<0.001
EQ-5D Health Utility Status (HUS, /1)	0.57 (0.38)	0.53 (0.37)	-0.01 (-0.06, 0.04)	0.63	0.53 (0.39)	-0.03 (-0.09, 0.03)	0.33	0.40 (0.40)	-0.12 (-0.16, -0.07)	<0.001
EQ-Visual Analogue Scale (EQ-VAS, /100)	63.7 (26.0)	61.4 (29.8)	0.16 (-3.54, 3.87)	0.93	61.2 (28.4)	-1.04 (-5.60, 3.52)	0.66	50.4 (30.1)	-9.58 (-13.42, -5.73)	<0.001
Verbal Fluency	11.7 (7.1)	10.7 (8.3)	-0.32 (-1.56, 0.91)	0.61	10.2 (7.1)	-0.71 (-2.16, 0.75)	0.34	9.6 (7.8)	-0.97 (-2.23, 0.30)	0.14
TICS-M	18.7 (8.8)	16.1 (10.0)	-1.49 (-3.05, 0.07)	0.06	15.9 (9.1)	-1.28 (-3.16, 0.61)	0.18	13.6 (9.7)	-2.92 (-4.55, -1.29)	<0.001
MMSE	14.0 (5.8)	12.0 (6.9)	-1.17 (-2.21, -0.13)	0.028	12.0 (6.5)	-0.97 (-2.24, 0.29)	0.13	10.5 (7.2)	-1.79 (-2.89, -0.69)	0.001

Data are n (%), mean (SD), median [interquartile range], mean difference (MD) or odds ratio (OR) with 95% confidence intervals. Comparison using logistic regression, multiple regression or ordinal regression with <30% stenosis as reference group. Adjusted for age, sex, baseline mRS, history of previous stroke, history of diabetes mellitus, TACS, nitrate use, baseline SSS, thrombolysis, feeding status, time to randomisation, baseline SBP, GTN/no GTN and continue/stop. *=ordinal logistic regression.

Table 5.3: Clinical outcomes by GTN vs. no GTN by degree of ipsilateral carotid stenosis

	Stenosis 30-<50%				Stenosis 50-<70%				Stenosis ≥70%			
	GTN	**No GTN**	**OR/MD (95% CI)**	**p**	**GTN**	**No GTN**	**OR/MD (95% CI)**	**p**	**GTN**	**No GTN**	**OR/MD (95% CI)**	**p**
Number of Participants	102	122	-	-	77	71	-	-	94	119	-	-
SBP change day 0-1 (mmHg)*	-13.8 (19.8)	-3.2 (17.9)	-11.0 (-15.7, -6.3)	<0.001	-10.9 (18.1)	-5.6 (15.7)	-3.9 (-9.6, 18)	0.18	-8.9 (17.5)	-3.1 (17.1)	-6.5 (-11.0, -2.0)	0.005
Primary outcome	102	122	-	-	77	71	-	-	93	119	-	-
mRS (/6)[§]	2 [3]	3 [2]	0.77 (0.47, 1.27)	0.31	3 [2]	3 [2]	0.71 (0.39, 1.31)	0.28	3 [2]	4 [2]	0.56 (0.34, 0.93)	0.024
Secondary outcomes												
Day 7 (or Discharge)												
Death, by cause (%)	0 (0)	0 (0)	-	-	1 (1.3)	0 (0)	-	-	3 (3.2)	2 (1.7)	-	-
Symptomatic recurrent stroke (%)	1 (1.0)	3 (2.5)	0.09 (0.00, 11.08)	0.33	0 (0)	1 (1.4)	-	-	3 (3.2)	3 (2.5)	0.87 (0.09, 8.21)	0.90
Ischaemic	1 (1.0)	2 (1.6)	0.18 (0.00, 11.17)	0.41	0 (0)	1 (1.4)	-	-	3 (3.2)	3 (2.5)	0.87 (0.09, 8.21)	0.90
NIH Stroke Scale (/42), calculated	7.3 (6.2)	7.6 (6.0)	-0.41 (-1.46, 0.64)	0.44	6.6 (5.0)	6.6 (5.8)	-0.75 (-1.96, 0.46)	0.22	8.9 (6.3)	9.2 (5.8)	-0.55 (-1.75, 0.66)	0.37
Clinical deterioration (%)	6 (5.9)	9 (7.4)	0.69 (0.19, 2.49)	0.57	3 (3.9)	4 (5.6)	0.53 (0.06, 4.46)	0.56	10 (10.6)	13 (11.0)	0.89 (0.35, 2.27)	0.89
Neurological deterioration (%)	3 (2.9)	6 (4.9)	0.23 (0.03, 2.12)	0.20	1 (1.3)	3 (4.2)	-	-	11 (11.7)	7 (5.9)	2.33 (0.79, 6.83)	0.13
Headache (%)	12 (11.8)	8 (6.6)	2.03 (0.70, 5.94)	0.19	16 (20.8)	7 (9.9)	2.73 (0.96, 7.72)	0.059	19 (20.2)	11 (9.2)	2.80 (1.18, 6.65)	0.019

Hypotension (%)	3 (2.9)	0 (0)	-	-	3 (3.9)	0 (0)	-	-	2 (2.1)	0 (0)	-	-
Hypertension (%)	6 (5.9)	8 (6.6)	0.93 (0.26, 3.40)	0.91	3 (3.9)	4 (5.6)	162.4 (0.03, 925848)	0.25	5 (5.3)	7 (5.9)	0.76 (0.21, 2.82)	0.68
Death or discharge to institution (%)	22 (21.6)	28 (23.0)	0.84 (0.41, 1.75)	0.65	13 (16.9)	17 (23.9)	0.64 (0.25, 1.62)	0.34	27 (28.7)	45 (37.8)	0.51 (0.26, 1.00)	0.050
Day 90												
Death (%)	7 (6.9)	14 (11.5)	0.43 (0.14, 1.32)	0.14	3 (3.9)	8 (11.3)	0.18 (0.30, 1.03)	0.054	9 (9.7)	16 (13.4)	0.63 (0.23, 1.75)	0.37
Barthel Index	75.2 (34.4)	70.8 (36.2)	5.78 (-2.37, 13.94)	0.16	74.9 (31.5)	69.7 (35.8)	7.86 (-2.24, 17.95)	0.13	65.7 (38.0)	57.9 (37.9)	7.87 (-0.10, 16.74)	0.08
Zung Depression Scale (ZDS, /100)	56.5 (22.2)	57.8 (23.3)	-0.89 (-7.20, 5.43)	0.78	51.1 (19.2)	56.8 (24.6)	-7.33 (-14.93, 0.27)	0.059	59.7 (22.6)	62.1 (22.6)	-3.78 (-9.87, 2.32)	0.22
EQ-5D Health Utility Status (HUS, /1)	0.54 (0.38)	0.53 (0.36)	0.00 (-0.08, 0.09)	0.94	0.53 (0.39)	0.53 (0.40)	0.05 (-0.07, 0.17)	0.44	0.44 (0.39)	0.37 (0.40)	0.07 (-0.02, 0.17)	0.11
EQ-Visual Analogue Scale (EQ-VAS, /100)	62.4 (28.6)	60.5 (30.8)	2.12 (-5.81, 10.05)	0.60	62.6 (26.0)	59.7 (30.9)	5.57 (-3.96, 15.10)	0.25	54.3 (29.6)	47.4 (30.4)	6.91 (-1.04, 14.85)	0.09
Verbal Fluency	11.3 (8.1)	10.2 (8.5)	0.78 (-1.97, 3.52)	0.58	10.6 (6.5)	9.8 (7.6)	0.96 (-1.54, 3.47)	0.45	9.5 (7.0)	9.7 (8.5)	0.23 (-2.48, 2.94)	0.87
TICS-M	15.8 (9.2)	16.3 (10.6)	-0.88 (-4.16, 2.41)	0.60	18.4 (7.5)	13.3 (10.0)	4.92 (1.36, 8.49)	0.008	14.7 (9.8)	12.8 (9.6)	2.34 (-1.20, 5.88)	0.19
MMSE	12.2 (6.4)	11.9 (7.2)	0.26 (-1.94, 2.45)	0.82	14.0 (5.2)	10.1 (7.1)	4.11 (1.63, 6.59)	0.002	11.3 (4.0)	10.1 (7.4)	1.34 (-1.25, 3.94)	0.31

Data are n (%), mean (SD), median [interquartile range], mean difference (MD) or odds ratio (OR) with 95% confidence intervals. Comparison using logistic regression, multiple regression or ordinal regression. Adjusted for age, sex, baseline mRS, history of previous stroke, history of diabetes mellitus, TACS, nitrate use, baseline SSS, thrombolysis, feeding status, time to randomisation, baseline SBP and continue/stop. *=adjusted for baseline SBP only; §=ordinal logistic regression

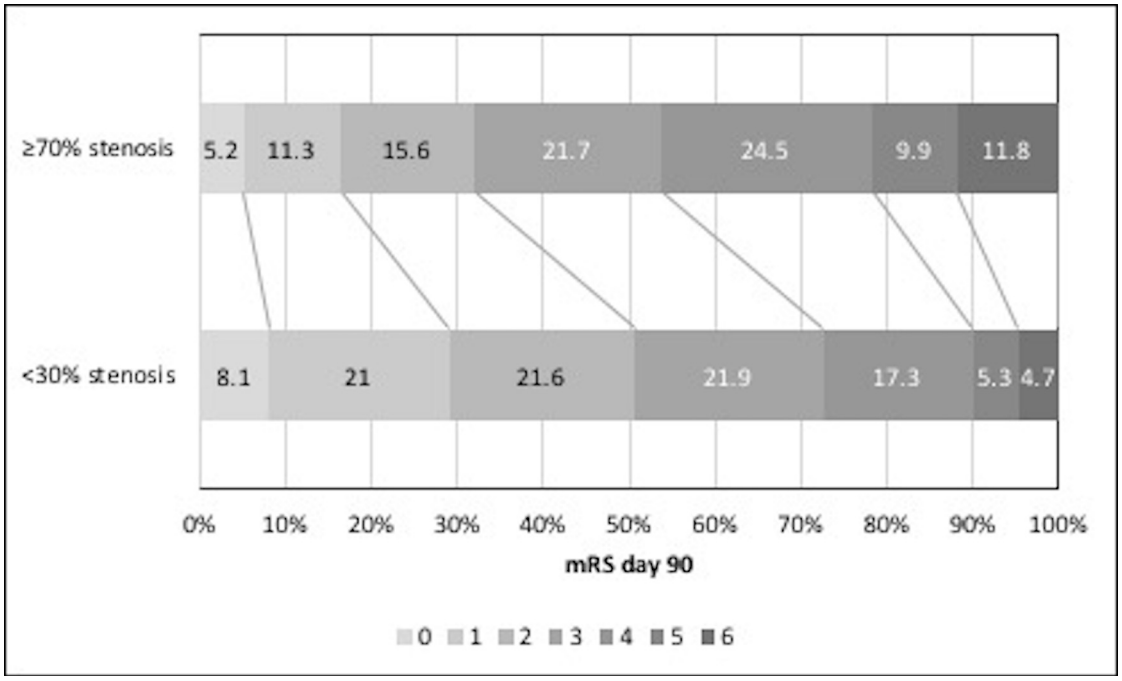


Figure 5.1: mRS at day 90 <30% vs. ≥70% ipsilateral stenosis

Shift in mRS at day 90 between those with <30% vs. ≥70% ipsilateral stenosis between those randomised to GTN vs. no GTN, irrespective of treatment allocation.

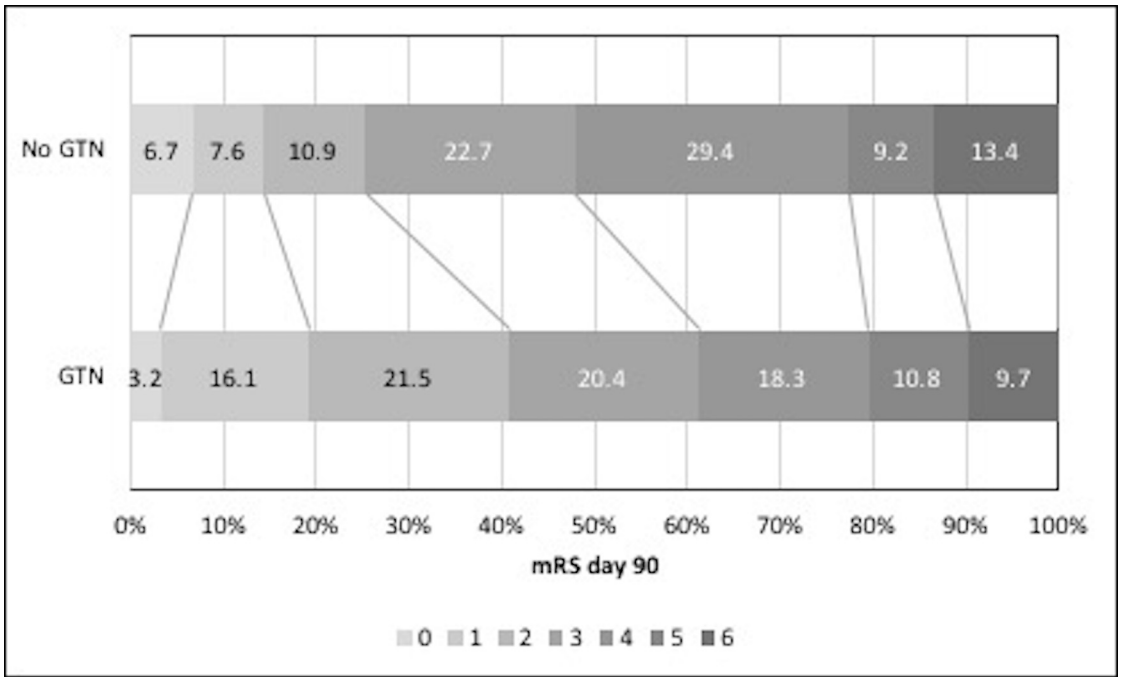


Figure 5.2: mRS at day 90 in those with ≥70% ipsilateral stenosis GTN vs. no GTN

Shift in mRS at day 90 in those with ≥70% ipsilateral stenosis between those randomised to GTN vs. no GTN.

Effects of continuing vs. stopping pre-stroke antihypertensives

In those with 50-<70% ipsilateral stenosis, continuing pre-stroke antihypertensives was associated with more depression, worse disability and quality of life, and poorer cognitive score (TICS-M) at day 90 compared with stopping pre-stroke antihypertensives, independent of GTN allocation. No effect on mRS was seen nor were these effects replicated in those with more severe (≥70%) ipsilateral stenosis. Safety outcomes did not differ between randomised groups (Table 5.4).

Bilateral carotid stenosis

Only 97/2023 (4.8%) had ≥50% bilateral carotid stenosis. There were no significant associations across degrees of bilateral carotid stenosis with clinical outcome measures at either 7 or 90 days (Table 5.5). Neither GTN nor continuing pre-stroke antihypertensives influenced outcome in participants with bilateral carotid stenosis (Tables 5.6 and 5.7).

Blood pressure change and carotid stenosis

The largest fall in BP was seen from baseline to day 1 in those randomised to GTN vs. no GTN (7/3 mmHg overall) and did not significantly differ across degrees of ipsilateral (Table 5.3, interaction p=0.22) or bilateral carotid stenosis (Table 5.6, interaction p=0.19). When assessed across degrees of ipsilateral carotid stenosis, change in BP from baseline to day 1 did not influence mRS at day 90 (Table 5.8). There were no significant interactions between treatment with

GTN or continuing pre-stroke antihypertensives in relation to BP change and outcome. Similarly, across degrees of bilateral carotid stenosis, change in BP from baseline to day 1 did not influence mRS at day 90 (Table 5.8).

Over 7 days of treatment, tachyphylaxis associated with GTN was seen by day 4; the difference in BP between treatment groups diverged by day 1, narrowed over time and at day 7 there was no significant difference in BP between GTN vs. no GTN regardless of severity of ipsilateral carotid stenosis (<30%: GTN 149.5 [SD 22.4] / 83.5 [14.7] mmHg vs. no GTN 150.8 [22.0] / 83.7 [13.6 mmHg, p=0.31 / 0.89; ≥70% stenosis: GTN 144.3 [22.6] / 78.4 [13.1] mmHg vs. no GTN 149.9 [24.3] / 81.6 [13.3] mmHg, p=0.11/0.12, Figure 5.3).

In those who continued vs. stopped their pre-stroke antihypertensives, BP diverged from day 1 up to day 7 across all degrees of ipsilateral carotid stenosis (<30% stenosis: continue 145.8 [24.2] / 80.6 [15.3] mmHg vs. stop 153.9 [22.4] / 85.3 [13.7] mmHg, p<0.001/<0.001; ≥70% stenosis: continue 143.4 [21.2] / 79.8 [11.7] mmHg vs. stop 154.3 [26.2] / 83.1 [13.8] mmHg, p=0.027/0.20, Figure 5.4).

No precipitous changes in BP were seen in bilateral carotid disease treated with GTN vs. no GTN (Figure 5.5) or continue vs. stop pre-stroke antihypertensives (Figure 5.6).

Table 5.4: Clinical outcomes by stop vs. continue antihypertensives by degree of ipsilateral carotid stenosis

	Stenosis 30-<50%				Stenosis 50-<70%				Stenosis ≥70%			
	Cont	**Stop**	**OR/MD (95% CI)**	**p**	**Cont**	**Stop**	**OR/MD (95% CI)**	**p**	**Cont**	**Stop**	**OR/MD (95% CI)**	**p**
Number of Participants	65	68	-	-	52	47	-	-	57	57	-	-
SBP change day 0-1 (mmHg)*	-8.7 (17.3)	-6.0 (21.0)	-3.48 (-9.91, 2.96)	0.29	-8.4 (17.3)	-6.0 (18.0)	-4.12 (-11.39, 3.15)	0.26	-5.8 (16.6)	-6.4 (14.6)	0.01 (-5.76, 5.77)	0.99
Primary outcome	65	68	-	-	52	47	-	-	56	57	-	-
mRS (/6)§	2 [3]	2.5 [3]	0.93 (0.48, 1.80)	0.82	3 [2]	3 [2]	1.92 (0.86, 4.27)	0.11	4 [3]	4 [3]	1.46 (0.73, 2.93)	0.29
Secondary outcomes												
Day 7 (or Discharge)												
Death, by cause (%)	0 (0)	0 (0)	-	-	1 (1.9)	0 (0)	-	-	1 (1.8)	1 (1.8)	-	-
Symptomatic recurrent stroke (%)	2 (3.1)	2 (2.9)	1.05 (0.05, 22.98)	0.97	0 (0)	1 (2.1)	-	-	2 (3.5)	2 (3.5)	-	-
Ischaemic	2 (3.1)	1 (1.5)	5.13 (0.08, 338.19)	0.44	0 (0)	1 (2.1)	-	-	2 (3.5)	2 (3.5)	-	-
NIH Stroke Scale (/42), calculated	7.6 (6.4)	8.0 (6.6)	0.63 (-0.98, 2.23)	0.44	7.3 (5.7)	6.7 (4.8)	0.45 (-1.17, 2.07)	0.58	8.6 (6.2)	9.2 (5.5)	-0.66 (-2.30, 0.97)	0.42
Clinical deterioration (%)	6 (9.2)	5 (7.5)	1.95 (0.40, 9.51)	0.41	5 (9.8)	1 (2.1)	10.60 (0.63, 177.41)	0.10	6 (10.7)	5 (8.8)	2.05 (0.38, 11.08)	0.40
Neurological deterioration (%)	4 (6.2)	2 (2.9)	-	-	2 (3.8)	0 (0)	-	-	4 (7.0)	2 (3.5)	2.00 (0.19, 21.11)	0.57
Headache (%)	4 (6.2)	7 (10.3)	0.53 (0.10, 2.77)	0.45	9 (17.3)	7 (14.9)	1.03 (0.29, 3.63)	0.96	7 (12.3)	8 (14.0)	1.04 (0.20, 5.41)	0.96
Hypotension (%)	1 (1.5)	1 (1.5)	-	-	0 (0)	3 (6.4)	-	-	0 (0)	0 (0)	-	-
Hypertension (%)	2 (3.1)	6 (8.8)	0.38 (0.04, 3.35)	0.38	1 (1.9)	5 (10.6)	-	-	1 (1.8)	6 (10.5)	0.09 (0.01, 1.34)	0.08

Death or discharge to institution (%)	17 (26.2)	17 (25.0)	1.37 (0.51, 3.65)	0.53	10 (19.2)	8 (6.5)	2.47 (0.66, 9.18)	0.18	21 (36.8)	16 (28.1)	1.39 (0.54, 3.61)	0.50
Day 90												
Death (%)	9 (13.8)	7 (10.3)	1.59 (0.42, 6.01)	0.49	6 (11.5)	1 (2.1)	81.00 (1.08, 6093.98)	0.046	12 (21.4)	6 (10.5)	3.11 (0.85, 11.44)	0.09
Barthel Index	70.2 (38.3)	71.3 (36.3)	-2.48 (-14.27, 9.32)	0.68	67.5 (36.1)	74.9 (27.1)	-13.20 (-26.29, -0.11)	0.048	58.7 (37.8)	50.3 (42.5)	10.73 (-24.40, 2.94)	0.12
Zung Depression Scale (ZDS, /100)	61.2 (24.6)	60.0 (22.1)	1.20 (-7.55, 9.96)	0.79	60.9 (23.3)	48.8 (17.8)	13.18 (3.80, 22.56)	0.007	67.6 (23.9)	61.6 (22.4)	10.21 (1.10, 19.33)	0.029
EQ-5D Health Utility Status (HUS, /1)	0.49 (0.41)	0.51 (0.36)	-0.03 (-0.15, 0.09)	0.62	0.48 (0.39)	0.56 (0.33)	-0.16 (-0.30, -0.01)	0.036	0.33 (0.43)	0.35 (0.39)	-0.06 (-0.20, 0.07)	0.37
EQ-Visual Analogue Scale (EQ-VAS, /100)	57.0 (31.5)	59.5 (29.3)	-2.04 (-12.69, 8.62)	0.71	54.0 (32.3)	68.1 (20.4)	-17.20 (-29.41, -4.99)	0.006	44.1 (33.8)	48.3 (27.0)	-6.22 (-18.56, 6.13)	0.32
Verbal Fluency	8.5 (7.5)	10.5 (7.7)	-2.08 (-5.71, 1.55)	0.26	7.6 (6.0)	11.3 (5.0)	-2.69 (-5.61, 0.22)	0.07	6.7 (6.2)	9.7 (8.6)	-3.11 (-6.84, 0.62)	0.10
TICS-M	12.9 (9.4)	15.8 (10.0)	-2.38 (-7.28, 2.52)	0.34	13.2 (9.9)	19.3 (7.8)	-5.66 (-10.40, -0.92)	0.021	11.1 (10.2)	13.1 (9.3)	-3.39 (-8.45, 1.67)	0.18
MMSE	10.1 (7.1)	11.7 (6.9)	-1.30 (-4.76, 2.16)	0.45	10.1 (7.4)	13.9 (5.1)	-2.71 (-6.57, 1.15)	0.16	8.3 (7.8)	10.4 (6.7)	-2.80 (-6.52, 0.92)	0.14

Data are n (%), mean (SD), median [interquartile range], mean difference (MD) or odds ratio (OR) with 95% confidence intervals. Comparison using logistic regression, multiple regression or ordinal regression. Adjusted for age, sex, baseline mRS, history of previous stroke, history of diabetes mellitus, TACS, nitrate use, baseline SSS, thrombolysis, feeding status, time to randomisation, baseline SBP and GTN/no GTN. *=adjusted for baseline SBP only; §=ordinal logistic regression.

Table 5.5: Clinical outcomes by degree of bilateral carotid stenosis

	Stenosis <30%	Stenosis 30-<50%			Stenosis ≥50%		
		n (%) / mean (SD)	OR/MD (95% CI)	p	n (%) / mean (SD)	OR/MD (95% CI)	p
Number of Participants	1287	115	-	-	97	-	-
Primary outcome	1282	115	-	-	96	-	-
mRS (/6)*	2 [3]	2 [3]	0.91 (0.65, 1.29)	0.61	3 [2]	0.99 (0.60, 1.61)	0.95
Secondary outcomes							
Day 7 (or Discharge)							
Death, by cause (%)	9 (0.7)	0 (0)	-	-	1 (1.0)	1.72 (0.18, 16.35)	0.64
Symptomatic recurrent stroke (%)	10 (0.8)	2 (1.7)	1.85 (0.38, 9.04)	0.45	1 (1.0)	0.89 (0.10, 7.84)	0.91
Ischaemic	9 (0.7)	1 (0.9)	1.08 (0.13, 9.13)	0.95	1 (1.0)	0.86 (0.09, 8.19)	0.90
NIH Stroke Scale (/42), calculated	6.8 (5.4)	6.9 (6.3)	-0.06 (-0.69, 0.58)	0.85	6.8 (5.1)	-0.15 (-0.84, 0.55)	0.68
Clinical deterioration (%)	58 (4.5)	9 (7.9)	1.54 (0.72, 3.28)	0.27	5 (5.2)	0.95 (0.36, 2.53)	0.91
Neurological deterioration (%)	52 (4.0)	6 (5.2)	1.20 (0.49, 2.92)	0.69	2 (2.1)	0.47 (0.11, 1.98)	0.30
Headache (%)	179 (13.9)	11 (9.6)	0.82 (0.42, 1.61)	0.57	11 (11.3)	0.95 (0.48, 1.87)	0.88
Hypotension (%)	20 (1.6)	1 (0.9)	0.52 (0.07, 4.10)	0.53	2 (2.1)	1.06 (0.23, 4.85)	0.94
Hypertension (%)	73 (5.7)	8 (7.0)	1.32 (0.61, 2.88)	0.49	6 (6.2)	1.19 (0.48, 2.95)	0.70
Death or discharge to institution (%)	327 (25.4)	28 (24.3)	0.85 (0.53, 1.37)	0.50	24 (24.7)	0.80 (0.48, 1.34)	0.40
Day 90							
Death (%)	62 (4.8)	11 (9.6)	1.94 (0.93, 4.06)	0.08	7 (7.3)	1.47 (0.61, 3.52)	0.39
Barthel Index	76.9 (31.6)	75.0 (35.8)	0.65 (-4.41, 5.71)	0.80	70.8 (33.7)	-3.78 (-9.32, 1.77)	0.18
Zung Depression Scale (ZDS, /100)	52.4 (20.7)	57.2 (23.1)	3.91 (-0.10, 7.92)	0.056	55.6 (20.8)	2.56 (-1.80, 6.92)	0.25
EQ-5D Health Utility Status (HUS, /1)	0.56 (0.38)	0.56 (0.36)	0.01 (-0.05, 0.07)	0.74	0.51 (0.37)	-0.04 (-0.11, 0.03)	0.23

EQ-Visual Analogue Scale (EQ-VAS, /100)	63.4 (26.2)	63.4 (30.2)	1.76 (-3.14, 6.66)	0.48	59.0 (28.3)	-3.42 (-9.02, 2.18)	0.23
Verbal Fluency	11.8 (7.2)	11.2 (8.0)	0.18 (-1.39, 1.75)	0.82	10.6 (7.6)	-0.68 (-2.44, 1.09)	0.45
TICS-M	18.7 (8.7)	16.7 (9.6)	-0.68 (-2.61, 1.26)	0.49	15.8 (8.4)	-1.96 (-4.16, 0.24)	0.08
MMSE	13.9 (5.8)	12.5 (6.5)	-0.44 (-1.72, 0.84)	0.50	12.6 (6.3)	-0.77 (-2.25, 0.70)	0.30

Data are n (%), mean (SD), median [interquartile range], mean difference (MD) or odds ratio (OR) with 95% confidence intervals. Comparison using logistic regression, multiple regression or ordinal regression. Adjusted for age, sex, baseline mRS, history of previous stroke, history of diabetes mellitus, TACS, nitrate use, baseline SSS, thrombolysis, feeding status, time to randomisation, baseline SBP, GTN/no GTN and continue/stop. *=ordinal logistic regression.

Table 5.6: Clinical outcomes by GTN vs. no GTN by degree of bilateral carotid stenosis

	Stenosis <30%				Stenosis 30-<50%				Stenosis ≥50%			
	GTN	**No GTN**	**OR/MD (95% CI)**	**p**	**GTN**	**No GTN**	**OR/MD (95% CI)**	**p**	**GTN**	**No GTN**	**OR/MD (95% CI)**	**p**
Number of Participants	650	637	-	-	52	63	-	-	53	44	-	-
SBP change day 0-1 (mmHg)*	-12.1 (18.2)	-4.0 (17.7)	-7.78 (-9.67, -5.89)	<0.001	-12.8 (19.6)	0.4 (17.2)	-13.17 (-19.70, -6.65)	<0.001	-10.8 (19.8)	-6.0 (18.9)	-4.99 (-12.73, 2.76)	0.20
Primary outcome	648	634	-	-	52	63	-	-	52	44	-	-
mRS (/6)§	2 [3]	3 [3]	1.01 (0.83, 1.23)	0.94	2 [3]	2 [2]	0.98 (0.46, 2.08)	0.95	3 [2]	3 [2]	0.98 (0.46, 2.16)	0.96
Secondary outcomes												
Day 7 (or Discharge)												
Death, by cause (%)	4 (0.6)	5 (0.8)	0.81 (0.19, 3.48)	0.78	0 (0)	0 (0)	-	-	1 (1.9)	0 (0)	-	-
Symptomatic recurrent stroke (%)	6 (0.9)	4 (0.6)	1.64 (0.44, 6.08)	0.46	1 (1.9)	1 (1.6)	-	-	0 (0)	1 (2.3)	-	-
Ischaemic	6 (0.9)	3 (0.5)	2.37 (0.56, 10.02)	0.24	1 (1.9)	0 (0)	-	-	0 (0)	1 (2.3)	-	-
NIH Stroke Scale (/42), calculated	6.5 (5.3)	7.1 (5.6)	-0.31 (-0.67, 0.05)	0.10	6.9 (6.8)	7.0 (5.9)	0.11 (-1.45, 1.67)	0.89	6.6 (5.0)	7.1 (5.2)	-0.17 (-1.67, 1.33)	0.82
Clinical deterioration (%)	27 (4.2)	31 (4.9)	0.83 (0.48, 1.43)	0.49	5 (9.8)	4 (6.3)	0.32 (0.01, 7.94)	0.48	1 (1.9)	4 (9.1)	-	-
Neurological deterioration (%)	28 (4.3)	24 (3.8)	1.11 (0.63, 1.96)	0.72	3 (5.8)	3 (4.8)	-	-	0 (0)	2 (4.5)	-	-
Headache (%)	135 (20.8)	44 (6.9)	3.49 (2.41, 5.07)	<0.001	5 (9.6)	6 (9.5)	0.50 (0.06, 3.87)	0.50	10 (18.9)	1 (2.3)	6213.3 (0.08, 517340458)	0.13
Hypotension (%)	17 (2.6)	3 (0.5)	6.53 (1.87, 22.77)	0.003	1 (1.9)	0 (0)	-	-	2 (3.8)	0 (0)	-	-
Hypertension (%)	35 (5.4)	38 (6.0)	0.81 (0.49, 1.33)	0.40	3 (5.8)	5 (7.9)	2.09 (0.24, 18.29)	0.51	3 (5.7)	3 (6.8)	0.45 (0.00, 9001.7)	0.88

Death or discharge to institution (%)	159 (24.5)	168 (26.4)	1.02 (0.77, 1.34)	0.91	11 (21.2)	17 (27.0)	0.64 (0.17, 2.34)	0.49	12 (22.6)	12 (27.3)	0.31 (0.08, 1.28)	0.11
Day 90												
Death (%)	26 (4.0)	36 (5.7)	0.71 (0.41, 1.26)	0.24	6 (11.5)	5 (7.9)	2.27 (0.18, 28.69)	0.53	4 (7.7)	3 (6.8)	-	-
Barthel Index	78.8 (29.5)	74.9 (33.6)	2.28 (-0.57, 5.13)	0.12	75.5 (38.1)	74.7 (34.2)	-2.59 (-14.53, 9.36)	0.67	74.6 (33.1)	66.4 (34.2)	3.58 (-9.50, 16.66)	0.59
Zung Depression Scale (ZDS, /100)	51.9 (19.5)	53.0 (21.9)	-0.71 (-3.03, 1.61)	0.55	57.6 (24.5)	56.9 (22.2)	3.96 (-5.46, 13.37)	0.41	56.9 (21.4)	53.8 (20.1)	6.25 (-3.04, 15.54)	0.18
EQ-5D Health Utility Status (HUS, /1)	0.58 (0.36)	0.55 (0.40)	0.02 (-0.02, 0.06)	0.30	0.58 (0.37)	0.54 (0.35)	-0.01 (-0.13, 0.12)	0.94	0.55 (0.34)	0.45 (0.41)	0.07 (-0.08, 0.21)	0.36
EQ-Visual Analogue Scale (EQ-VAS, /100)	65.1 (25.4)	62.8 (27.1)	0.83 (-1.99, 3.65)	0.56	63.3 (32.7)	63.4 (28.4)	-5.52 (-17.44, 6.40)	0.36	61.8 (28.5)	55.3 (28.0)	3.03 (-10.63, 16.68)	0.66
Verbal Fluency	11.9 (7.1)	11.6 (7.3)	-0.03 (-0.97, 0.92)	0.95	12.0 (8.0)	10.6 (8.1)	0.59 (-3.29, 4.46)	0.76	9.8 (6.8)	11.8 (8.7)	-2.12 (-5.94, 1.70)	0.27
TICS-M	18.9 (8.4)	18.4 (9.1)	0.03 (-1.17, 1.23)	0.96	15.7 (9.5)	17.5 (9.7)	-4.64 (-9.29, 0.01)	0.051	16.4 (8.4)	14.8 (8.6)	1.23 (-3.48, 5.93)	0.60
MMSE	14.1 (5.5)	13.7 (6.1)	0.19 (-0.61, 0.98)	0.64	12.2 (6.9)	12.8 (6.3)	-2.56 (-5.46, 0.33)	0.08	13.1 (6.3)	12.0 (6.4)	0.55 (-3.01, 4.12)	0.75

Data are n (%), mean (SD), median [interquartile range], mean difference (MD) or odds ratio (OR) with 95% confidence intervals. Comparison using logistic regression, multiple regression or ordinal regression. Adjusted for age, sex, baseline mRS, history of previous stroke, history of diabetes mellitus, TACS, nitrate use, baseline SSS, thrombolysis, feeding status, time to randomisation, baseline SBP and continue/stop. *=adjusted for baseline SBP only; §=ordinal logistic regression.

Table 5.7: Clinical outcomes by continue vs. stop antihypertensives by degree of bilateral carotid stenosis

	Stenosis <30%				Stenosis 30-<50%				Stenosis ≥50%			
	Cont	**Stop**	**OR/MD (95% CI)**	**p**	**Cont**	**Stop**	**OR/MD (95% CI)**	**p**	**Cont**	**Stop**	**OR/MD (95% CI)**	**p**
Number of Participants	317	311	-	-	31	34	-	-	32	33	-	-
SBP change day 0-1 (mmHg)*	-8.2 (19.8)	-7.2 (17.5)	-1.49 (-4.33, 1.35)	0.30	-5.4 (17.0)	-1.0 (19.7)	-5.25 (-14.33, 3.83)	0.25	-13.5 (21.9)	-2.7 (15.8)	-14.35 (-23.81, -4.88)	0.004
Primary outcome	315	310	-	-	31	34	-	-	31	33	-	-
mRS (/6)§	3 [2]	3 [3]	0.97 (0.73, 1.28)	0.82	2 [3]	2 [2]	1.18 (0.43, 3.25)	0.75	2 [2]	3 [2]	0.75 (0.25, 2.21)	0.60
Secondary outcomes												
Day 7 (or Discharge)												
Death, by cause (%)	3 (0.9)	1 (0.3)	6.80 (0.14, 333.09)	0.34	0 (0)	0 (0)	-	-	1 (3.1)	0 (0)	-	-
Symptomatic recurrent stroke (%)	5 (1.6)	0 (0)	-	-	1 (3.2)	1 (2.9)	-	-	0 (0)	1 (3.0)	-	-
Ischaemic	5 (1.6)	0 (0)	-	-	1 (3.2)	0 (0)	-	-	0 (0)	1 (3.0)	-	-
NIH Stroke Scale (/42), calculated	7.9 (6.1)	6.8 (5.5)	0.63 (0.09, 1.17)	0.023	7.1 (7.3)	7.3 (6.1)	0.90 (-1.61, 3.41)	0.47	5.9 (4.5)	7.1 (5.0)	-0.81 (-2.81, 1.19)	0.42
Clinical deterioration (%)	21 (6.6)	10 (3.2)	2.08 (0.95, 4.57)	0.07	4 (12.9)	3 (9.1)	7.49 (0.11, 495.42)	0.35	2 (6.3)	1 (3.0)	-	-
Neurological deterioration (%)	18 (5.7)	8 (2.6)	2.15 (0.91, 5.11)	0.08	2 (6.5)	2 (5.9)	-	-	0 (0)	0 (0)	-	-
Headache (%)	43 (13.6)	33 (10.6)	1.29 (0.76, 2.17)	0.34	2 (6.5)	4 (11.8)	-	-	4 (12.5)	4 (12.1)	-	-
Hypotension (%)	8 (2.5)	3 (1.0)	3.24 (0.82, 12.75)	0.09	0 (0)	0 (0)	-	-	0 (0)	2 (6.1)	-	-
Hypertension (%)	19 (6.0)	19 (6.1)	1.10 (0.54, 2.26)	0.79	2 (6.5)	3 (8.8)	1.74 (0.11, 28.24)	0.70	1 (3.1)	5 (15.2)	-	-

Death or discharge to institution (%)	107 (33.8)	72 (23.2)	1.74 (1.18, 2.57)	0.006	11 (35.5)	9 (26.5)	3.10 (0.56, 17.13)	0.20	8 (25.0)	8 (24.2)	1.90 (0.36, 989)	0.45
Day 90												
Death (%)	18 (5.7)	19 (6.1)	0.71 (0.35, 1.46)	0.36	5 (16.1)	4 (11.8)	-	-	5 (16.1)	1 (3.0)	-	-
Barthel Index	72.5 (34.5)	75.0 (33.0)	-0.45 (-4.90, 4.00)	0.84	68.5 (40.8)	76.0 (36.5)	-4.58 (-22.35, 13.19)	0.61	60.6 (23.9)	50.9 (18.2)	-0.61 (-18.39, 17.18)	0.95
Zung Depression Scale (ZDS, /100)	53.6 (20.6)	54.0 (21.6)	-0.65 (-4.12, 2.82)	0.71	62.2 (25.4)	60.1 (22.6)	-0.81 (-13.16, 11.54)	0.90	60.6 (23.9)	50.9 (18.2)	5.15 (-7.24, 17.54)	0.41
EQ-5D Health Utility Status (HUS, /1)	0.53 (0.39)	0.55 (0.39)	0.00 (-0.06, 0.05)	0.97	0.46 (0.41)	0.58 (0.32)	-0.08 (-0.26, 0.11)	0.41	0.53 (0.35)	0.50 (0.39)	-0.04 (-0.22, 0.15)	0.68
EQ-Visual Analogue Scale (EQ-VAS, /100)	60.8 (26.3)	63.5 (26.8)	-1.65 (-5.85, 2.56)	0.44	55.7 (33.0)	62.0 (31.0)	-3.29 (-18.85, 12.27)	0.67	55.0 (32.7)	61.1 (24.5)	-6.35 (-24.95, 12.25)	0.49
Verbal Fluency	11.0 (7.0)	10.7 (7.0)	0.20 (-1.17, 1.56)	0.78	10.1 (8.4)	9.7 (6.3)	0.40 (-4.50, 5.30)	0.87	7.6 (5.9)	12.9 (8.7)	-2.94 (-9.09, 3.22)	0.33
TICS-M	16.8 (9.3)	17.4 (9.1)	-0.61 (-2.53, 1.31)	0.53	13.8 (9.5)	16.0 (10.4)	0.23 (-7.12, 7.57)	0.95	12.6 (10.0)	19.2 (7.4)	-4.57 (-11.59, 2.45)	0.19
MMSE	12.6 (6.4)	13.2 (6.1)	-0.58 (-1.86, 0.71)	0.38	10.6 (7.1)	11.8 (6.8)	0.54 (-4.13, 5.22)	0.81	10.2 (8.1)	14.1 (5.1)	-3.03 (-8.74, 2.69)	0.28

Data are n (%), mean (SD), median [interquartile range], mean difference (MD) or odds ratio (OR) with 95% confidence intervals. Comparison using logistic regression, multiple regression or ordinal regression. Adjusted for age, sex, baseline mRS, history of previous stroke, history of diabetes mellitus, TACS, nitrate use, baseline SSS, thrombolysis, feeding status, time to randomisation, baseline SBP and GTN/no GTN. *=adjusted for baseline SBP only; §=ordinal logistic regression.

Table 5.8: Change in BP from baseline to day 1 and association with mRS at day 90 by degree of ipsilateral and bilateral carotid stenosis and treatment allocation

Carotid stenosis (%)		Overall			GTN vs. no GTN			Stop vs. Continue		
	ΔSBP	OR (95% CI)	p	$P_{Interaction}$	OR (95% CI)	p	$p_{Interaction}$	OR (95% CI)	p
Ipsilateral										
<30	-8.0 (18.7)	0.95 (0.90, 1.00)	0.07	0.37	1.05 (0.87, 1.28)	0.61	0.88	0.96 (0.73, 1.26)	0.77
30-50	-8.1 (19.5)	0.93 (0.81, 1.07)	0.31	0.63	0.84 (0.50, 1.42)	0.52	0.94	0.91 (0.46, 1.80)	0.79
50-70	-8.3 (17.1)	0.80 (0.66, 0.97)	0.026	0.66	0.77 (0.41, 1.45)	0.41	0.44	1.93 (0.85, 4.41)	0.12
≥70	-5.7 (17.5)	1.05 (0.90, 1.22)	0.55	0.34	0.53 (0.32, 0.90)	0.019	0.98	1.44 (0.72, 2.91)	0.31
Bilateral										
<30	-8.1 (18.4)	0.95 (0.90, 1.01)	0.09	0.10	1.02 (0.83, 1.26)	0.83	0.66	0.94 (0.70, 1.25)	0.66
30-50	-5.6 (19.4)	0.83 (0.67, 1.03)	0.09	0.22	1.27 (0.57, 2.83)	0.56	0.22	1.05 (0.37, 3.01)	0.92
≥50	-8.6 (19.5)	1.95 (0.74, 1.21)	0.65	0.21	1.01 (0.44, 2.32)	0.99	0.59	0.54 (0.16, 1.86)	0.33

ΔSBP = SBP day 0 - SBP day 1 (mmHg). Data are n (%), mean (SD), median [interquartile range], mean difference (MD) or odds ratio (OR) with 95% confidence intervals. Comparison using ordinal regression. Adjusted for age, sex, baseline mRS, history of previous stroke, history of diabetes mellitus, TACS, nitrate use, baseline SSS, thrombolysis, feeding status, time to randomisation, baseline SBP, stop/continue antihypertensives and GTN/no GTN. Overall ORs per 10mmHg reduction in BP.

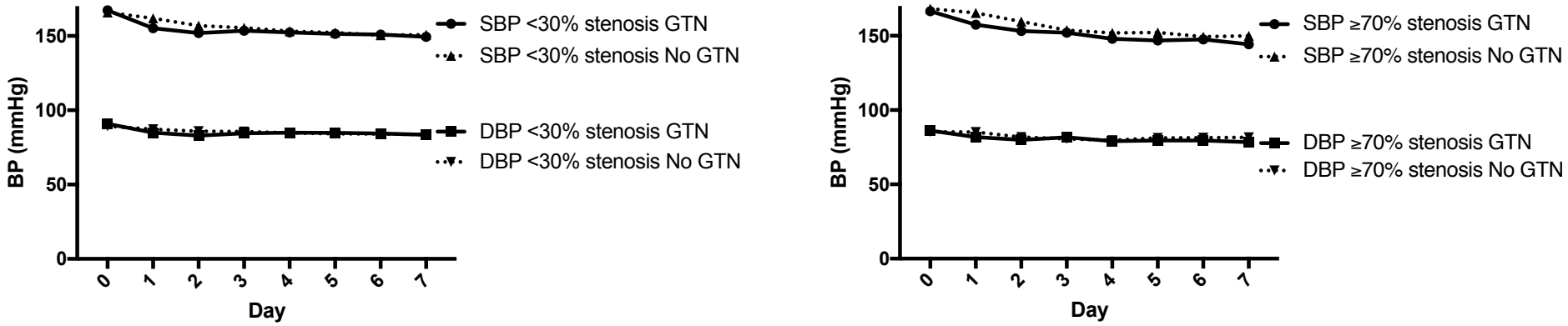


Figure 5.3: Mean BP over days 0-7 by degree of ipsilateral carotid stenosis and GTN vs. no GTN

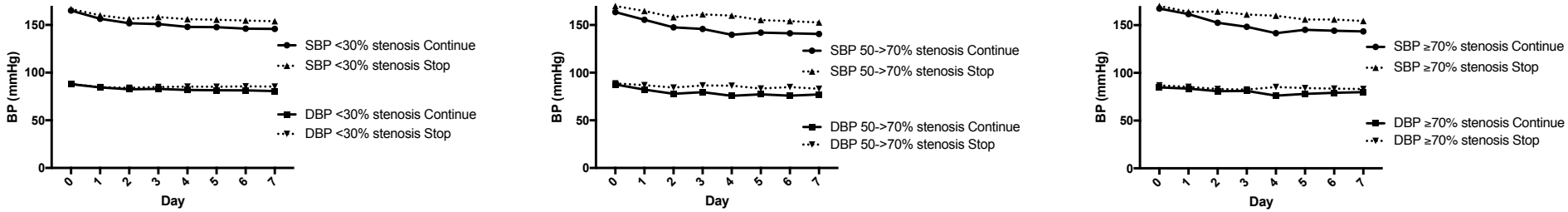


Figure 5.4: Mean BP over days 0-7 by degree of ipsilateral carotid stenosis and Continue vs. Stop pre-stroke antihypertensives

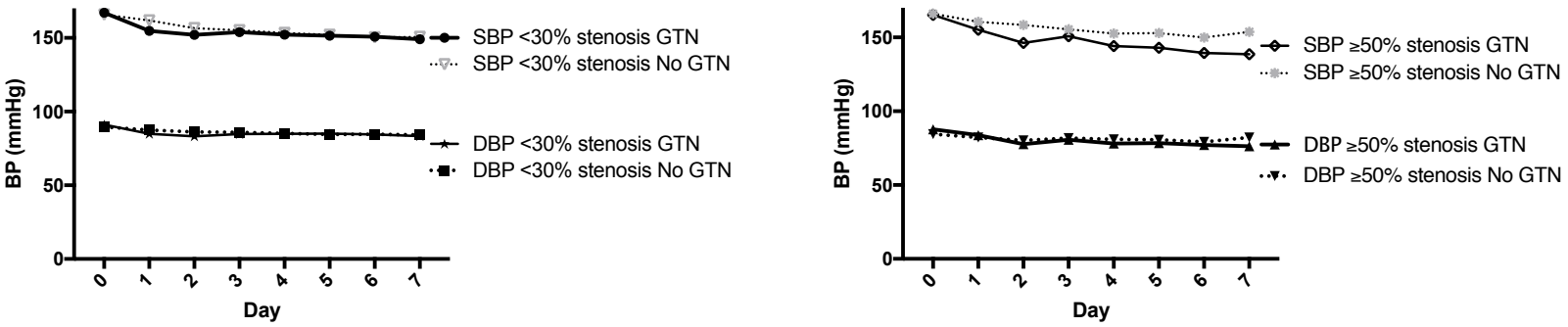


Figure 5.5: Mean BP over days 0-7 by degree of bilateral carotid stenosis and GTN vs. no GTN

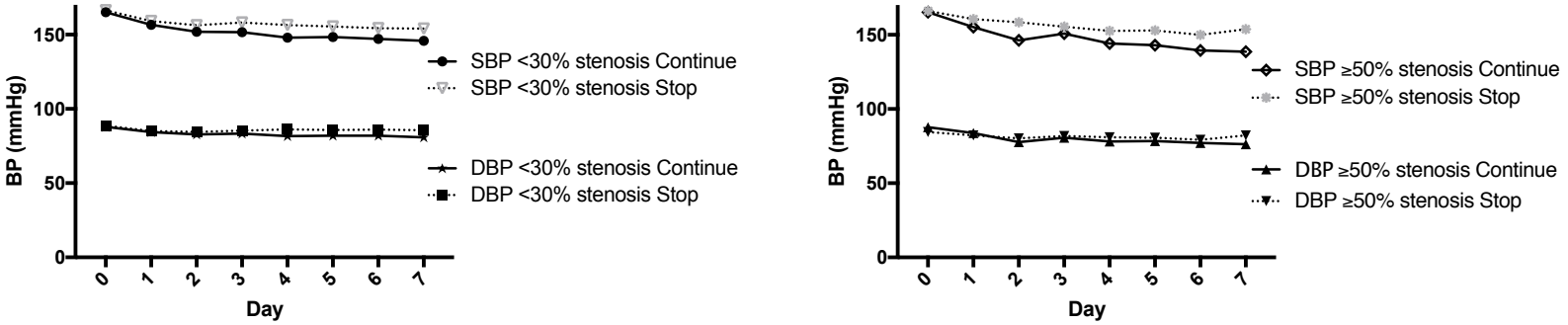


Figure 5.6: Mean BP over days 0-7 by degree of bilateral carotid stenosis and Continue vs. Stop pre-stroke antihypertensives

5.5 DISCUSSION

In this pre-planned ENOS substudy,(232) taking all patients irrespective of treatment allocation, the presence of severe (≥70%) ipsilateral symptomatic carotid stenosis was associated with unfavourable clinical outcomes both early and late after acute stroke. However, treatment with GTN vs. no GTN was associated with a significant shift to less death or dependency at 90 days in those participants with ≥70% ipsilateral stenosis. Continuing vs. stopping pre-stroke antihypertensives was associated with worse secondary outcomes in those with 50-<70% ipsilateral stenosis. Modest BP lowering was safe in acute stroke patients in the context of unilateral and bilateral carotid stenosis. Therefore, the null hypothesis can be rejected: transdermal GTN is safe in acute stroke patients with ipsilateral carotid stenosis.

It has long been established that higher degrees of symptomatic carotid stenosis are associated with early stroke recurrence and subsequent dependency after minor stroke and transient ischaemic attack; carotid endarterectomy is therefore recommended.(239) This dataset suggests that patients with more severe strokes with large artery disease who may not have been eligible for carotid revascularisation have both poor early and late clinical outcomes as expected. These findings are likely to be even stronger in clinical practice as the 32.9% patients with ischaemic stroke who did not

have carotid imaging had more severe strokes and therefore worse clinical outcome than those with imaging.

Although pathophysiological data have demonstrated dysfunctional cerebral autoregulation in severe carotid stenosis,(240-242) and cerebral blood flow can become dependent upon systemic BP,(51) there are limited prospective data assessing BP lowering in acute stroke with carotid stenosis. Previous blinded analysis of ENOS performed during recruitment revealed that BP lowering in the context of carotid stenosis was safe.(232) We have reinforced that finding here with no evidence to suggest modest BP lowering is associated with stroke recurrence or other poor outcomes. A *post-hoc* analysis of the Scandinavian Candesartan Acute Stroke Trial (SCAST) found no clear evidence to suggest that BP lowering was detrimental in patients with carotid stenosis, but there were non-significant tendencies towards increased stroke progression and poor functional outcome with candesartan.(153) As in the present substudy, the observed BP lowering effect in SCAST was modest (5/2 mmHg) and therefore differing drug class effects may explain the differences observed between the present analysis and SCAST. Further, continuing pre-stroke antihypertensives in those with modest ipsilateral carotid stenosis (50-<70%) was associated with poorer outcomes across several secondary domains, but not in those with ≥70% ipsilateral stenosis. This may be due to those with modest stenosis having greater baseline comorbidity (higher mRS, more hypertension, diabetes, previous stroke, ischaemic heart disease and

hyperlipidaemia) than those with ≥70% stenosis. This imbalance may represent selection bias, whereby patients with more severe stroke and severe stenosis were not imaged as doing so would not change management. It is unclear whether there are drug class-specific mechanisms that may be harmful in the context of carotid stenosis early after stroke, but we add to evidence from the main ENOS trial that routinely continuing pre-stroke antihypertensives should perhaps be avoided until the patient is neurologically stable.(84) Whether larger precipitous drops in BP are safe is beyond the scope of this substudy, but given the uncertainty this practice should perhaps be avoided.

The shift in mRS to less death or dependency with GTN seen in those with ≥70% ipsilateral carotid stenosis may be related to its effects outwith BP lowering. As a vasodilator, GTN did not reduce cerebral blood flow in acute stroke patients despite lowering peripheral and central BP.(183, 243) Although it is unclear whether GTN improves cerebral blood flow,(243) it can be hypothesised that GTN may improve collateral blood supply via surface pial collaterals (244) and thereby maintain blood flow to the ischaemic penumbra in the context of carotid stenosis when given early.

Bilateral carotid stenosis is uncommon (4.8% in this analysis) and therefore data regarding the safety of BP lowering in acute stroke patients in this context are scanty.(152) Impaired cerebral perfusion is more common in this population than in unilateral carotid stenosis

and although we found no evidence that modest BP lowering was unsafe, further data are required to address this question.

The strengths of this planned substudy include its large population in the context of a completed randomised controlled trial with near total follow-up. However, there are limitations. First, not all patients with ischaemic stroke had carotid imaging studies performed, a deficiency most prominent in patients with severe stroke. Second, the analyses across degrees of carotid stenosis are sub-groups analyses and, so the results may represent chance. Third, the median time to randomisation in ENOS was 26 hours and so the effect of BP lowering in the context of carotid stenosis within the first few hours of ischaemic stroke remains unclear. Fourth, no adjustment was made for multiplicity of testing due to the exploratory nature of the study. Fifth, imaging information on carotid stenosis was provided by investigators at site with unknown imaging modality and reporting criteria (NASCET,(9) European Carotid Surgery Trial [ECST],(245) and Carotid and Vertebral Artery Transluminal Angioplasty Study [CAVATAS](246)) and were not centrally adjudicated. However, data was validated and checked for accuracy. Sixth, it was not possible to adjust cognitive outcome data for cognition at baseline and many of the differences seen at three months in association with degrees of stenosis (apart from allocated trial treatment differences) likely reflect baseline status. Seventh, ENOS assessed mild to moderate BP lowering and the results presented here do not provide information on the effects of intensive BP lowering. Last, data on carotid

endarterectomy was not captured prospectively in ENOS and was therefore unavailable for the population studied, although in a population of patients with mostly moderate to severe stroke it is unlikely that many patients underwent endarterectomy.

In summary, this ENOS substudy has demonstrated that severe ipsilateral carotid stenosis is associated with worse clinical outcomes at 7 and 90 days after acute stroke irrespective of treatment allocation. Transdermal GTN improved functional outcome at 90 days in those with ≥70% ipsilateral stenosis and was safe across all degrees of carotid stenosis whether unilateral or bilateral. Further, modest BP lowering with GTN was safe in the context of carotid stenosis although continuing pre-stroke antihypertensives was associated with poorer secondary outcomes in those with 50-<70% ipsilateral carotid stenosis.

Future studies should establish whether GTN and other BP lowering therapies have specific mechanistic properties that may be of benefit in acute stroke in the context of carotid stenosis.

CHAPTER 6:

THE ASSOCIATIONS OF IMAGING MARKERS OF SMALL VESSEL DISEASE AND 'BRAIN FRAILTY' WITH CLINICAL OUTCOME, AND EFFECTS OF GLYCERYL TRINTIRATE, IN PATIENTS WITH ACUTE LACUNAR STROKE

Publications (under review) contributing to this chapter:

Appleton JP, Woodhouse LJ, Adami A, Becker JL, Cala LA, Casado AM, et al. The association of imaging markers of small vessel disease and 'brain frailty' with clinical outcome, and effects of glyceryl trinitrate, in patients with acute lacunar stroke: a secondary analysis of the Efficacy of Nitric Oxide in Stroke (ENOS) trial.

Presentations contributing to this chapter:

Glyceryl trinitrate improves early neurological outcome in acute stroke patients presenting with lacunar syndromes and acute lacunar infarction. UK Stroke Forum, The Arena and Convention Centre, Liverpool (November 2017)

The association of imaging markers of small vessel disease and 'brain frailty' with clinical outcome following acute stroke. UK Stroke Forum, The International Centre, Telford (accepted for presentation in December 2018)

6.1 ABSTRACT

Background

Cerebral small vessel disease (SVD) is a common cause of lacunar stroke, cognitive impairment and dementia. We assessed the association of baseline imaging markers of SVD and 'brain frailty' with functional and cognitive outcomes after acute stroke, including lacunar stroke, in the Efficacy of Nitric Oxide in Stroke (ENOS) trial, and the effect of glyceryl trinitrate (GTN) in these patients.

Methods

ENOS randomised 4011 patients with acute stroke (<48 hours of onset) to transdermal GTN or no GTN for 7 days. The primary outcome was functional outcome (modified Rankin Scale, mRS) at day 90. Stroke syndrome was classified using the Oxfordshire Community Stroke Project classification. Brain imaging was adjudicated masked to clinical information and treatment, and assessed for SVD (leukoaraiosis, old lacunar infarcts/lacunes, atrophy) and 'brain frailty' (leukoaraiosis, atrophy, old vascular lesions). Ordered categorical data were analysed using ordinal logistic regression with adjustment for prognostic variables.

Results

In all participants, and in 1397/4011 (34.8%) participants with lacunar syndromes (LACS), baseline imaging features of SVD and 'brain frailty' on CT were common and independently associated with

unfavourable shifts in mRS at day 90 in all participants (SVD score: OR 1.15, 95% CI 1.07-1.24; 'brain frailty' score: OR 1.25, 95% CI 1.17-1.34) and in those with LACS (SVD score: OR 1.30, 95% CI 1.15-1.47; 'brain frailty' score: OR 1.28, 95% CI 1.14-1.44). 'Brain frailty' was associated with poorer cognitive scores at 90 days, in all, and in LACS participants. GTN did not influence three-month outcomes in participants with LACS.

Conclusion

Baseline imaging features of SVD and 'brain frailty' were common in lacunar stroke and all stroke, predicted worse prognosis after all acute stroke with a stronger effect in lacunar stroke, and may aid future clinical decision-making. Whether the vascular or neurodegenerative features are more associated with cognitive impairment requires further testing.

6.2 INTRODUCTION

Cerebral small vessel disease (SVD) is a common cause of lacunar ischaemic stroke, haemorrhagic stroke, vascular cognitive impairment and dementia.(7) The pathophysiology differs from other stroke subtypes and is thought to reflect intrinsic damage to small perforating arterioles manifest as endothelial dysfunction, blood-brain barrier breakdown and inflammation.(247) Imaging markers of SVD include white matter hyperintensities or leukoaraiosis, microbleeds prominent perivascular spaces and lacunes in addition to acute lacunar infarcts and intracerebral haemorrhage.(248) All are visible on magnetic resonance imaging (MRI), whilst microbleeds and perivascular spaces are not visible on the more commonly available computed tomography (CT) scanning.

Recently, instead of considering each individual SVD feature separately, a total sum score of SVD features was found to be sensitive to risk factor associations,(249) associations with cognition(250) and mobility.(251) Several large trials have reviewed the association between imaging markers of SVD and outcome.(252-254) Some analyses focused on features seen on MRI,(249, 254) whilst others identified general pre-stroke features visible on CT (including SVD-specific) that were associated independently with poor outcome (leukoaraiosis, cerebral atrophy and old vascular lesions) suggesting that these might represent markers of 'brain frailty'.(252)

Few trials or large observational studies have focused on outcomes after acute lacunar stroke, largely because such patients have better clinical outcomes compared to other ischaemic stroke subtypes.(255-257) Three trials have reported outcomes following acute lacunar stroke in a total of 835 participants,(255, 257) with 422 being the largest trial population of acute lacunar stroke patients to date.(257) There are also modest studies using non-randomised data from thrombolysis registers.(255, 256) In contrast, the Efficacy of Nitric Oxide in Stroke (ENOS) trial assessed the safety and efficacy of transdermal glyceryl trinitrate (GTN) in 4011 patients with acute stroke of whom a large proportion (1397, 35%) were of lacunar subtype.(84)

The aims of the present analysis were to assess the influence of imaging markers of SVD and 'brain frailty' at baseline on functional and cognitive outcome in patients with acute lacunar stroke in the ENOS trial, and the effect of GTN in these patients.

6.3 METHODS

Hypotheses tested in this chapter

- Transdermal GTN is safe in acute stroke patients with lacunar strokes and small vessel disease.
- Transdermal GTN improves clinical outcomes when given within 6 hours of stroke onset in patients with lacunar strokes and small vessel disease.

ENOS trial

Details pertaining to the ENOS trial protocol, statistical analysis plan, baseline characteristics and main results are available elsewhere.(84, 189, 208, 209) In summary, ENOS recruited 4011 patients in 173 centres in 23 countries within 48 hours of stroke onset with high systolic BP (140-220 mmHg) and randomised them to GTN 5 mg patch or no patch for 7 days. Participants on antihypertensive medication prior to their index event were also randomised to continue or stop these medications for 7 days. Patients or relatives/carers provided written consent. ENOS was registered (ISRCTN99414122) and approved by ethics committees/competent authorities in all participating countries.

Study population

Stroke syndrome was assessed at baseline using the Oxfordshire Community Stroke project (OCSP) clinical classification.(17) We incorporated imaging findings to create a hierarchy of increasing specificity of definitions of acute lacunar stroke(258) as follows:

Non-lacunar syndrome patients (n=2614)

Clinical lacunar syndrome (LACS) patients (n=1397)

LACS with compatible scan (n=623): i.e. LACS with an adjudicated acute lacunar infarct, or if no acute infarct visible then no alternative acute non-lacunar infarct or other pathology seen to explain the presentation

LACS with corresponding acute lacunar infarction on imaging (n=143)

Imaging

CT or MRI brain scans were performed at baseline, usually prior to randomisation. A second CT brain scan was performed at day 7 (end of treatment) where feasible. Uncompressed DICOM, JPEG, PNG, or GIF image files were sent to the coordinating centre, either uploaded via a secure website or on a compact disc. Images sent on film were digitised using a VICOM digitiser. Scans were adjudicated using a set proforma encompassing validated scales(252) by trained neuroradiologists or neurologists (AA, JLB, LAC, AC, RAD, PK, JMW) blind to clinical details and randomisation allocation (see appendix 10.2.2). These assessments documented the location, size and swelling associated with any acute ischaemic or haemorrhagic lesion and the presence of pre-stroke changes including atrophy, leukoaraiosis and old vascular lesions:

Atrophy, assessed in cortical and central regions, was defined as 0=absent, 1=moderate or 2=severe compared against a standard template,(252) thus providing a maximum score of 4.

Leukoaraiosis was assessed anteriorly and posteriorly:(259) 0=no lucency, 1=lucency restricted to region adjoining ventricles, 2=lucency covering entire region from lateral ventricle to cortex; providing a maximum score of 4.
Old vascular lesions were classified by location (e.g. cortical, striatocapsular, borderzone, lacunar).

In addition to individual imaging markers of SVD, we also applied scores adapted for CT scanning as follows:
SVD score comprises 1 point each for severe leukoaraiosis (score=2 anteriorly and/or posteriorly as above), any old lacunar infarcts/lacunes, and severe atrophy (max 3/3)(254)
SVD score excluding atrophy (max 2/2) was also assessed as atrophy, although related, is not specific to SVD.
'Brain frailty' score comprises 1 point each for leukoaraiosis (score=1 or 2 anteriorly and/or posteriorly) , cerebral atrophy (score=1 or 2 cortically and/or centrally), and old vascular lesions (max 3/3). Although there is no accepted definition of brain frailty, we used the individual features used in IST-3, which were individually shown to be associated with poor clinical outcome after acute stroke.(252)

Clinical outcomes

The primary outcome in ENOS was the modified Rankin Scale (mRS),(234) a 7-level ordered categorical scale (0=independent, 6= dead), measured at day 90. Secondary outcomes at day 90 included disability (Barthel Index), quality of life (Health utility status

calculated using European Quality of Life 5-dimensions 3-level, and visual analogue scale), mood (Zung depression scale) and cognition (telephone mini-mental state examination [t-MMSE],(237) modified telephone interview for cognitive status [TICS-M],(238) and verbal fluency). In essence, t-MMSE and TICS-M assess attention and memory, whilst verbal fluency assesses executive function. Participants who died by day 90 were assigned a worst score for these outcomes. Safety outcomes included all-cause mortality, early neurological deterioration (reduction of ≥5 points or reduction in the consciousness domain >2 points from baseline to day 7 on the Scandinavian Stroke Scale [SSS]), and symptomatic hypotension. Day 90 outcomes were assessed by trained blinded assessors via telephone at national coordinating centres.

Statistical analysis

Data were analysed by intention-to-treat.(189) Data are number (%), median [interquartile range, IQR], or mean (standard deviation, SD). Differences in baseline characteristics were assessed using X^2 for categorical variables and one-way analysis of variance (ANOVA) for continuous variables.

Differences between treatment group (GTN vs. no GTN) effects on outcome were assessed using binary logistic regression, multiple linear regression, ordinal logistic regression or Cox proportional hazard regression. Associations between baseline imaging characteristics and mRS and cognitive outcomes at day 90 were

assessed using ordinal logistic regression and multiple linear regression respectively. Statistical models were adjusted for baseline prognostic covariates including age, sex, baseline mRS, history of previous stroke, history of diabetes mellitus, final diagnosis, nitrate use, baseline systolic BP, baseline SSS score, thrombolysis, feeding status, time to randomisation and treatment allocation (GTN vs. no GTN and/or continue vs. stop pre-stroke antihypertensives). Results are reported as odds ratio (OR) or mean difference (MD) and associated 95% confidence intervals (CI), with significance defined as $p \leq 0.05$. Analyses were performed using SPSS version 24 (Chicago, IL).

6.4 RESULTS

A total of 1397/4011 (34.8%) patients with LACS were recruited into ENOS. Baseline characteristics of LACS differed from those presenting with non-LACS (Table 6.1): LACS were younger (67.9 [12.0] years); more were male (61.1%), recruited in Asia, had diabetes or were smokers; they were less dependent at baseline, with less pre-stroke hypertension, transient ischaemic attack (TIA), hyperlipidaemia, ischaemic heart disease, peripheral arterial disease or atrial fibrillation, but more diabetes, than non-LACS participants. LACS participants also had milder index events (mean SSS 42 vs. 29 $p<0.001$), a longer time to randomisation and fewer received thrombolysis than non-LACS. There was no difference in 7-day compliance with GTN between LACS and non-LACS participants (74% vs. 75%) with a median treatment duration of 7 days in both populations. Most participants (92%) were imaged using CT. At baseline there were more acute lacunar infarctions and fewer haemorrhages in those with LACS than non-LACS, whilst background changes – leukoaraiosis, cerebral atrophy and old vascular lesions – did not differ between the groups (Table 6.2). In LACS participants, 50% had moderate or severe cerebral atrophy, 41% had some degree of leukoaraiosis and 61% had an old vascular lesion.

SVD and 'brain frailty' scores were moderately positively correlated: Spearman's correlation coefficient 0.626, $p<0.001$. Thus, although they measure similar imaging markers, the differences in severity of

the individual markers included provide two different ways of assessing brain health.

Table 6.1: Baseline clinical characteristics. Comparison of non-LACS vs. LACS.

	All	Non LACS	LACS				LACS & compatible scan			LACS & acute lacunar infarct		
			All	p	GTN	No GTN	All	GTN	No GTN	All	GTN	No GTN
Number of patients	4011	2614	1397		695	702	623	308	315	143	71	72
Age (years)	70.3 (12.2)	71.6 (12)	67.9 (12)	**<0.001**	67.8 (12.2)	68.1 (12.1)	68.7 (11.6)	68.2 (11.7)	69.0 (11.5)	66.8 (10.9)	65.5 (11.5)	68.0 (10.2)
Sex, male (%)	2297 (57.3)	1444 (55.2)	853 (61.1)	**<0.001**	424 (61)	429 (61.1)	395 (63.4)	185 (60.1)	210 (66.7)	84 (58.7)	41 (57.7)	43 (59.7)
Geographical region (%)												
Asia	559 (13.9)	276 (10.6)	283 (20.3)	**<0.001**	140 (20.1)	143 (20.4)	107 (17.2)	57 (18.5)	50 (15.9)	46 (32.2)	23 (32.4)	23 (31.9)
Europe	647 (16.1)	409 (15.6)	238 (20.3)	0.25	118 (17)	120 (17.1)	125 (20.1)	62 (20.1)	63 (20.0)	17 (11.9)	10 (14.1)	7 (9.7)
United Kingdom	2545 (63.5)	1741 (66.6)	804 (57.6)	**<0.001**	399 (57.4)	405 (57.7)	359 (57.6)	172 (55.8)	187 (59.4)	75 (52.4)	35 (49.3)	40 (55.6)
Other	260 (6.5)	188 (7.2)	72 (5.2)	**0.012**	38 (5.5)	34 (4.8)	32 (5.1)	17 (5.5)	15 (4.8)	5 (3.5)	3 (4.2)	2 (2.8)
mRS >0 (%)	1026 (25.6)	733 (28)	293 (21)	**<0.001**	134 (19.3)	159 (22.6)	130 (20.9)	62 (20.1)	68 (21.6)	28 (19.6)	14 (19.7)	14 (19.4)
Medical history												
Hypertension	2607 (65)	1768 (67.6)	839 (60.1)	**<0.001**	405 (58.3)	434 (61.8)	364 (58.4)	175 (56.8)	189 (60.0)	80 (55.9)	41 (57.7)	39 (54.2)
Treated hypertension	2138 (53.3)	1499 (57.3)	639 (45.7)	**<0.001**	318 (45.8)	321 (45.7)	293 (47.0)	152 (49.4)	141 (44.8)	65 (45.5)	37 (52.1)	28 (38.9)

Diabetes	699 (17.4)	427 (16.3)	272 (19.5)	**0.013**	134 (19.3)	138 (19.7)	137 (22.0)	68 (22.1)	69 (21.9)	36 (25.2)	22 (31)	14 (19.4)
Atrial fibrillation	762 (19)	626 (23.9)	136 (9.7)	**<0.001**	63 (9.1)	73 (10.4)	54 (8.7)	29 (9.4)	25 (7.9)	4 (2.8)	3 (4.2)	1 (1.4)
Prior stroke	594 (14.8)	406 (15.5)	188 (13.5)	0.08	96 (13.8)	92 (13.1)	79 (12.7)	38 (12.3)	41 (13.0)	19 (13.3)	10 (14.1)	9 (12.5)
Prior TIA	544 (13.6)	387 (14.8)	157 (11.2)	**0.001**	83 (11.9)	74 (10.5)	77 (12.4)	40 (13.0)	37 (11.7)	16 (11.2)	9 (12.7)	7 (9.7)
Prior IHD	669 (16.7)	467 (17.9)	202 (14.5)	**0.002**	97 (14)	105 (15)	96 (15.4)	47 (15.3)	49 (15.6)	17 (11.9)	7 (9.9)	10 (13.9)
Prior PAD	117 (2.9)	79 (3)	38 (2.7)	**0.040**	16 (2.3)	22 (3.1)	19 (3.0)	9 (2.9)	10 (3.2)	3 (2.1)	1 (1.4)	2 (2.8)
Hyperlipidaemia	1098 (27.4)	759 (29)	339 (24.3)	**<0.001**	173 (24.9)	166 (23.6)	155 (24.9)	83 (26.9)	72 (22.9)	34 (23.8)	16 (22.5)	18 (25)
Smoking, current	945 (23.6)	566 (21.7)	379 (27.1)	**<0.001**	186 (26.8)	193 (27.5)	181 (29.1)	90 (29.2)	91 (28.9)	50 (35)	23 (32.4)	27 (37.5)
past	1222 (30.5)	795 (30.4)	427 (30.6)	**<0.001**	218 (31.4)	209 (29.8)	188 (30.2)	87 (28.2)	101 (32.1)	37 (25.9)	15 (21.1)	22 (30.6)
never	1679 (41.9)	1111 (42.5)	568 (40.7)	**<0.001**	283 (40.7)	285 (40.6)	243 (39.0)	127 (41.2)	116 (36.8)	55 (38.5)	33 (30.6)	22 (30.6)
Nitrate	154 (3.8)	115 (4.4)	39 (2.8)	**0.012**	22 (3.2)	17 (2.4)	10 (1.6)	8 (2.6)	2 (0.6)	1 (0.7)	0	1 (1.4)
Alcohol >21 units per week	294 (7.3)	185 (7.1)	109 (7.8)	0.40	56 (8.1)	53 (7.5)	43 (6.9)	19 (6.2)	24 (7.6)	10 (7)	4 (5.6)	6 (8.3)
Qualifying event												
Ischaemic stroke	3342 (83.3)	2162 (82.7)	1180 (84.5)	0.16	584 (84)	596 (84.9)	623 (100)	308 (100)	315 (100)	143 (100)	71 (100)	72 (100)

Haemorrhagic stroke	629 (15.7)	429 (16.4)	200 (14.3)	0.08	100 (14.4)	100 (14.2)	0	0	0	0	0	0
Side of lesion, right (%)	2091 (52.1)	1239 (47.4)	852 (61)	**<0.001**	419 (60.3)	433 (61.7)	349 (56.0)	177 (57.5)	172 (54.6)	81 (56.6)	46 (64.8)	35 (48.6)
SSS (/58)	33.7 (13.2)	29.4 (13.5)	41.7 (7.9)	**<0.001**	41.9 (8.0)	41.6 (7.7)	42.9 (7.4)	43.1 (7.5)	42.8 (7.3)	42.4 (7.9)	42.9 (7.4)	42.0 (8.4)
NIHSS (/42), calculated	11.2 (5.7)	13 (5.8)	7.7 (3.4)	**<0.001**	7.7 (3.4)	7.8 (3.3)	7.2 (3.2)	7.2 (3.2)	7.3 (3.2)	7.4 (3.4)	7.3 (3.2)	7.6 (3.6)
GCS < 15 (%)	1229 (30.6)	1145 (43.8)	84 (6)	**<0.001**	48 (6.9)	36 (5.1)	25 (4.0)	14 (4.5)	11 (3.5)	7 (4.9)	3 (4.2)	4 (5.6)
Haemodynamics												
Systolic BP (mmHg)	167.2 (19)	167.4 (18.9)	166.9 (19.1)	0.43	167.4 (19.2)	166.4 (18.9)	166.8 (18.8)	167.8 (18.8)	165.8 (18.8)	170.8 (18.7)	171.4 (18.1)	170.3 (19.4)
Diastolic BP (mmHg)	89.5 (13.1)	89.1 (13.3)	90.3 (12.9)	**0.004**	90.4 (12.9)	90.3 (12.9)	89.9 (13.1)	90.1 (13.4)	89.7 (12.8)	92.8 (13.0)	93.4 (14.0)	92.2 (12.0)
Heart rate (bpm)	77.5 (14.7)	78.2 (15.2)	76.2 (13.7)	**<0.001**	76.5 (13.6)	75.8 (13.7)	76.0 (13.5)	76.1 (13.3)	75.9 (13.7)	75.1 (12.5)	75.3 (12.4)	74.9 (12.7)
Systolic BP variability[+]	7.3 (5.5)	7.4 (5.7)	7.1 (5.3)	0.10	7.3 (5.5)	6.8 (5.0)	7.0 (5.3)	7.2 (5.5)	6.8 (5.7)	7.6 (5.5)	6.9 (4.7)	8.3 (6.2)
Time to randomisation (hours)	26.0 (12.9)	25.4 (13.1)	27.1 (12.4)	**<0.001**	27.1 (12.7)	27.1 (12.2)	27.3 (12.6)	27.8 (13.0)	26.9 (12.2)	32.2 (10.9)	30.8 (11.3)	33.6 (10.4)
Thrombolysis (%)	425 (10.6)	335 (12.8)	90 (6.4)	**<0.001**	37 (5.3)	53 (7.5)	46 (7.4)	18 (5.8)	28 (8.9)	4 (2.8)	3 (4.2)	1 (1.4)

BP: blood pressure; GCS: Glasgow coma scale; GTN: glyceryl trinitrate; IHD: ischaemic heart disease; LACS: lacunar syndrome; mRS: modified Rankin Scale; NIHSS: national institutes for health stroke scale; PAD: peripheral arterial disease; SSS: Scandinavian stroke scale; TIA: transient ischaemic attack. LACS vs. non-LACS assessed using X^2 for categorical variables and one-way analysis of variance (ANOVA) for continuous variables. Bold represents $p<0.05$.

Table 6.2: Baseline radiological characteristics. Comparison of non-LACS vs. LACS.

	All	Non LACS	LACS				LACS & compatible scan			LACS & acute lacunar infarct		
			All	p	GTN	No GTN	All	GTN	No GTN	All	GTN	No GTN
Baseline scan (%)	3857 (96.2)	2516 (96.3)	1341 (96)	0.68	674 (97)	667 (95)	623 (100)	308 (100)	315 (100)	143 (100)	71 (100)	72 (100)
Time to scan (hours)	17.0 (23.7)	15.3 (21.7)	20.3 (26.7)	**<0.001**	20.0 (27.3)	20.7 (26.2)	21.1 (28.7)	21.8 (30.9)	20.5 (26.4)	31.9 (34.6)	33.4 (41.0)	30.5 (27.0)
Visible acute infarction	2041 (50.9)	1383 (52.9)	658 (47.1)	**<0.001**	328 (47.2)	330 (47)	144 (23.1)	71 (23.1)	73 (23.2)	143 (100)	71 (100)	72 (100)
Lacunar	241 (6.0)	98 (3.7)	143 (10.2)	**<0.001**	71 (10.2)	72 (10.3)	143 (23.0)	71 (23.1)	72 (22.9)	143 (100)	71 (100)	72 (100)
Thalamus	20 (1)	8 (0.6)	12 (1.8)	**0.016**	8 (2.4)	4 (1.2)	12 (8.3)	8 (11.3)	4 (5.5)	12 (9.4)	8 (12.9)	4 (6.2)
Centrum semiovale	67 (3.3)	26 (1.9)	41 (6.2)	**<0.001**	23 (7)	18 (5.5)	41 (28.5)	23 (32.4)	18 (24.7)	41 (32.3)	23 (37.1)	18 (27.7)
Internal capsule /lentiform	121 (5.9)	52 (3.8)	69 (10.5)	**<0.001**	30 (9.1)	39 (11.8)	69 (47.9)	30 (42.3)	39 (53.4)	69 (54.3)	30 (48.4)	39 (60)
Internal border zone	5 (0.2)	3 (0.2)	2 (0.3)	0.79	1 (0.3)	1 (0.3)	2 (1.4)	1 (1.4)	1 (1.4)	2 (1.6)	1 (1.6)	1 (1.5)
Visible haemorrhage	673 (16.8)	476 (18.2)	197 (14.1)	**0.003**	98 (14.1)	99 (14.1)	0	0	0	0	0	0
Intracerebral haemorrhage	587 (14.6)	404 (15.4)	184 (13.2)	**0.040**	97 (14)	87 (12.4)	0	0	0	0	0	0

Lobar or cerebellar§	79 (13.5)	54 (13.4)	25 (13.7)	0.60	14 (14.4)	11 (12.8)	0	0	0	0	0	0
Deep§	507 (86.5)	349 (86.6)	158 (86.3)	0.09	83 (85.6)	75 (87.2)	0	0	0	0	0	0
Cerebral atrophy (%)	3229 (80.5)	2126 (81.3)	1103 (79)	0.13	553 (79.6)	550 (78.3)	522 (83.8)	255 (82.8)	267 (84.8)	104 (72.7)	48 (67.6)	56 (77.8)
Moderate	1454 (36.3)	968 (37)	486 (34.8)		249 (35.8)	237 (33.8)	219 (35.2)	108 (35.1)	111 (35.2)	69 (48.3)	33 (46.5)	36 (50.0)
Severe	609 (15.2)	400 (15.3)	209 (15)		100 (14.4)	109 (15.5)	124 (19.9)	58 (18.8)	66 (21.0)	28 (19.6)	11 (15.5)	17 (23.6)
Leukoaraiosis	1644 (41)	1075 (41.1)	569 (40.7)	0.95	265 (38.1)	304 (43.3)	268 (43.0)	119 (38.6)	149 (47.3)	84 (58.7)	36 (50.7)	48 (66.7)
Leukoaraiosis score (/4)	1.0 (1.3)	1.0 (1.3)	1.0 (1.3)	0.55	0.9 (1.3)	1.1 (1.4)	1.0 (1.3)	0.9 (1.3)	1.1 (1.3)	1.4 (1.4)	1.2 (1.4)	1.5 (1.4)
Old infarcts (n, %)	2326 (60.5)	1520 (60.5)	806 (60.5)	0.98	409 (61.0)	397 (59.9)	395 (63.4)	193 (62.7)	202 (64.1)	74 (51.7)	38 (53.5)	36 (50.0)
Striatocapsular	585 (14.6)	375 (14.3)	210 (15)	0.50	99 (14.2)	111 (15.8)	114 (18.3)	55 (18.7)	59 (18.7)	14 (9.8)	8 (11.3)	6 (8.3)
Borderzone	74 (1.8)	48 (1.8)	26 (1.9)	0.93	9 (1.3)	17 (2.4)	9 (1.4)	4 (1.3)	5 (1.6)	4 (2.8)	3 (4.2)	1 (1.4)
Lacunar	1423 (35.5)	909 (34.8)	514 (36.8)	0.15	267 (38.4)	247 (35.2)	249 (40.0)	124 (40.3)	125 (39.7)	68 (47.6)	35 (49.3)	33 (45.8)
At least 1 of above	1619 (42.2)	1031 (41.0)	588 (44.1)	0.07	304 (45.4)	284 (42.8)	284 (45.6)	144 (46.8)	140 (44.4)	71 (49.7)	38 (53.5)	33 (45.8)
SVD score (/3)~	1.0 (0.8)	1.0 (0.8)	1.0 (0.8)	0.69	1.0 (0.9)	1.0 (0.8)	1.1 (0.9)	1.0 (0.9)	1.1 (0.8)	0.9 (0.9)	0.9 (0.9)	1.0 (0.9)

Brain frailty score (/3)#	1.9 (0.9)	1.9 (0.9)	1.9 (0.9)	0.52	1.8 (1.0)	1.9 (0.9)	1.9 (0.9)	1.8 (1.0)	2.0 (0.9)	1.8 (1.0)	1.7 (1.1)	1.9 (1.0)
No lesion seen (%)	94 (2.3)	47 (1.8)	47 (3.4)	**0.002**	25 (3.6)	22 (3.1)	37 (5.9)	20 (6.5)	17 (5.4)	0	0	0
Non-stroke lesion (%)	30 (0.7)	20 (0.8)	10 (0.7)	**0.009**	6 (0.9)	4 (0.6)	3 (0.5)	2 (0.6)	1 (0.3)	1 (0.7)	1 (1.4)	0

GTN: glyceryl trinitrate; LACS: lacunar syndrome; LACS & compatible scan: acute lacunar infarct or no acute infarct seen; SVD: small vessel disease. §=Lobar: borderzone, cerebellar or brainstem, ACA, PCA, and MCA excluding striatocapsular regions; Deep: lacunar, MCA including striatocapsular regions); ~= SVD score comprises severe anterior or posterior leukoaraiosis, any old lacunar infarcts, severe central or cortical atrophy (max 3/3). #=Brain frailty score comprises leukoaraiosis, cerebral atrophy, old vascular lesions (max 3/3). LACS vs. non-LACS assessed using X^2 for categorical variables and one-way analysis of variance (ANOVA) for continuous variables. Bold represents $p<0.05$.

Imaging and functional outcome

The associations between baseline imaging characteristics and mRS at day 90 were assessed in all participants (n=3995), in participants with LACS (n=1392), in LACS and a compatible scan (n=623) and LACS with acute lacunar infarction (n=143) (Table 6.3). In the whole population, visible infarction, cerebral atrophy and its severity, leukoaraiosis and its severity, old lacunar infarct/lacune, and old striatocapsular infarct were individually associated with unfavourable shifts in mRS at day 90. In LACS participants, parenchymal haemorrhage, cerebral atrophy and its severity, leukoaraiosis and its severity, old lacunar infarct/lacune, and old striatocapsular infarct were individually associated with unfavourable shifts in mRS at day 90. In those with LACS and a compatible scan, cerebral atrophy, moderate leukoaraiosis, old lacunar infarct/lacune, and old striatocapsular infarct were individually associated with unfavourable shifts in mRS at day 90. In the small population with LACS and acute lacunar infarction, no individual imaging features were associated with mRS at day 90.

SVD score was associated with mRS at day 90 in the whole ENOS population, those with LACS, and those with LACS and a compatible scan, with increasing SVD burden associated with unfavourable shifts in mRS at day 90. Increasing effect sizes were seen with increasing specificity of lacunar stroke syndrome: whole ENOS population OR 1.15, 95% CI 1.07-1.24; LACS OR 1.30, 95% CI 1.15-1.47; LACS and a compatible scan OR 1.43, 95% CI 1.19-1.72; LACS with acute

lacunar infarction OR 1.45, 95% CI 1.00-2.11. Hence, a one-point increase in SVD score was associated with increased odds of shift in the mRS to more death or dependency of 1.15, 1.30 and 1.43-1.45 (respectively) as the specificity of lacunar stroke diagnosis increased. Removing atrophy from the SVD score resulted in comparable associations with outcome (data not shown).

Increased 'brain frailty' features were associated with worse functional outcome at 90 days (Figure 6.1) in the whole ENOS population (OR 1.25, 95% CI 1.17-1.34), in LACS (OR 1.28, 95% CI 1.14-1.44), and LACS and a compatible scan (OR 1.29, 95% CI 1.08-1.53), but not in the small group of LACS with an acute lacunar infarct. Hence, a one-point increase in 'brain frailty' score was associated with a similar increased odds of shift to more death or dependency across the three populations.

MRI data were available for 109 LACS participants. Only old lacunar infarct/lacune was associated with an unfavourable shift in mRS at day 90 in this small population (Table 6.4).

Table 6.3: Associations between baseline imaging characteristics and primary outcome (mRS at day 90).

	Overall			LACS			LACS & compatible scan			LACS & acute lacunar infarct		
	n (%)	**OR (95% CI)**	**p**	**n (%)**	**OR (95% CI)**	**p**	**n (%)**	**OR (95% CI)**	**p**	**n (%)**	**OR (95% CI)**	**p**
n	3995	-	**-**	1392	-	-	623	-	-	143	-	-
Visible infarction	2041 (50.9)	1.14 (1.01, 1.28)	**0.030**	658 (47.1)	1.17 (0.94, 1.45)	0.15	144 (24.1)	0.98 (0.69, 1.38)	0.91	143 (100)	-	-
Lacunar	241 (6.4)	0.83 (0.66, 1.05)	0.13	143 (11.0)	0.83 (0.60, 1.13)	0.24	143 (24.0)	0.97 (0.68, 1.37)	0.85	143 (100)	-	-
Thalamus	20 (0.5)	1.15 (0.52, 2.51)	0.73	12 (0.9)	1.18 (0.43, 3.28)	0.75	12 (2.0)	1.39 (0.50, 3.89)	0.53	12 (8.4)	1.64 (0.55, 4.50)	0.37
Centrum semiovale	67 (1.8)	1.00 (0.65, 1.54)	0.99	41 (3.1)	0.88 (0.50, 1.53)	0.64	41 (6.9)	1.01 (0.57, 1.78)	0.98	41 (28.7)	1.02 (0.53, 1.96)	0.95
Internal capsule/ lentiform	121 (3.2)	0.73 (0.53, 1.01)	0.057	69 (5.7)	0.72 (0.47, 1.11)	0.14	69 (11.6)	0.83 (0.53, 1.31)	0.43	69 (48.3)	0.74 (0.41, 1.36)	0.33
Internal border zone	5 (0.1)	0.77 (0.16, 3.70)	0.75	2 (0.2)	1.42 (0.12, 16.98)	0.78	2 (0.3)	1.66 (0.14, 20.09)	0.06	2 (1.4)	1.24 (0.10, 16.25)	0.87
Visible haemorrhage	673 (16.8)	1.10 (0.95, 1.29)	0.20	197 (14.1)	1.22 (0.93, 1.61)	0.15	0	-	-	0	-	-
Parenchymal haemorrhage	587 (14.6)	1.11 (0.95, 1.31)	0.19	184 (14.1)	1.38 (1.01, 1.89)	**0.041**	0	-	-	0	-	-
Lobar or cerebellar	79 (2.1)	1.46 (0.98, 2.18)	0.07	25 (1.9)	0.84 (0.41, 1.71)	0.63	0	-	-	0	-	-
Deep	507 (13.4)	1.06 (0.89, 1.25)	0.52	158 (12.1)	1.34 (0.99, 1.82)	0.057	0	-	-	0	-	-

Cerebral atrophy score (/4), median [IQR]	2 [2]	1.14 (1.09, 1.21)	**<0.001**	2 [2]	1.23 (1.13, 1.35)	**<0.001**	2 [2]	1.16 (1.02, 1.32)	**0.028**	2 [3]	1.12 (0.88, 1.42)	0.83
Leukoaraiosis score (/4), median [IQR]	0 [2]	1.11 (1.07, 1.16)	**<0.001**	0 [2]	1.11 (1.03, 1.19)	**0.007**	0 [2]	1.10 (0.98, 1.23)	0.11	1 [2]	1.15 (0.91, 1.45)	0.24
Old infarcts												
Striatocapsular	585 (15.2)	1.29 (1.10, 1.51)	**0.002**	210 (15.8)	1.51 (1.15, 1.97)	**0.003**	114 (18.3)	1.82 (1.25, 2.64)	**0.002**	14 (9.8)	1.69 (0.62, 4.65)	0.31
Borderzone	74 (1.9)	1.35 (0.89, 2.04)	0.15	26 (2.0)	0.69 (0.35, 1.39)	0.31	9 (1.4)	0.52 (0.16, 1.71)	0.28	4 (2.8)	0.26 (0.04, 1.65)	0.15
Lacunar	1423 (37.0)	1.19 (1.06, 1.35)	**0.003**	514 (38.6)	1.35 (1.11, 1.65)	**0.003**	249 (40.0)	1.70 (1.26, 2.29)	**0.001**	68 (47.6)	1.43 (0.77, 2.64)	0.26
At least 1 of above	1619 (42.1)	1.21 (1.08, 1.36)	**0.001**	588 (44.1)	1.35 (1.11, 1.65)	**0.003**	284 (45.6)	1.76 (1.31, 2.36)	**<0.001**	71 (49.7)	1.31 (0.71, 2.40)	0.38
SVD score (/3), median [IQR]	1 [2]	1.15 (1.07, 1.24)	**<0.001**	1 [2]	1.30 (1.15, 1.47)	**<0.001**	1 [2]	1.43 (1.19, 1.72)	**<0.001**	1 [2]	1.45 (1.00, 2.11)	0.051
Brain frailty score (/3), median [IQR]	2 [2]	1.25 (1.17, 1.34)	**<0.001**	2 [2]	1.28 (1.14, 1.44)	**<0.001**	2 [2]	1.29 (1.08, 1.53)	**0.005**	2 [2]	1.16 (0.84, 1.60)	0.35
No lesion seen	94 (2.4)	1.10 (0.76, 1.60)	0.61	47 (3.5)	0.74 (0.43, 1.27)	0.28	37 (5.9)	0.74 (0.39, 1.39)	0.35	0	-	-

The OR represents an ordinal logistic regression analysis of shift in the mRS with adjustment for age, sex, baseline SSS, time to randomisation. CI: confidence interval; LACS: lacunar syndrome; mRS: modified Rankin Scale; OR: odds ratio; SD: standard deviation; SSS: Scandinavian Stroke Scale; SVD: small vessel disease. Data are n (%) or median (interquartile range [IQR]). SVD score comprises severe anterior or posterior leukoaraiosis, any old lacunar infarcts, severe central or cortical atrophy (max 3/3). Brain frailty score comprises leukoaraiosis, cerebral atrophy, old vascular lesions (max 3/3).

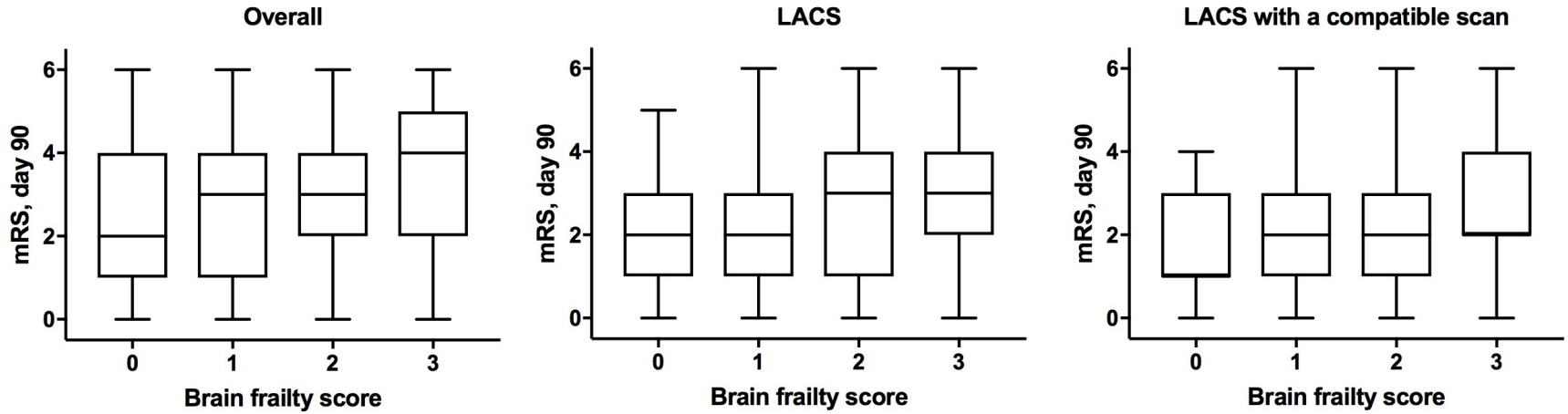


Figure 6.1: mRS at day 90 by 'Brain frailty' score on baseline imaging

Boxplots of mRS at day 90 by 'brain frailty' score in the whole ENOS population (n=3995), in LACS (n=1392) and LACS with compatible scan participants (n=623).

Table 6.4: Associations between baseline imaging characteristics and primary outcome (mRS at day 90) in participants with MR imaging

	Overall			Non-LACS			LACS		
	n (%)	**OR (95% CI)**	**p**	**n (%)**	**OR (95% CI)**	**p**	**n (%)**	**OR (95% CI)**	**p**
n (%)	267			158			109		
Time to scan (hrs)	72.2 (78.0)			72.4 (80.8)			71.9 (74.0)		
Visible infarction	218 (81.6)	0.87 (0.34, 2.19)	0.76	132 (83.5)	0.82 (0.23, 2.88)	0.76	86 (78.9)	1.12 (0.28, 4.50)	0.87
Lacunar	95 (35.7)	1.22 (0.76, 1.94)	0.41	44 (28.0)	1.57 (0.82, 3.01)	0.18	51 (46.8)	0.88 (0.44, 1.75)	0.71
Thalamus	5 (1.9)	0.88 (0.17, 4.46)	0.88	2 (1.3)	0.29 (0.02, 3.87)	0.35	3 (2.8)	1.18 (0.14, 9.81)	0.88
Centrum semiovale	36 (13.5)	1.11 (0.59, 2.11)	0.74	16 (10.2)	1.50 (0.58, 3.87)	0.40	20 (18.3)	0.81 (0.34, 1.96)	0.64
Internal capsule/ lentiform	47 (17.7)	1.10 (0.62, 1.95)	0.75	22 (14.0)	1.55 (0.67, 3.59)	0.31	25 (22.9)	0.84 (0.37, 1.88)	0.67
Internal border zone	4 (1.5)	3.40 (0.58, 20.03)	0.18	2 (1.3)	5.41 (0.43, 67.56)	0.19	2 (1.8)	2.11 (0.16, 28.33)	0.57
Visible haemorrhage	40 (15.0)	0.69 (0.37, 1.30)	0.25	20 (12.7)	0.75 (0.30, 1.84)	0.53	20 (18.3)	0.73 (0.29, 1.80)	0.49
Parenchymal haemorrhage	32 (12.0)	0.69 (0.15, 3.23)	0.50	16 (10.1)	0.69 (0.15, 3.23)	0.64	16 (14.7)	1.09 (0.21, 5.58)	0.92
Cerebral atrophy score (/4), median [IQR]	2 [2]	0.94 (0.78, 1.13)	0.51	2 [2]	0.92 (0.73, 1.17)	0.51	2 [1]	0.93 (0.68, 1.29)	0.67

Leukoaraiosis score (/4), median [IQR]	1 [2]	1.06 (0.90, 1.26)	0.49	1 [2]	1.16 (0.93, 1.45)	0.19	1 [2]	0.95 (0.72, 1.25)	0.72
Old infarcts									
Striatocapsular	10 (3.7)	2.48 (0.80, 7.72)	0.12	5 (3.2)	1.83 (0.37, 9.03)	0.46	5 (4.6)	3.68 (0.72, 18.90)	0.12
Borderzone	26 (9.7)	1.36 (0.65, 2.85)	0.41	18 (11.4)	1.53 (0.62, 3.76)	0.36	8 (7.3)	1.04 (0.28, 3.92)	0.95
Lacunar	86 (32.2)	1.61 (1.01, 2.56)	**0.046**	54 (34.2)	1.30 (0.71, 2.37)	0.39	32 (29.4)	2.44 (1.14, 5.23)	**0.022**
At least 1 of above	102 (38.2)	1.46 (0.94, 2.29)	0.10	63 (39.9)	1.33 (0.74, 2.89)	0.34	39 (35.8)	1.86 (0.90, 3.83)	0.09
SVD score (/3), median [IQR]	1 [1]	1.21 (0.93, 1.58)	0.15	1 [1]	1.19 (0.84, 1.69)	0.32	0 [1]	1.26 (0.93, 1.48)	0.29
Brain frailty score (/3), median [IQR]	2 [2]	1.18 (0.93, 1.48)	0.17	2 [2]	1.23 (0.90, 1.68)	0.19	2 [2]	1.13 (0.79, 1.62)	0.51
No lesion seen	4 (1.5)	1.48 (0.25, 8.81)	0.67	3 (1.9)	0.90 (0.11, 7.68)	0.93	1 (0.9)	1.54 90.04, 53.09)	0.81

CI: confidence interval; LACS: lacunar syndrome; mRS: modified Rankin Scale; OR: odds ratio; SVD: small vessel disease. Ordinal logistic regression with adjustment for age, gender, baseline SSS, time to randomisation. SVD score comprises severe anterior or posterior leukoaraiosis, any old lacunar infarcts, severe central or cortical atrophy (max 3/3). Brain frailty score comprises leukoaraiosis, cerebral atrophy, old vascular lesions (max 3/3).

Imaging and cognitive outcomes

Day 90 cognitive outcomes were available in about half of the overall population: t-MMSE in 1949 (49%, Table 6.5); TICS-M in 1930 (48%, Table 6.6); and verbal fluency in 2269 (57%, Table 6.7). Participants alive with no cognitive data at 90 days were older (73 [16] vs. 71 [18] years) with more severe strokes (baseline mean SSS 35 vs. 32, $p<0.001$) than those with at least one telephone cognitive assessment. Overall, visible infarction, cerebral atrophy score and its severity, and leukoaraiosis and its severity were independently associated with worse cognitive scores on t-MMSE and TICS-M at 90 days (Figures 6.2 and 6.3). In addition to those imaging features, acute lacunar infarction, parenchymal and deep haemorrhage, and old lacunar infarct/lacune were each independently associated with worse verbal fluency scores at 90 days. 'Brain frailty' was associated with worse scores on all three cognitive measures (Figure 6.4), whilst SVD score was associated with poorer verbal fluency only (Figure 6.5).

In participants with LACS, leukoaraiosis and its severity was associated with worse cognitive scores on all three measures, whilst cerebral atrophy and its severity was associated with worse scores on TICS-M and verbal fluency, but the association with t-MMSE did not reach statistical significance. In addition, deep parenchymal haemorrhage and old lacunar infarct/lacune were independently associated with poorer verbal fluency scores only. 'Brain frailty' score was associated with worse cognitive outcomes across all three domains, whilst SVD score was not (Tables 6.5, 6.6 and 6.7).

In those with LACS and a compatible scan, leukoaraiosis and its severity was associated with worse scores across all three cognitive measures, whilst old lacunar infarct/lacune was associated with worse verbal fluency scores only. 'Brain frailty' score was associated with worse t-MMSE and TICS-M scores but was not significantly associated with verbal fluency. SVD scores were not associated with cognitive outcomes in this small population.

There were insufficient data to perform analyses on cognitive outcomes in those with LACS and an acute lacunar infarction (n=60).

Table 6.5: Associations between baseline imaging characteristics and t-MMSE at day 90.

	Overall			LACS			LACS & compatible scan		
	n (%)	**MD (95% CI)**	**p**	**n (%)**	**MD (95% CI)**	**p**	**n (%)**	**MD (95% CI)**	**p**
n	1949	-	**-**	719	-	-	313	-	-
Visible infarction	996 (51.1)	-0.75 (-1.29, -0.22)	**0.006**	327 (47.3)	-0.24 (-1.06, 0.58)	0.56	39 (12.6)	-0.35 (-2.23, 1.53)	0.71
Lacunar	81 (4.2)	-0.70 (-2.06, 0.67)	0.32	39 (5.6)	0.06 (-1.84, 1.96)	0.95	39 (12.6)	-0.35 (-2.23, 1.53)	0.71
Thalamus	4 (0.2)	-0.15 (-6.80, 6.50)	0.97	2 (0.3)	-0.40 (-7.79, 6.99)	0.92	2 (0.6)	-0.74 (-7.82, 6.33)	0.84
Centrum semiovale	27 (1.4)	-1.01 (-3.25, 1.23)	0.38	16 (2.3)	1.15 (-1.58, 3.87)	0.41	16 (5.2)	0.86 (-1.79, 3.51)	0.52
Internal capsule/ lentiform	41 (2.1)	-0.16 (-2.44, 2.13)	0.89	20 (2.9)	0.27 (-2.67, 3.20)	0.86	20 (6.5)	-0.11 (-2.94, 2.72)	0.94
Internal border zone	3 (0.2)	0.68 (-6.38, 7.73)	0.85	0	-	-	0	-	-
Visible haemorrhage	336 (17.1)	-0.61 (-1.34, 0.13)	0.11	90 (12.9)	-0.95 (-2.20, 0.30)	0.64	0	-	-
Parenchymal haemorrhage	296 (15.2)	-0.42 (-1.20, 0.36)	0.29	84 (12.2)	-0.62 (-1.91, 0.66)	0.34	0	-	-
Lobar or cerebellar	52 (2.7)	-0.05 (-1.67, 1.57)	0.95	15 (2.2)	1.03 (-1.85, 3.92)	0.48	0	-	-
Deep	243 (12.5)	-0.42 (-1.30, 0.45)	0.34	68 (9.8)	-1.08 (-2.50, 0.33)	0.13	0	-	-
Cerebral atrophy score (/4), median [IQR]	2 [2]	-	**0.006**	2 [2]	-	0.14	2 [1]	-	0.82
Leukoaraiosis score (/4), median [IQR]	0 [2]	-	**<0.001**	0 [2]	-	**0.001**	0 [2]	-	**0.014**
Old infarcts									
Striatocapsular	316 (16.1)	0.31 (-0.42, 1.04)	0.41	129 (18.6)	0.07 (-1.01, 1.15)	0.90	66 (21.1)	-0.69 (-2.21, 0.82)	0.37
Borderzone	35 (1.8)	-0.72 (-2.80, 1.35)	0.49	11 (1.6)	-0.94 (-4.27, 2.39)	0.58	3 (1.0)	0.40 (-5.52, 6.32)	0.89
Lacunar	764 (38.8)	-0.26 (-0.81, 0.29)	0.36	282 (40.6)	0.03 (-0.84, 0.90)	0.95	123 (39.3)	0.16 (-1.16, 1.47)	0.82
At least 1 of above	859 (43.7)	-0.14 (-0.68, 0.41)	0.62	323 (46.5)	-0.05 (-0.90, 0.80)	0.91	145 (46.3)	-0.58 (-1.86, 0.69)	0.37

SVD score (/3), median [IQR]	1 [2]	-	0.28	1 [2]	-	0.76	1 [1]	-	0.95
Brain frailty score (/3), median [IQR]	2 [2]	-	**0.001**	2 [2]	-	**0.020**	2 [2]	-	**0.011**
No lesion seen	42 (2.1)	0.30 (-1.57, 2.18)	0.75	17 (2.4)	0.54 (-2.12, 3.19)	0.69	15 (4.8)	0.62 (-2.22, 3.46)	0.67

Multiple linear regression of mean difference (MD) between t-MMSE scores with adjustment for age, sex, baseline SSS, time to randomisation. CI: confidence interval; LACS: lacunar syndrome; MD: mean difference; SD: standard deviation; SSS: Scandinavian Stroke Scale; SVD: small vessel disease; t-MMSE: telephone mini-mental state examination. Data are mean (standard deviation [SD]). SVD score comprises severe anterior or posterior leukoaraiosis, any old lacunar infarcts, severe central or cortical atrophy (max 3/3). Brain frailty score comprises leukoaraiosis, cerebral atrophy, old vascular lesions (max 3/3).

Table 6.6: Associations between baseline imaging characteristics and TICS-M at day 90

	Overall			LACS			LACS & compatible scan		
	n (%)	**MD (95% CI)**	**p**	**n (%)**	**MD (95% CI)**	**p**	**n (%)**	**MD (95% CI)**	**p**
n	1930	-	**-**	686	-	-	308	-	-
Visible infarction	987 (51.1)	-0.84 (-1.60, -0.08)	**0.031**	326 (47.5)	-0.23 (-1.45, 0.98)	0.71	38 (12.3)	0.88 (-1.98, 3.73)	0.55
Lacunar	80 (4.1)	-0.57 (-2.51, 1.38)	0.57	38 (5.5)	1.20 (-1.62, 4.02)	0.41	38 (12.3)	0.88 (-1.98, 3.73)	0.55
Thalamus	4 (0.2)	0.35 (-9.09, 9.80)	0.94	2 (0.3)	0.18 (-10.74, 11.09)	0.98	2 (0.6)	-0.17 (-10.84, 10.50)	0.98
Centrum semiovale	28 (1.5)	-0.66 (-3.79, 2.46)	0.68	16 (2.3)	2.12 (-1.90, 6.15)	0.30	16 (5.2)	1.88 (-2.11, 5.88)	0.36
Internal capsule/ lentiform	39 (2.0)	0.52 (-2.75, 3.78)	0.76	19 (2.8)	2.45 (-1.90, 6.81)	0.27	19 (6.2)	2.14 (-2.16, 6.43)	0.33
Internal border zone	3 (0.2)	-0.88 (-10.89, 9.14)	0.86	0	-	-	0	-	-
Visible haemorrhage	331 (17.0)	-0.72 (-1.77, 0.33)	0.18	88 (12.8)	-0.55 (-2.40, 1.31)	0.56	0	-	-
Parenchymal haemorrhage	292 (15.1)	-0.35 (-1.46, 0.76)	0.53	82 (12.0)	-0.11 (-2.03, 1.80)	0.91	0	-	-
Lobar or cerebellar	52 (2.7)	0.70 (-1.60, 2.99(	0.55	15 (2.2)	1.53 (-2.74, 5.80)	0.48	0	-	-
Deep	239 (12.4)	-0.56 (-1.80, 0.68)	0.38	66 (9.6)	-0.66 (-2.78, 1.45)	0.54	0	-	-
Cerebral atrophy score (/4), median [IQR]	2 [2]	-	**0.001**	2 [2]	-	**0.015**	2 [1]	-	0.56
Leukoaraiosis score (/4), median [IQR]	0 [2]	-	**<0.001**	0 [2]	-	**0.001**	0 [2]	-	**0.035**

Old infarcts									
Striatocapsular	310 (15.9)	0.29 (-0.76, 1.33)	0.59	126 (18.3)	-0.42 (-2.09, 1.19)	0.61	64 (20.6)	-1.41 (-3.71, 0.89)	0.23
Borderzone	35 (1.8)	-0.79 (-3.73, 2.15)	0.60	11 (1.6)	-1.89 (-6.79, 3.01)	0.45	3 (1.0)	1.03 (-7.85, 9.91)	0.82
Lacunar	758 (38.9)	-0.55 (-1.33, 0.24)	0.17	279 (40.4)	-0.52 (-1.81, 0.77)	0.43	121 (38.9)	-0.14 (-2.13, 1.86)	0.89
At least 1 of above	850 (43.6)	-0.38 (-1.16, 0.39)	0.33	319 (46.2)	-0.54 (-1.80, 0.72)	0.40	143 (46.0)	-0.85 (-2.78, 1.07)	0.38
SVD score (/3), median [IQR]	1 [2]	-	0.20	1 [2]	-	0.21	1 [1]	-	0.93
Brain frailty score (/3), median [IQR]	2 [2]	-	**<0.001**	2 [2]	-	**0.001**	2 [2]	-	**0.003**
No lesion seen	41 (2.1)	0.23 (-2.46, 2.91)	0.87	17 (2.5)	0.77 (-3.14, 4.68)	0.70	15 (4.8)	1.09 (-3.17, 5.35)	0.62

CI: confidence interval; LACS: lacunar syndrome; MD: mean difference; SD: standard deviation; SSS: Scandinavian Stroke Scale; SVD: small vessel disease; TICS-M: modified telephone interview for cognitive status. Data are mean (standard deviation [SD]). Multiple linear regression with adjustment for age, sex, baseline SSS, time to randomisation. SVD score comprises severe anterior or posterior leukoaraiosis, any old lacunar infarcts, severe central or cortical atrophy (max 3/3). Brain frailty score comprises leukoaraiosis, cerebral atrophy, old vascular lesions (max 3/3).

Table 6.7: Associations between baseline imaging characteristics and verbal fluency at day 90.

	Overall			LACS			LACS & compatible scan		
	n (%)	**MD (95% CI)**	**p**	**n (%)**	**MD (95% CI)**	**p**	**n (%)**	**MD (95% CI)**	**p**
n	2269	-	-	818	-	-	366	-	-
Visible infarction	1176 (51.8)	-0.72 (-1.29, -0.15)	**0.013**	395 (48.3)	-0.79 (-1.82, 0.23)	0.13	60 (16.4)	-1.97 (-4.11, 0.17)	0.07
Lacunar	118 (5.2)	-1.32 (-2.60, -0.04)	**0.043**	60 (7.3)	-1.13 (-3.09, 0.84)	0.26	60 (16.4)	-1.97 (-4.11, 0.17)	0.07
Thalamus	8 (0.4)	-0.37 (-7.43, 6.70)	0.92	5 (0.6)	-0.96 (-8.94, 7.02)	0.81	5 (1.4)	-1.15 (-9.40, 7.10)	0.78
Centrum semiovale	38 (1.7)	-1.72 (-3.89, 0.45)	0.12	22 (2.7)	-0.43 (-3.52, 2.66)	0.79	22 (6.0)	-1.02 (-4.26, 2.22)	0.54
Internal capsule/ lentiform	61 (2.7)	-1.09 (-3.03, 0.86)	0.27	31 (3.8)	-0.82 (-3.68, 2.04)	0.57	31 (8.5)	-1.66 (-4.67, 1.35)	0.28
Internal border zone	4 (0.2)	-0.69 (-8.32, 6.93)	0.86	1 (0.1)	-6.00 (-20.12, 8.21)	0.41	1 (0.3)	-6.12 (-20.71, 8.47)	0.41
Visible haemorrhage	406 (17.7)	-0.97 (-1.73, -0.21)	**0.013**	115 (14.0)	-1.39 (-2.88, 0.10)	0.07	0	-	-
Parenchymal haemorrhage	361 (15.9)	-0.87 (-1.67, -0.07)	**0.033**	108 (13.2)	-1.28 (-2.81, 0.26)	0.10	0	-	-
Lobar or cerebellar	57 (2.5)	1.15 (-0.62, 2.92)	0.20	16 (2.0)	2.35 (-1.51, 6.20)	0.23	0	-	-
Deep	303 (13.4)	-1.22 (-2.10, -0.34)	**0.006**	91 (11.1)	-2.00 (-3.65, -0.36)	**0.017**	0	-	-
Cerebral atrophy score (/4), median [IQR]	2 [2]	-	**<0.001**	2 [2]	-	**0.006**	2 [2]	-	0.21
Leukoaraiosis score (/4), median [IQR]	0 [2]	-	**<0.001**	0 [2]	-	**<0.001**	0 [2]	-	**0.009**
Old infarcts									

Striatocapsular	347 (15.2)	-0.34 (-1.13, 0.45)	0.39	139 (16.9)	-0.11 (-1.49, 1.26)	0.87	71 (19.2)	-0.81 (-2.87, 1.24)	0.44
Borderzone	42 (1.8)	-1.49 (-3.66, 0.69)	0.18	14 (1.7)	-4.04 (-8.03, -0.06)	**0.047**	5 (1.4)	-5.12 (-11.69, 1.45)	0.13
Lacunar	867 (37.9)	-0.95 (-1.54, -0.37)	**0.001**	322 (39.1)	-1.16 (-2.23, -0.08)	**0.035**	142 (38.4)	-1.94 (-3.65, -0.23)	**0.026**
At least 1 of above	968 (42.3)	-0.84 (-1.42, -0.27)	**0.004**	365 (44.3)	-1.02 (-2.07, 0.03)	0.058	165 (44.6)	-1.96 (-3.62, -0.29)	**0.022**
SVD score (/3), median [IQR]	1 [2]	-	**0.013**	1 [2]	-	0.11	1 [1]	-	0.46
Brain frailty score (/3), median [IQR]	2 [2]	-	**<0.001**	2 [2]	-	**0.003**	2 [2]	-	0.08
No lesion seen	53 (2.3)	-0.09 (-2.01, 1.83)	0.93	23 (2.8)	2.28 (-0.86, 5.41)	0.15	21 (5.7)	0.73 (-2.73, 4.18)	0.68

Multiple linear regression of mean difference (MD) in scores, with adjustment for age, sex, baseline SSS, time to randomisation. CI: confidence interval; LACS: lacunar syndrome; MD: mean difference; SD: standard deviation; SSS: Scandinavian Stroke Scale; SVD: small vessel disease. Data are mean (standard deviation [SD]). SVD score comprises severe anterior or posterior leukoaraiosis, any old lacunar infarcts, severe central or cortical atrophy (max 3/3). Brain frailty score comprises leukoaraiosis, cerebral atrophy, old vascular lesions (max 3/3).

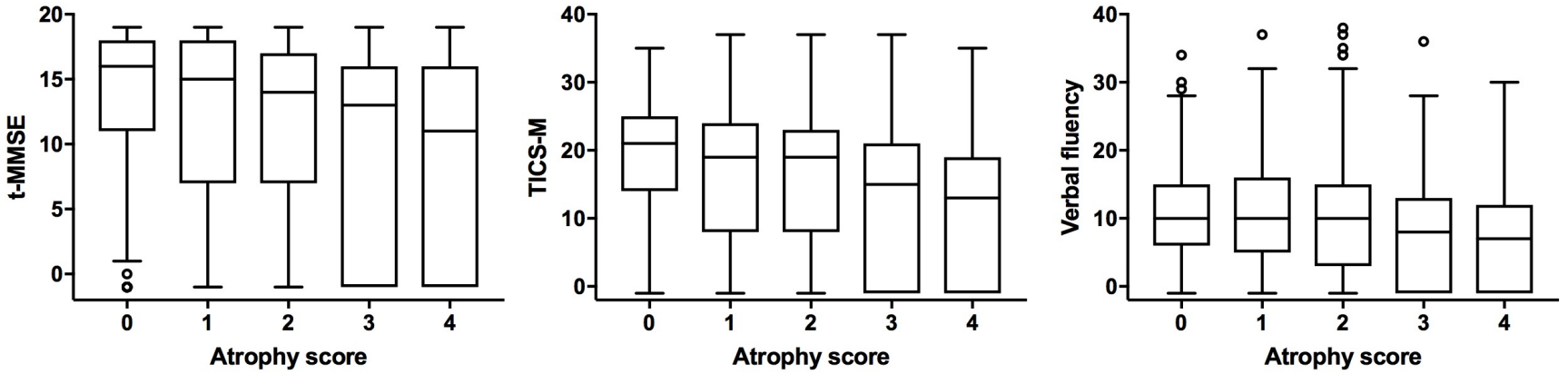


Figure 6.2: Cognitive scores at day 90 by atrophy score on baseline imaging

Boxplots of cognitive scores (t-MMSE: n=1949 , TICS-M: n=1930, verbal fluency: n=2269) at day 90 by atrophy score in the whole ENOS population.

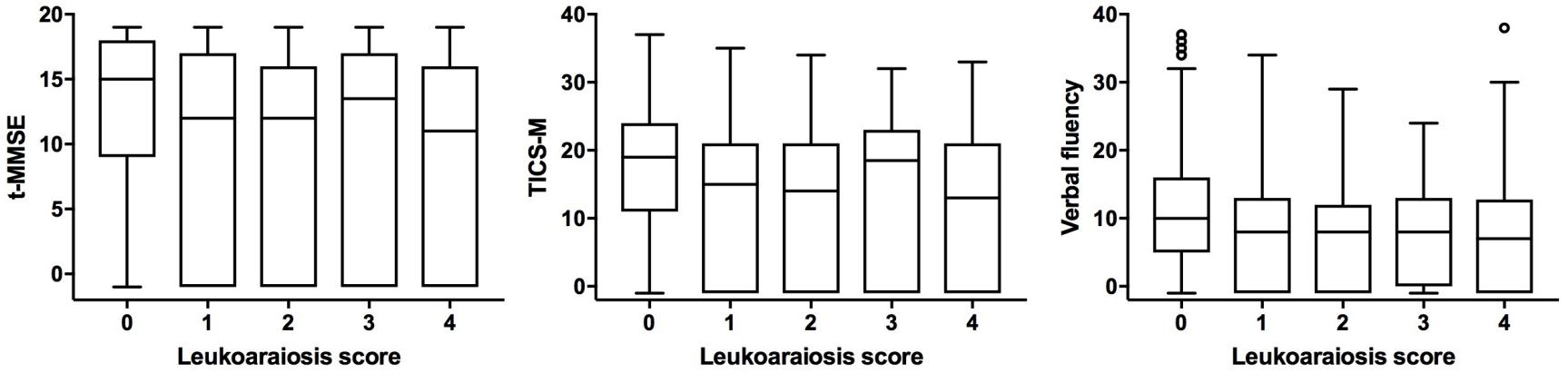


Figure 6.3: Cognitive scores at day 90 by leukoaraiosis score on baseline imaging

Boxplots of cognitive scores (t-MMSE: n=1949 , TICS-M: n=1930, verbal fluency: n=2269) at day 90 by leukoaraiosis score in the whole ENOS population.

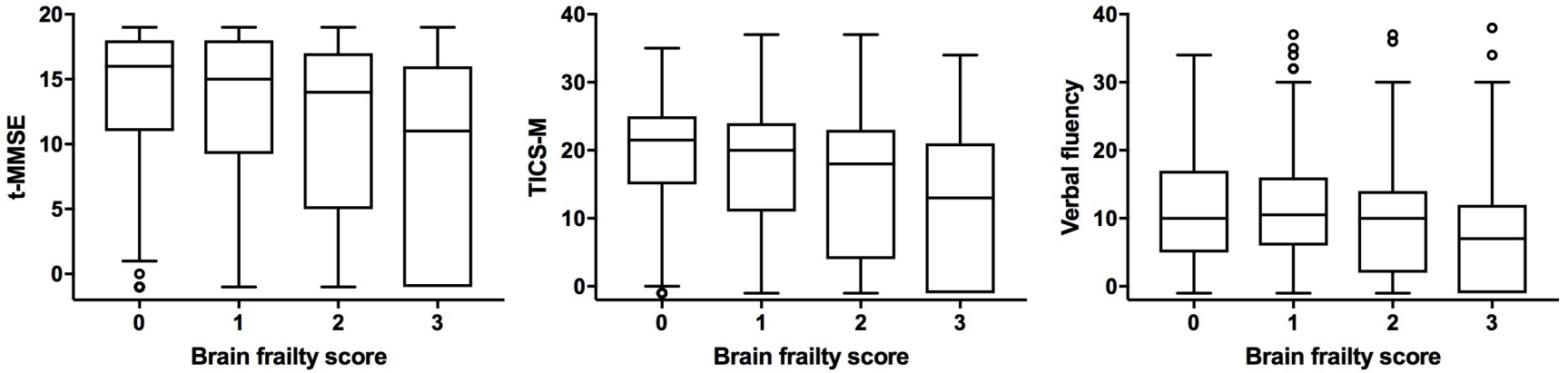


Figure 6.4: Cognitive scores at day 90 by 'Brain frailty' score on baseline imaging

Boxplots of cognitive scores (t-MMSE: n=1949 , TICS-M: n=1930, verbal fluency: n=2269) at day 90 by 'brain frailty' in the whole ENOS population.

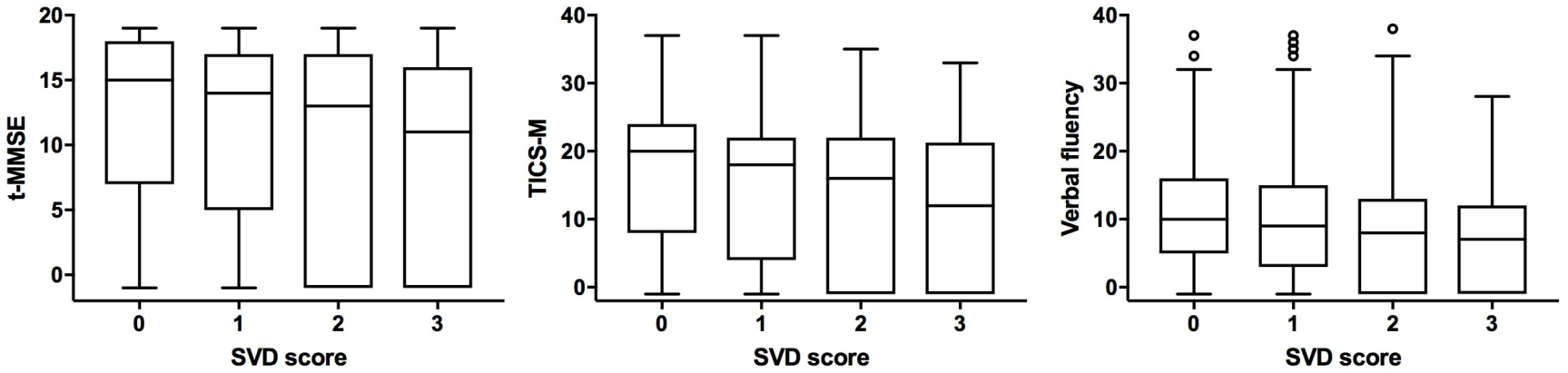


Figure 6.5: Cognitive scores at day 90 by SVD score on baseline imaging

Boxplots of cognitive scores (t-MMSE: n=1949 , TICS-M: n=1930, verbal fluency: n=2269) at day 90 by SVD score in the whole ENOS population.

GTN and lacunar stroke

Within LACS and non-LACS, baseline characteristics were well balanced between GTN vs. no GTN (Table 6.1). GTN had no effect on mRS at day 90 compared with no GTN in those with LACS (n=1392), LACS and compatible scan (n=623), or LACS and acute lacunar infarction (n=143) (Table 6.8). In those with LACS and an acute lacunar infarction, SSS at day 7 was significantly higher in those randomised to GTN vs. no GTN, indicating less neurological impairment. However, there was no difference between randomised groups in neurological deterioration by day 7. In those with LACS, symptomatic on-treatment hypotension was more common in those randomised to GTN vs. no GTN. GTN within 6 hours did not change any day 90 clinical outcomes in lacunar stroke populations (Table 6.9). Further, in 339 LACS participants GTN did not influence imaging markers at day 7 (data not shown).

Table 6.8: Outcomes by GTN vs. no GTN across the trial

	Overall	LACS				LACS & compatible scan				LACS & acute LACI			
		GTN	No GTN	OR/MD (95% CI)	p	GTN	No GTN	OR/MD (95% CI)	p	GTN	No GTN	OR/MD (95% CI)	p
Number	4011	695	702			308	315			71	72		
Primary outcome		693	699			308	315			71	72		
mRS (/6), ordinal	3 [2]	2 [3]	2 [2]	1.07 (0.88, 1.29)	0.51	2 [2]	2 [2]	1.09 (0.82, 1.45)	0.54	2 [2]	2 [2]	1.00 (0.53, 1.87)	0.99
Day 7 (or Discharge)													
Scandinavian Stroke Scale (SSS, /58)	38.8 (16.1)	46.6 (10.2)	46.5 (10.2)	-0.11 (-0.89, 0.67)	0.79	48.0 (9.6)	47.5 (9.9)	0.30 (-0.91, 1.50)	0.63	47.9 (8.8)	44.5 (12.6)	3.21 (0.22, 6.20)	**0.035**
Neurological deterioration (%)	254 (6.4)	29 (4.2)	21 (3)	1.33 (0.75, 2.38)	0.33	10 (3.2)	9 (2.9)	1.12 (0.42, 3.00)	0.82	2 (2.8)	5 (6.9)	0.01 (0.0, 1.23)	0.062
Headache (%)	530 (13.3)	154 (22.2)	48 (6.8)	3.96 (2.79, 5.62)	**<0.001**	64 (20.8)	23 (7.3)	3.30 (1.97, 5.55)	**<0.001**	17 (23.9)	3 (4.2)	11.57 (2.90, 46.24)	**0.001**
Hypotension (%)	68 (1.7)	16 (2.3)	2 (0.3)	8.00 (1.81, 35.51)	**0.006**	7 (2.3)	0	-	-	4 (5.6)	0	-	-
Day 90													
Death (%)	496 (12.4)	29 (4.2)	37 (5.3)	0.79 (0.47, 1.34)	0.38	10 (3.2)	15 (4.8)	0.79 (0.33, 1.86)	0.59	1 (1.4)	3 (4.2)	-	-
Barthel Index	64.4 (39.4)	79.7 (28.5)	78.4 (30)	0.54 (-2.15, 3.24)	0.69	82.8 (25.6)	81.5 (28.7)	0.35 (-3.61, 4.31)	0.86	85.5 (24.3)	78.4 (29.7)	6.00 (-2.19, 14.11)	0.15
Zung Depression Scale (ZDS, /100)	58.5 (24.1)	51.7 (19.3)	51.1 (20)	0.9 (-1.22, 3.0)	0.41	50.1 (18.7)	50.8 (19.6)	-0.26 (-3.46, 2.93)	0.87	49.8 (19.1)	48.7 (20.1)	3.25 (-4.03, 10.54)	0.38

EQ-5D Health Utility Status (HUS, /1)	0.46 (0.4)	0.58 (0.34)	0.59 (0.36)	-0.01 (-0.04, 0.02)	0.54	0.63 (0.33)	0.62 (0.35)	0.01 (-0.04, 0.06)	0.82	0.66 (0.33)	0.57 (0.38)	0.07 (-0.04, 0.18)	0.23
EQ-Visual Analogue Scale (EQ-VAS, /100)	56.1 (31.2)	64.2 (26)	64 (25.4)	-0.06 (-2.72, 2.6)	0.97	65.0 (26.1)	65.4 (24.8)	-0.43 (-4.41, 3.54)	0.83	67.5 (22.2)	62.8 (24.6)	2.54 (-6.21, 11.29)	0.57
Verbal Fluency	9.3 (7.8)	12.1 (7.5)	11.7 (7.3)	0.27 (-0.69, 1.23)	0.58	12.7 (7.4)	12.4 (7.7)	-0.06 (-1.61, 1.48)	0.94	11.7 (7.0)	9.8 (6.5)	1.21 (-2.93, 5.35)	0.56
TICS-M	14.7 (10.7)	19.2 (8.2)	18.3 (8.9)	0.57 (-0.59, 1.73)	0.33	19.7 (8.0)	18.3 (8.6)	0.59 (-1.17, 2.35)	0.51	22.7 (9.0)	15.9 (10.0)	4.42 (-2.70, 11.54)	0.21
t-MMSE	11 (7.6)	14.3 (5.4)	13.7 (6)	0.30 (-0.47, 1.08)	0.45	14.7 (4.9)	14.0 (5.9)	0.08 (-1.09, 1.24)	0.90	15.0 (4.7)	12.6 (7.0)	0.91 (-3.29, 5.11)	0.66

Data are n (%), mean (SD), median [interquartile range], mean difference (MD) or odds ratio (OR) with 95% confidence intervals. Comparison using logistic regression, multiple regression or ordinal regression. Adjusted for age, gender, baseline mRS, history of previous stroke, history of diabetes mellitus, final diagnosis, nitrate use, baseline SSS, thrombolysis, feeding status, time to randomisation, baseline SBP, continue/stop. CI: confidence interval; EQ-5D: European quality of life 5 dimensions; GTN: glyceryl trinitrate; LACS: lacunar syndrome; MD: mean difference; mRS: modified Rankin Scale; OR: odds ratio; SSS: Scandinavian stroke scale; t-MMSE: telephone mini-mental state examination; TICS-M: modified telephone interview for cognitive status.

Table 6.9: Outcomes by GTN vs. no GTN within 6 hours of randomisation

	Overall	LACS				LACS & compatible scan			
		GTN	No GTN	OR/MD (95% CI)	p	GTN	No GTN	OR/MD (95% CI)	p
Number of Participants	273	37	25			14	9		
Primary outcome	273	37	25			14	9		
mRS, ordinal (/6)	3 [3]	2 [2]	2 [2]	0.73 (0.25, 2.16)	0.57	2 [3]	2 [2]	0.26 (0.01, 7.06)	0.43
Day 7 (or discharge)									
SSS, /58	38.7 (17.5)	44.3 (12.5)	46.8 (11.5)	-6.20 (-5.78, 4.54)	0.81	49.4 (6.7)	50.8 (7.7)	5.76 (-4.04, 15.56)	0.21
Neurological deterioration (%)	27 (10.0)	0 (0)	0 (0)	-	-	0 (0)	0 (0)	-	-
Headache (%)	37 (13.7)	7 (18.9)	0 (0)	-	-	3 (21.4)	0 (0)	-	-
Hypotension (%)	9 (3.3)	0 (0)	0 (0)	-	-	0 (0)	0 (0)	-	-
Day 90									
Death (%)	37 (13.6)	1 (2.7)	1 (4)	0.00	1.00	0	0	-	-
Barthel Index	67.2 (38.2)	81.2 (29.1)	80.4 (30.4)	2.56 (-11.61, 16.73)	0.72	88.9 (15.3)	85.0 (26.7)	-1.47 (-36.08, 33.13)	0.92
Zung Depression Scale (ZDS, /100)	57 (24.8)	49.1 (16.9)	43.6 (18.6)	-0.27 (-9.80, 9.25)	0.95	47.3 (12.3)	41.1 (14.6)	11.19 (-8.08, 30.47)	0.20
EQ-5D Health Utility Status (HUS, /1)	0.49 (0.41)	0.60 (0.34)	0.65 (0.36)	0.00 (-0.19, 0.18)	0.99	0.69 (0.29)	0.68 (0.42)	0.04 (-0.40, 0.41)	0.98
EQ-Visual Analogue Scale (EQ-VAS, /100)	57.8 (32)	66.8 (26.9)	69.9 (24.7)	3.37 (-12.99, 19.73)	0.68	75 (22.9)	76.8 (22.4)	-4.79 (-33.55, 23.97)	0.70

Verbal Fluency	10.2 (7.4)	13.8 (7)	13 (6.4)	0.79 (-3.27, 4.85)	0.70	13.6 (6.3)	16.0 (6.8)	-3.00 (-18.38, 12.39)	0.62
TICS-M	16.2 (10.5)	20.7 (7.2)	19.8 (6.9)	1.30 (-3.33, 5.93)	0.57	21.5 (6.0)	22.0 (4.2)	-6.11 (-24.55, 12.34)	0.37
t-MMSE	12 (7.2)	15.5 (4.5)	15.8 (4.8)	0.61 (-2.08, 3.30)	0.65	16.4 (3.1)	17.9 (0.9)	-1.14 (-10.07, 7.80)	0.71

CI: confidence interval; EQ-5D: European quality of life 5 dimensions; MD: mean difference; mRS: modified Rankin Scale; OR: odds ratio; SSS: Scandinavian stroke scale; t-MMSE: telephone mini-mental state examination; TICS-M: modified telephone interview for cognitive status. Data are n (%), mean (SD), median [interquartile range], mean difference (MD) or odds ratio (OR) with 95% confidence intervals. Comparison using logistic regression, multiple regression or ordinal regression. Adjusted for age, gender, baseline mRS, history of previous stroke, history of diabetes mellitus, final diagnosis, nitrate use, baseline SSS, thrombolysis, feeding status, time to randomisation, baseline SBP, continue/stop

6.5 DISCUSSION

In this large population of patients presenting with lacunar stroke randomised into the ENOS trial, baseline imaging markers of SVD and 'brain frailty' were common and associated with poor functional and cognitive outcomes at 90 days, individually and in combination. The amount of SVD and 'brain frailty' was similar in lacunar as in non-lacunar stroke despite the lacunar stroke patients being younger. GTN was safe but did not alter functional outcome of acute stroke patients presenting with lacunar syndromes. Therefore, the null hypothesis can be rejected and transdermal GTN is safe in acute stroke patients with lacunar strokes and small vessel disease.

Pre-stroke TIA, ischaemic heart disease, peripheral arterial disease and atrial fibrillation were all less common in lacunar stroke participants than non-lacunar strokes, supporting previous studies demonstrating that large artery disease and cardioembolic sources are important risk factors for non-lacunar but less so for lacunar ischaemic strokes.(258, 260, 261) Whilst smoking and diabetes were more common in our lacunar than non-lacunar stroke population,(249) perhaps surprisingly, those with LACS had less hypertension and hyperlipidaemia than non-LACS, although this finding is in keeping with previous data demonstrating in stroke patients and healthy older populations, that traditional vascular risk factors combined account for less than 2% of the variance in SVD features.(261)

In line with our results, SVD scores and their component imaging findings have been associated with adverse clinical outcomes after stroke in four other smaller cohorts. Data from the Stroke Imaging Repository (STIR)/Virtual International Stroke Trials Archive (VISTA) showed that severe leukoaraiosis and total SVD score on MRI in 259 patients with ischaemic stroke treated with thrombolysis were associated with increased disability and functional dependency at 90 days.(254) In contrast, lacunes, cerebral atrophy and enlarged perivascular spaces were not individually associated with clinical outcome, probably due to a lack of power. A retrospective cohort involving 1,026 participants using MRI markers of SVD found an association between SVD score and all-cause and stroke-related mortality.(262) SVD burden on MRI has also been associated with worse quality of life scores three months after acute ischaemic stroke(263) and decreased cognitive function over four years in first-ever lacunar ischaemic stroke and hypertensive patients.(264) In the present analysis, SVD scores with and without atrophy showed similar associations with functional outcome at 90 days overall and in the lacunar stroke populations, suggesting that the main drivers on functional outcome were the vascular rather than the associated neurodegenerative imaging features. Interestingly, the strength of association of SVD score with worse functional outcome increased with increasing specificity of lacunar stroke diagnosis.

We confirm the important prognostic value of the three 'brain frailty' measures on CT of leukoaraiosis, atrophy and old vascular lesions that were each independently associated with poor outcome in IST-3.(252) In the current analysis, only 10% of LACS participants had no 'brain frailty' markers on baseline imaging. Further, we have demonstrated that pooling these imaging markers in a score including any old infarct (not just lacunes as in the SVD score) was associated with functional and cognitive outcomes 90 days after stroke; in contrast to the SVD score, 'brain frailty' showed a similar strength of association with poor functional outcome across non-LACS and LACS populations.

There were interesting differences in associations between individual imaging features, SVD score, 'brain frailty' score and performance in the different cognitive domains. In the whole ENOS population, several acute and pre-stroke imaging features were associated with impairment in all cognitive domains. However, the SVD score added little compared with leukoaraiosis alone, which tended to be associated with verbal fluency, whereas 'brain frailty' score was associated with all cognitive impairments. This may reflect the known effects of white matter lesions on processing speed, and of brain atrophy (as a sign of neurodegeneration) on memory, or a lack of power due to missing cognitive data. They further suggest that imaging signs may have differential effects on cognition and its domains in different stroke subtypes, emphasising the importance of testing all domains in future research but also that some cognitive

tests may be insensitive to the types of impairment specific to particular stroke types.

In any case, these markers are easy to detect on plain CT imaging in the acute stroke situation by physicians as well as radiologists and given their strong prognostic significance, may prove to be useful for predicting prognosis in addition to clinical markers in future clinical practice. Whether 'brain frailty' on imaging correlates with clinical frailty is unclear, although these features correlate with gait, balance(251) and cognitive impairments(250) implying that a correlation with clinical frailty is likely, and brain features may prove to be useful surrogate markers. For future acute stroke clinical trials, minimisation based upon baseline imaging markers of 'brain frailty' along with other strong prognostic variables could be important, in order to balance these prognostic variables between treatment groups.

The favourable effect of GTN given within 6 hours on stroke onset(83, 149) was not seen in this analysis of LACS participants, but this may have lacked power. Therefore, we accept the null hypothesis that transdermal GTN does not improve clinical outcomes when given within 6 hours of stroke onset in patients with lacunar strokes and small vessel disease. GTN improved neurological impairment at day 7 in those with LACS and an acute lacunar infarction, but not in less well-defined lacunar groups. The ongoing Rapid Intervention with Glyceryl trinitrate in Hypertensive stroke Trial-2 (RIGHT-2,

ISRCTN26986053) will provide further detail on whether GTN's effects vary between differing stroke aetiologies with imaging markers being key secondary outcomes.(188) In addition, the longer-term administration of isosorbide mononitrate (a long-acting nitrate) is being assessed for safety and efficacy in patients with lacunar ischaemic stroke and SVD in the ongoing Lacunar Intervention trial-2 (LACI-2, ISRCTN14911850).

The strengths of this ENOS analysis include the largest dataset of acute lacunar stroke patients to date from a high-fidelity randomised, controlled trial with near complete follow-up; blinded and standardised adjudication of imaging by trained observers using a standardised proforma; ordinal analysis of the mRS provided to increase statistical power; and generalisability to clinical practice through the predominant use of CT imaging. However, there are important limitations. First, no adjustment was made for multiplicity of testing. Therefore, some of the results may, in part, be due to chance although the strength of associations seen ($p<0.001$) mitigates the risks of multiple testing. Second, the mean age in ENOS was lower than that seen in the unselected clinical stroke population, although was typical for lacunar stroke. This may have attenuated the observed associations, since 'brain frailty' is likely to be even more prevalent in an older population. Third, ENOS recruited over a 12-year period in which clinical practice changed. Thus, the time from baseline imaging to stroke onset was longer than we would expect in current stroke clinical practice but is still common in patients with minor

stroke. Fourth, MRI, which is more sensitive to features of SVD, was performed in only a small proportion of ENOS participants. However, the predominant use of CT enabled associations between SVD features visible on CT and outcome to be assessed, which were found to be in keeping with MRI-based studies and immediately applicable in clinical practice. Fifth, clinical stroke syndrome classification using the OCSP was determined using clinical features entered on to a case report form by investigators and not adjudicated centrally. In addition, the OCSP is known to misclassify about 15% of lacunar strokes as partial anterior circulation syndrome (PACS) and cortical strokes as LACS adding 'noise' to the data. We accounted for this in our more specific populations of LACS with compatible scan and LACS with acute lacunar infarction; this adding to the generalisability of the dataset, and its findings, to clinical practice. Sixth, telephone cognition outcome data were available for about half of participants , mainly because of the severity of stroke in the population recruited. Last, although trained neuroradiologists adjudicated the imaging data, we cannot exclude inter- and intra-rater variability over the timescale of the trial and across the different imaging markers assessed.

In summary, we add to the increasing body of evidence that baseline imaging markers of SVD and 'brain frailty' are common and associated with worse functional and cognitive outcomes at 90 days individually and when amalgamated as scores. Whether the vascular or neurodegenerative features are more associated with cognitive impairments requires further testing. CT imaging features of 'brain

frailty’ and SVD predict prognosis, should be used for minimisation in clinical trials and may aid clinical decision-making in future.

CHAPTER 7:

EXPLORATORY INVESTIGATIONS

Presentations contributing to this chapter:

Serum amyloid protein is associated with outcome following acute ischaemic stroke: data from the Remote Ischaemic Conditioning After Stroke Trial (ReCAST). European Stroke Organisation Conference, Gothenburg, Sweden (May 2018)

Peripheral and central haemodynamics in patients on isosorbide mononitrate and/or cilostazol with lacunar ischaemic stroke: data from the LACI-1 trial. European Stroke Organisation Conference, Gothenburg, Sweden (May 2018)

Platelet and haemoglobin levels in patients on isosorbide mononitrate and/or cilostazol with lacunar ischaemic stroke: data from the LACI-1 trial. European Stroke Organisation Conference, Gothenburg, Sweden (May 2018)

7.1 INTRODUCTION

This chapter comprises investigations planned and carried out as part of thesis not pertaining to the other results chapters:

Pulse wave analysis and transcranial Doppler examinations of participants enrolled into the RIGHT-2 trial admitted to Nottingham University Hospitals NHS Trust.

Measurement of plasma nitric oxide levels in participants of the ReCAST-1 trial.

Pulse wave analysis and platelet function testing of participants enrolled into the LACI-1 trial at Nottingham and Edinburgh UK.

7.2 METHODS

7.2.1 Pulse wave analysis

Introduction

Peripheral BP measurements are different to central pressures the brain is subjected to. Therefore, measurement of central BP may be a more accurate predictor of cardiovascular and cerebrovascular risk. This can be measured using pulse wave analysis.

Although the arterial pressure wave during the cardiac cycle can be measured invasively, non-invasive assessment can be performed by compressing a peripheral artery against its underlying structures and coupled with the peripheral brachial BP, validated software calculates the aortic pressure and waveform from peripheral data (Sphygmocor, Australia).

Augmentation index

In addition to central BP, a measure of central arterial compliance, Augmentation index (AI), can be calculated. AI is the difference between peaks 2 and 1 of the aortic waveform, expressed as a percentage of PP (Figure 7.1):

$$AI = \frac{(P2 - P1)}{PP} \times 100$$

Another marker of arterial stiffness is pulse wave velocity (PWV, m/s), which can be calculated using electrocardiography and measurements taken at the carotid and radial or femoral arteries.

Increased arterial stiffness is more common with ageing and is associated with increased cardiovascular risk including stroke risk,(265) cardiovascular death, mortality after stroke,(266) cerebral SVD and cognitive impairment.(267)

Buckberg index

The Buckberg index or subendocardial viability ratio (a measure of cardiac subendocardial perfusion) can also be calculated using pulse wave analysis:

$$\text{Buckberg index (\%)} = \frac{\text{area under the diastolic curve}}{\text{area under the systolic curve}}$$

Higher heart rates can reduce Buckberg index and lead to significant variability in the measure. However, consistent lower values indicate the potential for aggravating subendocardial ischaemia, particularly in patients with known coronary artery disease.(268)

Method

A peripheral artery (radial, femoral or carotid) is compressed upon its underlying structures using a micromanometer probe, which detects the pulse waveform from the PP. With peripheral brachial BP readings,

the Syphgmocor software can calculate the aforementioned parameters.

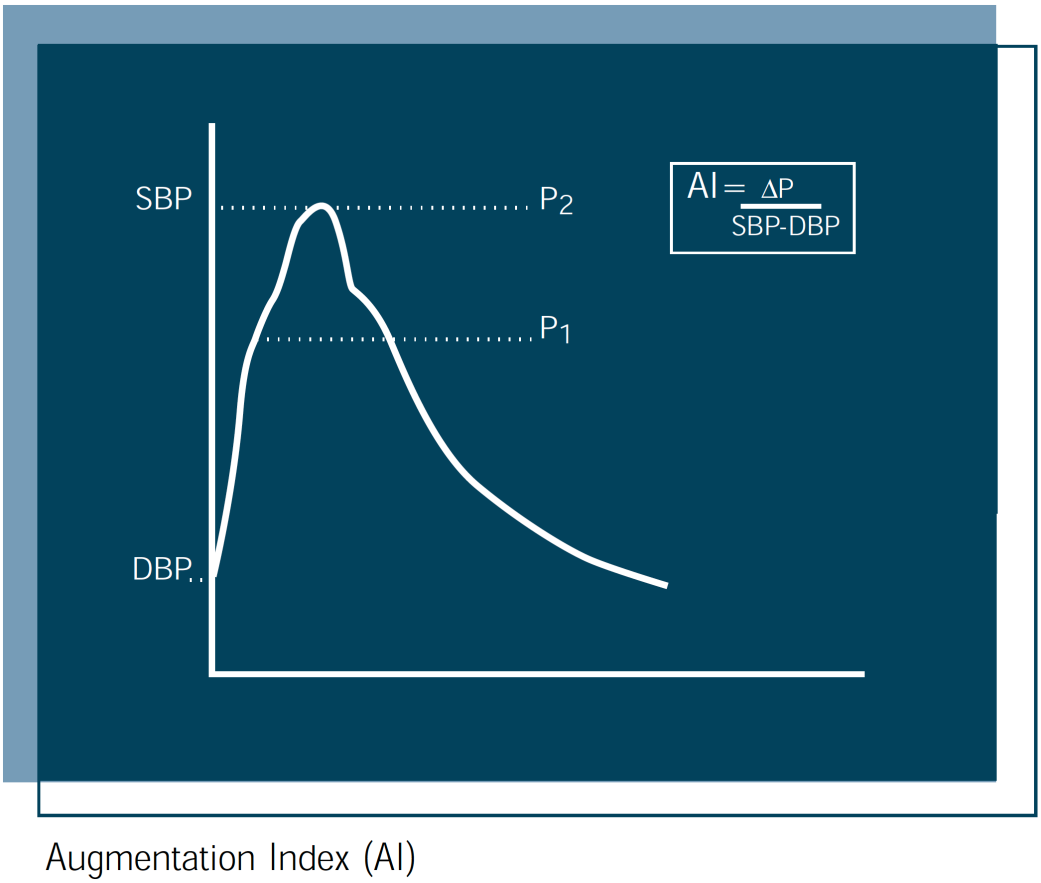


Figure 7.1: Aortic pressure wave demonstrating augmentation index calculation

(Pulse wave analysis, a clinical guide. SphygmoCor, USA)

Population

Participants recruited into the Rapid Intervention with Glyceryl trinitrate in Hypertensive stroke Trial-2 (RIGHT-2) and brought to Nottingham University Hospitals NHS Trust were recruited into the substudy. Presumed stroke patients were recruited by paramedics in the ambulance and randomised to GTN patch or no patch for 4 days.

Participants in the LACI-1 trial had pulse wave analysis and peripheral BP performed at weeks 0, 3 and 8. Patients with a lacunar ischaemic stroke were recruited at Edinburgh and Nottingham UK and randomised to cilostazol and/or isosorbide mononitrate (ISMN) in either isolation or combination for 9 weeks.

7.2.2 Transcranial Doppler

Introduction

Transcranial Doppler (TCD) uses ultrasound waves to examine CBF velocity within the cerebral arteries. High frequency sound waves (emitted by a transducer) are deflected by circulating erythrocytes within blood vessels. The transducer then records the shift in frequency of the reflected waves. TCD is a useful method of examining the dynamic flow of blood within the major intracranial blood vessels.

TCD measurements

The resultant computer output from the transducer contains both direct (peak systolic velocity, end diastolic velocity, direction of flow) and indirect (mean flow velocity, pulsatility index, resistance index) blood flow measurements. The pulsatility and resistance indices both provide information on vascular resistance distal to the point of measurement; they decrease as resistance decreases.

TCD is a sensitive method of detecting intracranial stenosis or occlusion; increased blood flow velocity, resistance flow pattern, or a dampened or absent signal are characteristic findings.(269)

The relationship between CBF velocity measured by TCD and true CBF is less clear. Whilst absolute velocity measured by TCD correlated with absolute CBF measured by Xenon CT in one study,(270) further studies have failed to replicate this.(271, 272) Similar contradictory

findings have been noted regarding regional CBF,(270, 272) hence CBF velocity is probably not a reliable indicator of true CBF.

Method

A 2 MHz probe connected to a Viasys Sonara TCD system was used. The patient was positioned supine with their head turned to the side. After the application of ultrasound gel, the probe was placed on the temporal bony window to view the main vessels comprising the circle of Willis (middle, anterior and posterior cerebral arteries). The signal depth, direction of flow and peak velocity were used to determine which vessel was visualised. Measurements were recorded via the temporal bone window of the middle cerebral artery at depths 50-65 mm in triplicate and then repeated on the contralateral side.

Population

Participants recruited into the Rapid Intervention with Glyceryl trinitrate in Hypertensive stroke Trial-2 (RIGHT-2) and brought to Nottingham University Hospitals NHS Trust were recruited into this substudy.

7.2.3 Measurement of plasma nitric oxide

Introduction

Nitric oxide (NO_x) in the form of nitrate (NO_{3-}) and nitrite (NO_{2-}) was measured in plasma samples using chemiluminescence (Sievers 280 nitric oxide analyser (NOA), Analytix Ltd, UK). Nitrate and nitrite are reduced to NO, which reacts with ozone to produce light detected by a sensitive photomultiplier tube. This is the most sensitive (0.5-1.0 pmol) and accurate method to measure NO.(273) Alternatives such as gas and liquid chromatography and mass spectrophotometry are not amenable to processing samples rapidly, whilst the Griess reaction has poor sensitivity (1-5 μM) and cannot detect nitrate.(274)

Method

The chemiluminescence system comprised a radical purge vessel with condenser and heating jacket, the NOA housing the reaction chamber, photomultiplier tube and ozone generator, and a vacuum pump (Figure 7.2). Nitrate and nitrite reduction occurred at 90°C using a water bath to supply water to the heating jacket. The condenser required a constant flow of cold water. Samples or standards (10 μL) were injected into the purge vessel containing 5 mL of filtered vanadium chloride, which reduced nitrate and nitrite in the samples or standards to NO. Bubbling nitrogen supplied by an external cylinder (pressure 0.2-0.5 psi) drove NO into the gas phase under vacuum (reaction cell pressure 8-12 torr). A gas bubbler containing 20 mL of 1M sodium hydroxide between the purge vessel and NOA prevented damage to the NOA by hydrochloric acid vapours (Figure 7.3). NO

delivered to the reaction cell chamber reacted with ozone produced from oxygen (external cylinder) and the light emitted was recorded. NOA calibration was performed by injecting sodium nitrate standards ranging from 100 nM to 100 μM in concentration. Plasma samples, deproteinised with ethanol, were injected in duplicate and the concentrations of plasma NO_x were calculated from the calibration curve using the liquid programme installed on a dedicated laptop.

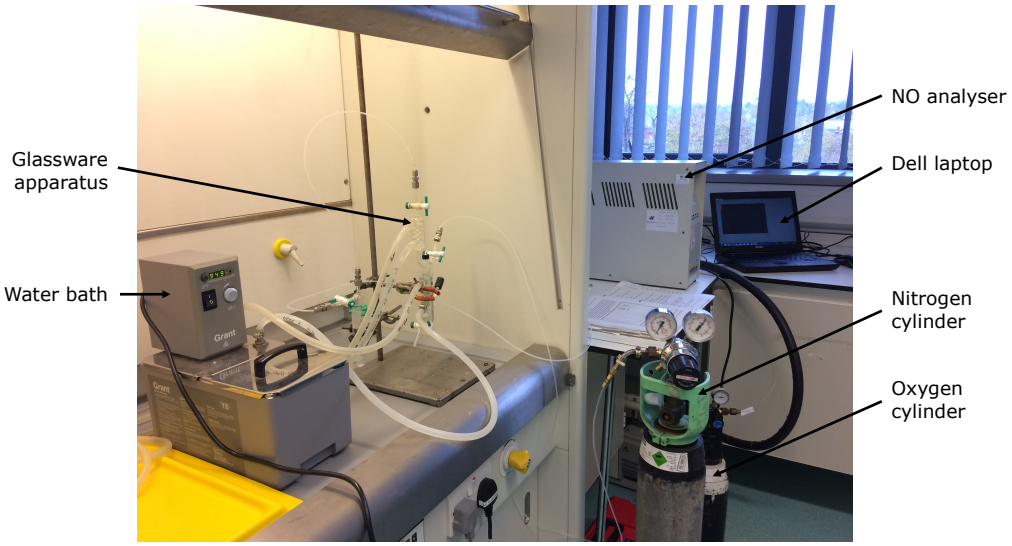


Figure 7.2: Nitric oxide analyser and associated apparatus

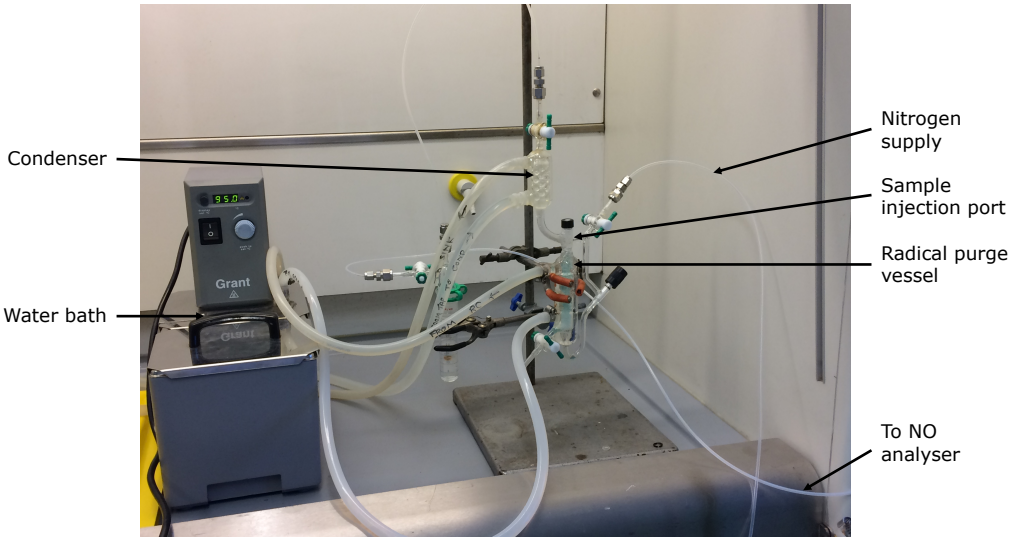


Figure 7.3: Nitric oxide glassware apparatus and water bath

Population

Participants in the Remote ischaemic Conditioning After Stroke Trial (ReCAST) had venous blood taken pre- and post-intervention and at day 4. Participants were randomised to remote ischaemic conditioning (RIC) or sham within 12 hours of ischaemic stroke onset.

7.2.4 Platelet function testing

P-selectin

P-selectin is derived from alpha-granules within platelets and becomes exposed on the platelet surface membrane when platelets are activated. Measurement of surface expression of P-selectin was performed using commercial kits sensitive to aspirin or clopidogrel (Platelet Solutions Ltd., Nottingham UK). Citrate anticoagulated blood was kept at 37°C once collected using a dry heat pad and insulation pouch, then incubated with platelet stimulants: arachidonic acid (AA)

for aspirin testing; adenosine diphosphate (ADP) for clopidogrel testing; and an unstimulated sample for baseline expression data. After 5 minutes incubating, a fixative (PAMFix, Platelet Solutions Ltd., Nottingham UK) was added and the fixed samples were transferred to the Nottingham flow cytometry laboratory for processing. Fixed blood was incubated with fluorescent antibodies to identify platelets (CD61) and P-selectin (CD62P). Median fluorescence (MF) was recorded for platelet surface expression of P-selectin for each sample.(275)

Vasodilator-stimulated phosphoprotein (VASP) phosphorylation

Intracellular cyclic adenosine monophosphate (cAMP) is a crucial marker of platelet function, but is difficult to measure. cAMP-dependent protein kinase A phosphorylates vasodilator-stimulated phosphoprotein (VASP), therefore VASP phosphorylation is an indirect measure of cAMP and has been used to assess platelet function and the response to antiplatelet agents including cilostazol, dipyridamole and clopidogrel.(276) Measurement of phosphorylated VASP was performed using a commercial kit (VASPFix, Platelet Solutions Ltd, Nottingham UK). A citrate blood sample at room temperature was incubated for 2 minutes with increasing concentrations of adenosine diphosphate. The stimulated samples were then fixed using VASPFix and then frozen prior to transfer to the Nottingham flow cytometry laboratory for processing. Thawed samples were resuspended in wash buffer prior to being analysed on the flow cytometer. For each sample, MF of phosphorylated VASP was recorded.(276)

Population

Participants in the LACI-1 trial had peripheral venous blood taken at weeks 0, 3 and 8. Patients were recruited at Edinburgh and Nottingham UK.

7.3 RESULTS

7.3.1 Pulse wave analysis and transcranial Doppler examinations of RIGHT-2 trial participants

Unfortunately, there were insufficient data to analyse from participants taken to Nottingham University Hospitals NHS Trust in the RIGHT-2 trial.

7.3.2 Measurement of plasma nitric oxide levels in ReCAST trial participants

Remote ischaemic conditioning (RIC), which involves repeated episodes of limb ischaemia and reperfusion was assessed in a small (n=26) pilot trial within 24 hours of ischaemic stroke onset.(277) Change in plasma NO levels did not differ between treatment groups pre- and post-intervention or at day 4 (Figure 7.4).(278)

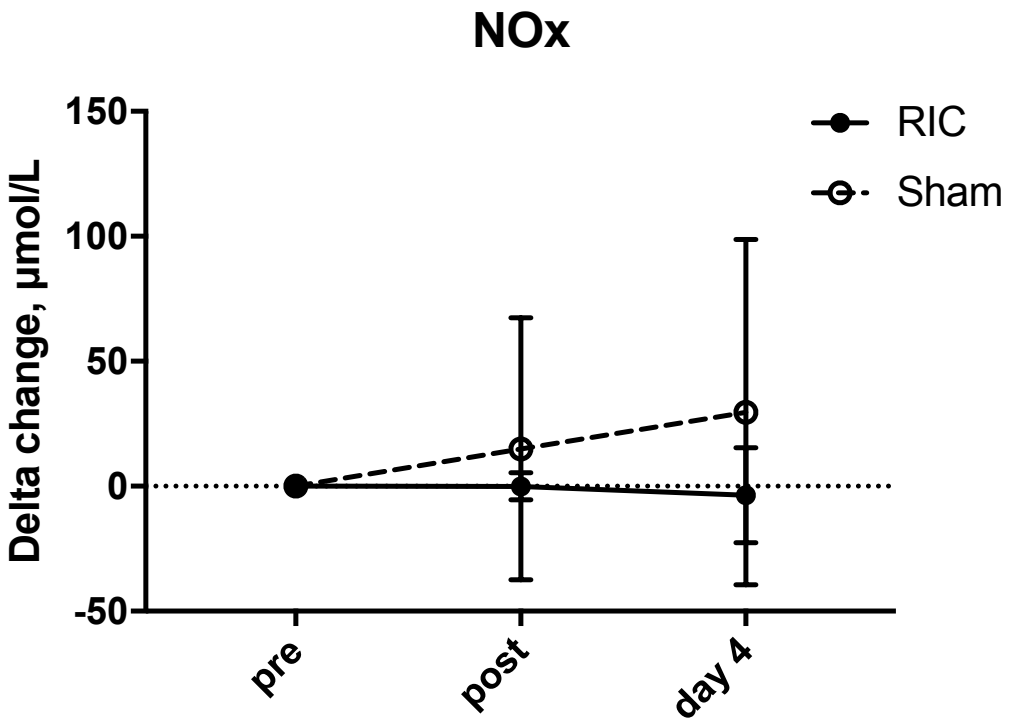


Figure 7.4: Change in plasma NO levels in RIC (n=13) and sham (n=13) patients from pre- to post-intervention and at day 4.

7.3.3 Pulse wave analysis and platelet function testing of LACI-1 trial participants

In a small pilot trial, cilostazol and isosorbide mononitrate (ISMN) – in isolation and combination – did not influence haemoglobin, platelet or platelet function levels over an 8 week period in 57 patients with previous lacunar ischaemic stroke. ISMN was safe with no significant changes in peripheral or central haemodynamics over the treatment period and there was a tendency towards improved arterial stiffness in those randomised to ISMN.

7.4 DISCUSSION

Disappointingly, there were insufficient data to establish the central haemodynamic and TCD outcomes in patients randomised to GTN vs. no GTN within the RIGHT-2 trial brought to Nottingham University Hospitals NHS Trust. Further mechanistic data would be helpful to establish whether GTN influences central haemodynamics and cerebral blood flow velocities in ultra-acute stroke.

There was no difference in change in NO levels between those randomised to RIC vs. sham in the ReCAST trial. Whilst this may mean that RIC does not influence NO levels in ischaemic stroke, it may suggest that the intervention occurred too late after stroke onset to influence NO levels, or may be underpowered. RIC is being assessed in larger studies involving ischaemic stroke patients within the thrombolysis window, and future studies should assess this therapy in the context of mechanical thrombectomy.

The longer-acting nitrate ISMN, in isolation or combination with cilostazol, did not influence haemoglobin, platelet or platelet function levels in this small pilot study. In addition, there were no significant changes in peripheral or central haemodynamics over the treatment period. Therefore, ISMN and cilostazol are currently being assessed for safety, tolerability and efficacy in the LACI-2 trial.

CHAPTER 8:

DISCUSSION

Presentations contributing to this chapter:

Glyceryl trinitrate lowers blood pressure and blood pressure variability in acute stroke patients presenting with lacunar syndromes. British and Irish Hypertension Society Annual Scientific Meeting. The Technology and Innovation Centre, University of Strathclyde, Glasgow (September 2017)

Glyceryl trinitrate lowers blood pressure and blood pressure variability in acute stroke patients presenting with lacunar syndromes. UK Stroke Forum, The Arena and Convention Centre, Liverpool (November 2017)

The effect of transdermal glyceryl trinitrate on imaging characteristics in acute ischaemic stroke: data from the Efficacy of Nitric Oxide in Stroke trial. World Stroke Conference, Montreal, Canada (October 2018)

8.1 INTRODUCTION

There are few effective treatments for acute stroke: intravenous thrombolysis and thrombectomy for ischaemic stroke; acute lowering of elevated BP for ICH; and stroke unit care for all stroke patients. High BP and its derivatives are independently associated with poor outcome after stroke but trials assessing BP lowering agents in ischaemic stroke have thus far been neutral. A subgroup of the ENOS trial – and pilot trial in the ambulance – have suggested that transdermal GTN may improve outcome when given within the first few hours following symptom onset. Transdermal GTN is a candidate treatment for acute stroke given its BP lowering, vasodilatory and neuroprotective properties. It is also easy to administer (and remove), inexpensive and can be given prior to imaging.

This thesis has sought to establish safety of this medication both overall in acute stroke and in important subgroups, whilst exploring potential mechanisms of action.

8.2 SAFETY AND EFFICACY OF GTN IN ACUTE STROKE

This thesis has confirmed the safety of transdermal GTN in 4197 patients with acute stroke, and its potential beneficial effects when administered early after symptom onset (Chapter 3). The updated Cochrane systematic review and meta-analysis involved unadjusted analyses confirming the analyses performed in a separate meta-analysis based on individual patient data and using adjusted analyses: transdermal GTN is safe in acute stroke and may improve clinical outcomes when administered within six hours of stroke onset.

Admittedly a large majority of patients (95.6%) were from the ENOS trial and all completed RCTs assessing transdermal GTN are from the same research group. Nevertheless, the potential time-dependent treatment benefit of GTN is currently being assessed in at least two large independent trials: RIGHT-2 (ISRCTN26986053) and MR-ASAP (ISRCTN99503308). Both are assessing transdermal GTN pre-hospital within the first few hours of stroke onset (four and three hours respectively), in different healthcare settings (UK and Netherlands) and should hopefully provide definitive evidence of whether or not GTN is efficacious in ultra-acute stroke.

8.3 HAEMODYNAMICS AND EFFECTS OF GTN IN ACUTE STROKE

The effects of transdermal GTN on haemodynamic parameters, and in turn their effects on outcome, in acute stroke were assessed in Chapter 3 using data from the ENOS trial. GTN lowered BP and RPP, whilst increasing HR modestly at day 1, suggesting that its effect on HR was negated by BP lowering. In addition, GTN reduced between-visit BP variability over days 1 to 7. Whether this statistically significant reduction is clinically meaningful is unclear and requires further work.

Increased between-visit BP variability over days 1-7 was associated with worse functional and cognitive outcomes, and increased death at day 90, independent of the trends with mean BP. This implies that the association with outcome is stronger for BP variability than absolute mean BP. Therefore, it may be that fluctuations in BP rather than absolute BP levels are more important at predicting outcome. This chapter supports previous analyses demonstrating that increased BP variability over the first days following stroke onset is associated with poor outcome.(204) We have added to this using data from one of the largest BP lowering trials in acute stroke – including both ischaemic and haemorrhagic stroke – and also shown that increased variability is associated with worse cognitive scores at 90 days. Increased BP variability hyperacutely in ICH patients has been associated with poor clinical outcomes;(217) a finding which is important to corroborate

with data from the RIGHT-2 trial in both ischaemic and haemorrhagic stroke. Importantly there is a lack of data on the effects of common BP medication on BP variability in acute stroke and further work is needed to establish whether BP variability is a modifiable target in acute stroke. If so, then medications that lower BP variability – such as GTN – may be of benefit.

8.4 SAFETY AND EFFICACY OF GTN IN ACUTE STROKE: SUBGROUP ANALYSES

This thesis also assessed safety and efficacy of transdermal GTN in important patient subgroups using data from the ENOS trial.

8.4.1 Transdermal GTN and blood markers of dehydration

In patients with blood marker evidence suggestive of dehydration, transdermal GTN did not cause precipitous drops in BP (Chapter 4). Overall, increased baseline urea was associated with poor functional outcome and increased death at day 90, with a suggestion that GTN vs. no GTN may lead to worse outcomes. This finding may represent chance as this was not seen for other blood markers and only ENOS participants recruited in Nottingham UK had available data for analysis. This therefore requires confirmation in larger datasets to ensure those with increased urea are not harmed by this medication. Nevertheless, it is reassuring that no precipitous drops in BP were seen in those treated with GTN, but it is unlikely that patients with serious dehydration would have been recruited into ENOS; hence, secondary analysis of the RIGHT-2 trial, involving a relatively unselected population with a significant proportion of stroke mimics, may prove illuminating.

8.4.2 Transdermal GTN and carotid stenosis

Modest BP lowering with transdermal GTN was safe in acute ischaemic stroke patients with carotid stenosis (Chapter 5). Across all degrees of ipsilateral and bilateral carotid stenosis, GTN did not cause harm; indeed, those with ≥70% ipsilateral stenosis who received GTN vs. no GTN had a favourable shift in mRS at 90 days. Although this finding might represent chance – there was no tendency towards improved outcomes in other carotid stenosis groups – it may suggest a potential mechanism of action. Transdermal GTN does not reduce CBF in acute stroke and so may preserve blood flow to ischaemic tissue in the context of severe ipsilateral stenosis through its arterial- and venous-dilatory effects. Previous CBF measurements using Xenon-CT(183) and MRI-perfusion(243) have shown that GTN does not reduce CBF despite lowering systemic BP. Further studies assessing the effects of commonly used BP lowering agents on CBF in acute stroke are needed.

8.4.3 Transdermal GTN, lacunar syndromes and small vessel disease

Chapter 6 assessed the safety and efficacy of GTN in lacunar syndromes using data from the ENOS trial. Although GTN did not influence outcome in lacunar syndromes overall, in those with a lacunar syndrome and acute lacunar infarct evident on imaging, GTN was associated with improved neurological impairment at day 7. Although GTN improved multiple outcomes when given within 6 hours

of stroke onset overall, it did not influence clinical outcomes in lacunar syndrome patients; this may suggest that GTN's effects differ across aetiologies of stroke. However, this analysis was underpowered to definitively answer this question.

Chapter 6 also demonstrated the prognostic importance of imaging markers of SVD and 'brain frailty'. When these features were amalgamated into scores, increasing burden of SVD was associated with worse functional outcome at 90 days, whilst increasing 'brain frailty' was associated with both poor functional outcome and worse cognition scores at day 90. Interestingly, the effect sizes for the association between SVD score and functional outcome increased with increasing specificity of lacunar stroke. These baseline imaging features are easy to detect on CT by physicians and radiologists and may be a helpful marker to aid prognostication following acute stroke in future. Future acute stroke trials should consider using these imaging markers as minimisation criteria given their prognostic importance.

8.5 LIMITATIONS

Limitations of the work presented have been discussed in each results chapter respectively.

All trials of transdermal GTN in acute stroke to date have been performed by the same stroke research group. It is therefore reassuring that ongoing and planned trials of transdermal GTN in ultra-acute stroke are being performed by other research groups.

The primary dataset utilised was from the ENOS trial, which despite being one of the largest, high-fidelity, randomised controlled trials in acute stroke patients has inherent limitations. Trial recruitment occurred over 12 years, during which time stroke practice changed significantly with the advent of thrombolysis, stroke unit care and adequate stroke secondary prevention. By systematically excluding patients via recruitment into a clinical trial, generalisability is reduced. However, as a pragmatic trial conducted across over 100 centres in 23 countries, ENOS provides real-world data across a variety of stroke populations. Analyses of cognitive data are limited by missing data in addition to the challenges of assessing cognition via telephone and the absence of baseline cognitive assessment. Carotid stenosis imaging was not performed in all ENOS participants and therefore resulting analyses are at risk of selection bias. However, this adds generalisability to clinical practice whereby patients with more severe stroke, unlikely to benefit from carotid revascularisation, would not have carotid imaging performed.

Unfortunately, there were insufficient data to provide novel insights into mechanistic central haemodynamic and CBF velocity effects of transdermal GTN in ultra-acute stroke. Future studies assessing the effects of antihypertensive medications in ultra-acute stroke on these markers are needed.

The safety of transdermal GTN in acute stroke requires confirmation by other research groups. Likewise, the efficacy of GTN within the first few hours of stroke onset requires verification as discussed.

8.6 FUTURE DIRECTIONS

This thesis has sought to address several questions pertaining to the role of transdermal GTN in acute stroke. In the process further questions have arisen and remain unanswered.

Although the haemodynamic properties of GTN in acute stroke have been explored and our knowledge expanded in this thesis, we do not know if GTN's haemodynamic effects differ by stroke aetiology. In ENOS, GTN lowered BP and between-visit BP variability overall and in those with lacunar syndromes, whilst BP variability was not reduced by GTN in the non-lacunar stroke population. However, the interaction between those with and without lacunar syndromes was non-significant ($p=0.23$) and therefore this finding may represent chance. Future research should assess whether the association between between-visit BP variability and clinical outcome differs by stroke aetiology. Further, it will be important to establish GTN's effects on between-visit BP variability when initiated in the first few hours of stroke and in turn whether this is influenced by the underlying stroke type and aetiology.

With the advent and expansion of mechanical thrombectomy in acute ischaemic stroke across the developed world, there is a need to maximise the target population, whilst also an opportunity to assess the potential of adjuvant therapies to further improve outcomes. GTN may have several roles here. Recent secondary analysis of the MR-CLEAN trial suggested a U-shaped curve of baseline BP in relation to

functional outcome at 90 days, with both high and low BP associated with worse outcome.(279) Therefore, studies assessing whether lowering of elevated BP in the context of large vessel occlusion with agents, including GTN, are warranted. Second, GTN's arterial- and venous-dilatory effects on collateral blood vessels may delay the process of infarction such that the ischaemic penumbra and mismatch is maintained, increasing the number of patients eligible for thrombectomy whilst also allowing more time for patients to be treated. Perfusion imaging studies are required to test this hypothesis. Third, the advent of revascularisation therapy may allow neuroprotective agents to be tested during and immediately after clot removal. Interventions that may be able to reduce or prevent reperfusion injury would ideally be tested in this setting also. NO plays a pivotal role in both neuroprotection and cerebral perfusion and so interventions that may increase NO levels, such as GTN, may be of benefit.

As well as potentially influencing collateral supply in ischaemic stroke, early treatment with GTN may reduce early imaging markers of ischaemia and infarct size in ischaemic stroke, and may attenuate haematoma expansion in ICH. These mechanisms are speculative and further imaging analysis of the ongoing trials is required to assess the on-treatment effects of GTN on imaging markers. Perfusion-based imaging studies are required to assess the effects of BP lowering agents within the first few hours of stroke onset, including in patients with carotid stenosis.

An alternative method postulated to improve collateral blood supply is to induce hypertension. Interestingly, recently presented data suggested that inducing hypertension (SBP 200 mmHg) using IV phenylephrine in acute IS patients within 24 hours of onset who were ineligible for revascularisation therapy improved neurological impairment by day 7 and was associated with less death and dependency at day 90. MRI perfusion data demonstrated that phenylephrine was associated with collateral enhancement at day 7.(280) Further work to establish mechanisms to improve or maintain collateral blood flow is needed.

Whilst the advent of exciting, effective and expensive treatments in acute stroke are revolutionising how we treat stroke patients in the developed world, treatments in developing countries remain limited. If GTN is found to be efficacious within the first few hours following acute stroke, then given its low cost (<£5 / patient) it should enable implementation in clinical practice in countries regardless of wealth. If transdermal GTN is efficacious, then what is the optimum treatment duration? ENOS and RIGHT both had 7-day treatment periods, whilst the ongoing trials have much shorter regimens: RIGHT-2 is four days, whilst MR-ASAP is using a single patch for 24 hours.

Given the potential benefit of GTN in acute stroke, interest has developed in the testing of longer-acting NO donors e.g. isosorbide mononitrate (ISMN) in secondary stroke prevention. Due to the

paucity of treatments for SVD and lacunar stroke, drug re-purposing has been suggested. NO donors have effects that may be of benefit in SVD and lacunar stroke, and so ISMN is currently being assessed for safety, tolerability and efficacy (LACI-2 trial: ISRCTN14911850).

The association of baseline imaging markers of 'brain frailty' with clinical outcome after acute stroke detailed in Chapter 6 requires validation in other datasets, as well as establishing whether these markers correlate with clinical frailty. The interesting differences in associations between individual imaging features, SVD and 'brain frailty' scores and cognition at day 90 warrant further exploration. Whilst 'brain frailty' was associated with worse cognitive scores across all three cognitive domains assessed, SVD score only influenced verbal fluency. This may reflect the known effects of leukoaraiosis on processing speed, and of brain atrophy on memory. Perhaps imaging markers have differential effects on cognition and its domains in different stroke types or that some cognitive tests are insensitive to different types of cognitive impairment that may occur in some stroke types. Future studies should assess all cognitive domains where possible (including processing speed) and consider analysing cognitive subdomains to establish whether certain imaging markers are associated with specific cognitive deficits following stroke.

8.7 CONCLUSIONS

In summary, this thesis has confirmed the safety of transdermal GTN in acute stroke both overall and in important subgroups. Mechanistic data suggest that GTN may reduce BP variability, which seems to be more strongly associated with outcome than absolute BP or trend in BP. Transdermal GTN is safe to be administered in acute stroke patients with elevated BP prior to blood markers of dehydration or carotid stenosis status being known. Primary and secondary analyses of ongoing trials of GTN in ultra-acute stroke will prove illuminating. Further mechanistic imaging data are required to establish the on-treatment effects of GTN in ultra-acute stroke. In addition, baseline imaging markers of SVD and 'brain frailty' predict clinical outcome and should be used as minimisation criteria in future acute stroke trials and may help guide future clinical decision-making.

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10. APPENDICES

10.1 APPENDIX 1: COCHRANE SEARCH STRATEGIES

10.1.1 EMBASE search strategy

1. Cerebrovascular disorders.mp. or cerebrovascular disease/

2. exp Brain ischemia/

3. Carotid artery diseases/ or Carotid artery thrombosis/

4. exp cerebrovascular accident/

5. exp brain hypoxia/ or exp brain ischemia

6. Cerebral artery disease/

7. exp occlusive cerebrovascular disease/

8. exp basal ganglion hemorrhage/

9. exp brain hemorrhage/

10. (stroke$ or cerebral vasc$ or cerebrovasc$ or cva or transient isch?emic attack$ or tia$).tw.

11. (brain or cerebr$ or cerebell$ or vertebrobasil$ or hemispher$ or intracran$ or intracerebral or infratentorial or supratentorial or middle cerebr$ or mca$ or anterior circulation).tw.

12. (isch?emi$ or infarct$ or thrombo$ or emboli$ or occlus$ or hypoxi$).tw.

13. 11 and 12

14. (brain or cerebral or intracranial).tw.

15. (h?emorrhage or h?ematoma or bleed$).tw.

16. 14 and 15

17. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 13 or 16

18. exp nitric oxide donor/

19. nitric oxide/

20. glyceryl trinitrate/

21. Nitric-Oxide Synthase/

22. arginine/

23. nitroprusside sodium/

24. (nitric$ or nitro$ or glyceryl trinitrat$ or GTN or arginine).tw.

25. or/18-24

26. 17 and 25

27. limit 26 to human

28. limit 27 to (clinical trial or randomized controlled trial or controlled clinical trial or multicentre study or phase 1 clinical trial or phase 2 clinical trial or phase 3 clinical trial or phase 4 clinical trial)

10.1.2 MEDLINE search strategy

1. Cerebrovascular disorders/

2. exp Brain ischemia/

3. Carotid artery diseases/ or Carotid artery thrombosis/

4. exp stroke/

5. exp Hypoxia-ischemia, brain/

6. Cerebral arterial diseases/ or Intracranial arterial diseases/

7. exp "Intracranial embolism and thrombosis"/

8. exp basal ganglia cerebrovascular disease/

9. exp intracranial hemorrhages/

10. (stroke$ or cerebral vasc$ or cerebrovasc$ or cva or transient isch?emic attack$ or tia$).tw.

11. (brain or cerebr$ or cerebell$ or vertebrobasil$ or hemispher$ or intracran$ or intracerebral or infratentorial or supratentorial or middle cerebr$ or mca$ or anterior circulation).tw.

12. (isch?emi$ or infarct$ or thrombo$ or emboli$ or occlus$ or hypoxi$).tw.

13. 11 and 12

14. (brain or cerebral or intracranial).tw.

15. (h?emorrhage or h?ematoma or bleed$).tw.

16. 14 and 15

17. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 13 or 16

18. exp nitric oxide donors/

19. nitric oxide/

20. nitroglycerin/

21. Nitric-Oxide Synthase/ai [Antagonists & Inhibitors]

22. arginine/

23. nitroprusside/

24. (nitric$ or nitro$ or glyceryl trinitrat$ or GTN or arginine).tw.

25. or/18-24

26. 17 and 25

27. limit 26 to human

10.1.3 Web of science / Science Citation Index search strategy

1. Cerebrovascular disorders.TI/TS

2. Brain ischemia.TI/TS

3. Carotid artery diseases or Carotid artery thrombosis.TI/TS

4. Stroke.TI/TS

5. Brain hypoxia.TI/TS

6. Cerebral artery disease.TI/TS

7. Occlusive cerebrovascular disease.TI/TS

8. Basal ganglia cerebrovascular disease.TI/TS

9. Intracranial hemorrhages.TI/TS

10. (stroke$ or cerebral vasc$ or cerebrovasc$ or cva or transient isch?emic attack$ or tia$).TI/TS

11. (brain or cerebr$ or cerebell$ or vertebrobasil$ or hemispher$ or intracran$ or intracerebral or infratentorial or supratentorial or middle cerebr$ or mca$ or anterior circulation).TI/TS

12. (isch?emi$ or infarct$ or thrombo$ or emboli$ or occlus$ or hypoxi$).TI/TS

13. 11 and 12

14. (brain or cerebral or intracranial).TI/TS

15. (h?emorrhage or h?ematoma or bleed$).TI/TS

16. 14 and 15

17. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 13 or 16

18. nitric oxide donor.TI/TS

19. nitric oxide.TI/TS

20. glyceryl trinitrate.TI/TS

21. Nitric-Oxide Synthase.TI/TS

22. arginine.TI/TS

23. nitroprusside sodium.TI/TS

24. (nitric$ or nitro$ or glyceryl trinitrat$ or GTN or arginine).TI/TS

25. or/18-24

26. 17 and 25

27. 26 Refined by: DOCUMENT TYPES: (CLINICAL TRIAL)

10.2 APPENDIX 2: MISCELLANEOUS

10.2.1 ENOS carotid stenosis working practice document

ENOS TRIAL

INTERNAL CAROTID ARTERY STENOSIS (ICA)

Completion of Hospital Event Information

PEAK SYSTOLIC VELOCITY (PSV)		% DIAMETER REDUCTION		ENTER ON HOSPITAL EVENT FORM Equivalent mid-point
1-109		0-29		14
110-130		30-49		39
>130		50-69		59
>210		70-95		82
"STRING FLOW"		96-99		98
"NO FLOW"		100		100

Both RIGHT and LEFT ICA needs entering

If the report states:

An exact percentage, then enter as reported

Between, or less than a certain percentage i.e. 0-29 or less than 29%, then enter the mid-point i.e. 14

If the report gives a velocity then find the equivalent mid-point percentage and enter this.

10.2.2 ENOS imaging adjudication proforma

Scan Assessment for Scan Number:

Supporting Data for Patient						
	Date of Stroke				History of previous strokes	
	Gender				Side of Weakness	
	Age				Final Diagnosis (HE Form)	

Scan Details		
1	Date and time of scan:	
2	Scan quality	☐Good ☐Moderate ☐Poor
	Scan Quality Comments	
3	Type of Scan	☐CT - With Contrast ☐CT - Without Contrast ☐MRI - With Contrast ☐MRI - Without Contrast
4	Image Source	☐Film ☐Electronic
5	Is the Scan completely normal?	☐Yes ☐No If 'Yes' then go to form sign-off/submission

Recent Stroke Changes		
6	Is there any sign of recent stroke change? If in doubt as to whether acute or old, code as acute *Bear in mind that ENOS allows entry into the trial up to 48 hours after the stroke onset and some patients may be scanned after entry into the trial. Therefore "acute" could include anything from an infarct that looks only a few hours old up to several days old*	☐Yes ☐No If NO then go to 15
7	Which side of the brain shows stroke change? *As per neuroradiology convention, the right side of the brain is the left side of the image; the left side of the brain is the right side of image*	☐Left ☐Right ☐Both
8	Please classify site and size of stroke lesion (most 'sites' will require one sub-terrority to be supplied) :	*"Site" should be used to record the general location and extent of both ischaemic and haemorrhagic strokes. Most primary haemorrhages will fit into the MCA territory, lacunar or ACA/PCA/brainstem/cerebellar classification. Further details about haemorrhage can be recorded in Section F*
a)	site	☐M = MCA ☐AS = Stroke of up to half of ACA territory ☐AL = Stroke of more than half of ACA territory ☐PS = Stroke of up to half of PCA territory ☐PL = Stroke of more than half of PCA territory ☐MAS = M+AS ☐MAL = M+AL ☐MPS = M+PS ☐MPL = M+PL ☐MAP = Stroke of whole MCA=>ACA and PCA territories ☐L = Lacune ☐B = Borderzone ☐C = Cerebellum ☐S = Brainstem ☐CS = Cerebellum and Brainstem
b)	sub-territory sites:	Sub-territory definitions (NB: Only codes 1-15 shown)
1	mca sub-territory codes:	☐8 = stroke of whole of MCA territory ☐7 = 6+stroke of lateral part of basal ganglia ☐6 = stroke of the whole of peripheral MCA territory ☐5 = stroke of the posterior half of peripheral MCA territory ☐4 = stroke of anterior half of peripheral MCA territory ☐3 = stroke of white matter lateral to the lateral ventricle ☐2 = basal ganglia stroke ☐1 = small cortical stroke
2	lacunar/borderzone sub-territory:	☐15 = posterior (mainly) border zone ☐14 = anterior (mainly) border zone ☐13 = lacune in brainstem, inc. Pons ☐12 = lacune in thalamus ☐11 = lacune in centrum semiovale ☐10 = lacune in internal border zone ☐9 = lacune in internal capsule/lentiform

3	cerebellum sub-territory:	☐18 = >1/2 hemisphere ☐17 = <1/2 hemisphere (medium) ☐16 = small cortical
4	brainstem sub-territory:	☐20 = extensive, i.e. pons + medulla ☐19 = small, i.e.<1/2 medulla
c)	degree of mass effect:	☐6 = 5+effacement of the basal cisterns ☐5 = 4+shift of the midline away from the side of the ventricle ☐4 = 1+effacement of the lateral and third ventricle ☐3 = 1+complete effacement of lateral ventricle ☐2 = 1+minor effacement of adjacent lateral ventricle ☐1 = effacement of the sulci overlying the stroke ☐0 = no swelling
9	Is the stroke ischaemic or primary haemorrhage?	☐Haemorrhage ☐Ischaemic If 'haemorrhage' then go to 17
10	Classify signs of ischaemic change in the main lesion (if more than one recent lesion)	
a)	Loss of grey/white matter cortex definition	☐Yes ☐No ☐Not Applicable
b)	Loss of basal ganglia outline	☐Yes ☐No ☐Not Applicable
c)	Hypodensity present (i.e. more than in a or b so that the lesion appears less dense than white matter)	☐Yes ☐No
d)	Mass effect (swelling) present	☐Yes ☐No
	If 10 d)=YES then please answer the following:	
1	sulcal effacement:	☐Yes ☐No
2	ventricular effacement:	☐Yes ☐No
3	midline shift:	☐Yes ☐No
4	uncal herniation:	☐Yes ☐No ☐Not Applicable
11	In your opinion does the new acute ischaemic change involve more than 1/3 of the MCA territory? *This only applies to MCA territorial strokes – i.e. not lacunar/borderzone/ACA or PCA territory ischaemic strokes or haemorrhages. For all of these, select "Not relevant".*	☐Yes ☐No ☐Not Relevant

ASPECTS for the MCA territory		
12	**ASPECT** Score: Assess the MCA territory in the ischaemic hemisphere (or the hemisphere most severely affected) Please indicate if each of the MCA areas listed below are normal (norm) or show some signs of an infarct (abnorm) *This only applies to MCA territorial strokes – i.e. not lacunar/borderzone/ACA or PCA territory ischaemic strokes or haemorrhages. For these tick "Not relevant"*	Aspect example (NB: Ignore areas A and P) ☐Not relevant If not relevant then go to Q13
a)	Caudate (C)	☐Normal ☐Abnormal ☐Not Applicable
b)	Lentiform (L)	☐Normal ☐Abnormal ☐Not Applicable
c)	Insula (I)	☐Normal ☐Abnormal ☐Not Applicable
d)	Internal Capsule (IC)	☐Normal ☐Abnormal ☐Not Applicable
e)	MCA1 (M1)	☐Normal ☐Abnormal ☐Not Applicable
f)	MCA2 (M2)	☐Normal ☐Abnormal ☐Not Applicable
g)	MCA3 (M3)	☐Normal ☐Abnormal ☐Not Applicable
h)	MCA4 (M4)	☐Normal ☐Abnormal ☐Not Applicable

i)	MCA5 (M5)	☐Normal ☐Abnormal ☐Not Applicable
j)	MCA6 (M6)	☐Normal ☐Abnormal ☐Not Applicable
13	Is there a second (discrete) recent ischaemic lesion?	☐Yes ☐No If NO then go to Q15
14	Describe second ischaemic lesion?	

Hyperdense Vessel Sign Hyperdense Vessel Sign Definitions		
15	Is there a hyperdense artery?	☐Yes ☐No If NO then go to Q17
16	Name hyperdense artery:	_____ _____

Haemorrhagic Changes				
17	Is there any haemorrhage anywhere?	☐Yes ☐No If NO then go to Q19		
18	Classify haemorrhage (if there are more than one haemorrhage, select all that are present and indicate the order of significance):	*For primary intracerebral haemorrhage, choose "C parenchymal haematoma" (or "E subdural", "F subarachnoid haemorrhage", or "H extradural", as appropriate)*		
			Order (Enter a number to indicate your estimate of the order of importance, 1 being most important)	Size (biggest diameter) of Haematoma
a)	petechial haemorrhage	☐Yes ☐No	☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8	
b)	significant haemorrhagic transformation of infarct (i.e. underlying infarct still visible)	☐Yes ☐No	☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8	☐<3cm ☐3-5cm ☐5-8cm ☐>8cm
c)	parenchymal haematoma (i.e. no infarct visible)	☐Yes ☐No	☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8	☐<3cm ☐3-5cm ☐5-8cm ☐>8cm
d)	parenchymal haematoma clearly remote from infarct	☐Yes ☐No	☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8	☐<3cm ☐3-5cm ☐5-8cm ☐>8cm
e)	subdural haematoma	☐Yes ☐No	☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8	
f)	subarachnoid haemorrhage	☐Yes ☐No	☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8	
g)	intraventricular haemorrhage	☐Yes ☐No	☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8	☐<3cm ☐3-5cm ☐5-8cm ☐>8cm
h)	extradural haemorrhage	☐Yes ☐No	☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8	

Reduction in Brain Tissue Volume		
19	Is there any cerebral atrophy/reduction in brain tissue volume?	☐Yes ☐NoIf NO then go to Q21
20	Classify atrophy:	
a)	Central	☐None ☐Moderate ☐Severe Central definitions

b)	Cortical	☐None ☐Moderate ☐Severe Cortical definitions

Periventricular Lucencies/Leukoariosis	Periventricular Lucencies definitions/examples	
21	Are there any periventricular lucencies?	☐Yes ☐No If NO then go to Q23
22	Classify extent of white matter lucency:	
a)	Anterior White Matter	☐2 = Lucency covering entire region from lateral ventricle to cortex ☐1 = Lucency restricted to region adjoining ventricles ☐0 = No lucency
b)	Posterior White Matter	☐2 = Lucency covering entire region from lateral ventricle to cortex ☐1 = Lucency restricted to region adjoining ventricles ☐0 = No lucency

Old Vascular Lesions		
Old Infarcts		
23	Are there any old vascular lesions (infarct)?	☐Yes ☐No If NO then go to Q25
24	Classify old infarct:	
a)	Old Cortical Infarct(s)	☐Yes ☐No
b)	Old Striatocapsular Infarct(s)	☐Yes ☐No
c)	Old Borderzone Infarct(s)	☐Yes ☐No
d)	Old Lacunar Infarct(s)	☐Yes ☐No
e)	Old Brainstem/Cerebellar Infarct(s)	☐Yes ☐No

Non-Stroke Lesions		
25	Is there a non-stroke lesion, which could have accounted for the patient's stroke syndrome?	☐Yes ☐No If NO then go to Q27
a)	Non-stroke lesion comments	
26	Classify non-stroke lesion:	
a)	Cerebral Tumor	☐Yes ☐No
b)	Encephalitis	☐Yes ☐No
c)	Cerebral Abscess	☐Yes ☐No
d)	Other (e.g. Contusion)	☐Yes ☐No
1	Specify Other:	

Comments		
27	Comments	

Date form completed (dd/mmm/yyyy)*: Please complete this date even if the scan is normal.	- Day - - Month - - Year -

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