The Efficacy of Nitric Oxide in Stroke (ENOS) Trial

A prospective, collaborative, international, multicentre, randomised, parallel-group, single and outcome blinded, controlled, factorial trial to investigate the safety and efficacy of treatment with transdermal glycercyl trinitrate, a nitric oxide donor, and of continuing or stopping temporarily pre-stroke antihypertensive therapy, in patients with acute stroke

Summary

Nitric oxide is a multimodal candidate treatment for acute stroke having a number of properties which may be beneficial in acute stroke, including lowering blood pressure, causing cerebral vasodilatation, and improving central and systemic haemodynamics. Nitric oxide donors are effective in experimental stroke, and pilot studies in patients with acute stroke suggest that glycercyl trinitrate can be delivered easily in a transdermal preparation.

Around half of all patients admitted with acute stroke are taking antihypertensive therapy immediately prior to their stroke. No data exist as to whether it is beneficial or safe to continue or temporarily stop this treatment during the acute phase.

ENOS is a prospective, collaborative, international, multicentre, randomised, parallel-group, single and outcome blinded, controlled, factorial trial designed to test two questions related to the management of blood pressure immediately post-stroke:

1. The safety and efficacy of nitric oxide, given as transdermal glycercyl trinitrate.
2. The safety and efficacy of continuing versus stopping temporarily pre-stroke antihypertensive medication.

Previously independent adult patients who are conscious and have residual limb weakness are eligible for enrolment. Patients will be randomised, centrally via the internet to daily glycercyl trinitrate patch or no patch; a gauze dressing will be placed over the patch or a similar area of skin to maintain patient blinding to the patch. Additionally, patients taking antihypertensive medication immediately prior to their stroke will be randomised to continue or temporarily stop this. Treatment must be initiated within 48 hours of stroke onset and is given for 7 days. A CT scan is required before enrolment or within 7 days of randomisation. Early follow-up is performed at the local centre over the 7 days of treatment, including recording of blood pressure, early stroke events, and serious adverse events. Central telephone follow-up by the National Co-ordinating Centre will be performed at 90 days. The primary outcome is combined death or dependency (modified Rankin Score >2).
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1. BACKGROUND

1.1 Acute treatment of stroke

Stroke is the third most common cause of death worldwide and is the leading cause of adult disability in many countries. Overall, one third of patients die in the first few months after stroke with a further third remaining dependent. While formal rehabilitation in a specialist unit significantly improves outcome, only two acute drug treatments have been shown to reduce combined ‘death or disability’ after acute ischaemic stroke. Aspirin has a small effect on outcome (absolute benefit 1.3%) but can be given to most patients; its effect is probably mediated through early secondary prevention. In contrast, intravenous alteplase is highly efficacious (absolute benefit 8-12%) although treatment has to be given within three hours of stroke onset and is not without hazard, particularly with respect to causing intracranial haemorrhage.

Preliminary evidence suggests that early treatment with ancrod (defibrinogenating agent), pro-uokinase (thrombolytic), or citocline (neuroprotectant) may be effective in patients with ischaemic stroke. Other potential neuroprotectants (e.g. aptiganel, eliprodil, enlimomab, gavestinel, lubeluzole, magnesium, nimodipine, NXY-059 selfotel, tirilazad) and anticoagulation strategies have proved ineffective, or even toxic.

No treatments have been found to be effective for primary intracerebral haemorrhage (PICH). In particular, no benefit was found in a large trial of surgery for PICH. The use of activated factor VIIa has shown initial promise in a recent phase II study and the results of larger trials are awaited. Hence, the need remains for an efficacious and safe treatment for both ischaemic and haemorrhagic stroke which can be given to a large proportion of patients.

Other major concerns for physicians treating acute stroke include the management of high blood pressure, hyperglycaemia, pyrexia, and intracerebral hypertension. This document describes the rationale and protocol of a trial investigating the management of blood pressure immediately post-stroke.

1.2 Blood pressure in acute stroke

High blood pressure is present in 75±9% of patients presenting with acute stroke, whether of ischaemic or haemorrhagic type. A number of factors contribute to this, including a previous history of hypertension, activation of neuro-endocrine hormones (e.g. sympathetic nervous systems), the Cushing reflex, and the stress of hospitalisation. Hypertension (and possibly hypotension) at admission are associated with a poor outcome after stroke. Hence, a ‘U’ or ‘J’ shaped curve appears to relate blood pressure and outcome, with the best outcome occurring with a systolic blood pressure of around 140-160 mmHg (figure 1a). Analysis of data from 17,398 patients with ischaemic stroke in the International Stroke Trial revealed that hypertension (systolic blood pressure > 180 mmHg) was associated with an increased risk of early re-infarction (figure 1b) and death from presumed cerebral oedema. A similar relationship was seen in the TAIST trial. Hypotension (systolic blood pressure < 120 mmHg) was associated with coronary heart disease events.
An obvious question is whether lowering blood pressure will improve outcome. However, no completed large randomised controlled trials (RCTs) have been reported which set out to assess the effect of lowering (or raising) blood pressure immediately after stroke. Hence, it remains unclear whether blood pressure should be actively altered during the acute phase of stroke. Indirect evidence argues both for and against lowering or elevating blood pressure (table 1).

Table 1. Evidence that lowering or raising blood pressure may be beneficial or detrimental (adapted from 25)

<table>
<thead>
<tr>
<th>Arguments in favour</th>
<th>Arguments against</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower BP</td>
<td>Cerebral autoregulation is lost after stroke; hence, perfusion might fall as blood pressure is decreased.</td>
</tr>
<tr>
<td>Case series in primary intracerebral haemorrhage.</td>
<td>Case series in ischaemic stroke</td>
</tr>
<tr>
<td>Observational study in ischaemic stroke.</td>
<td>β-adrenergic receptor antagonists and calcium channel blockers worsened outcome. Both drug classes are negatively inotropic, and calcium channel blockers can induce cardiac arrhythmias and steal, and reduce cerebral blood flow.</td>
</tr>
<tr>
<td>Blood pressure actively lowered in 'positive' alteplase trials.</td>
<td>Development of dextrophan, a NMDA ion channel blocker which lowers blood pressure, abandoned.</td>
</tr>
<tr>
<td>ACCESS trial of candesartan (angiotensin receptor antagonist) positive on secondary outcome (although treatment did not lower BP significantly).</td>
<td>Many antihypertensive drugs have mild-moderate antiplatelet activity. Such agents should probably be avoided in primary intracerebral haemorrhage.</td>
</tr>
<tr>
<td>Raise BP</td>
<td>Cerebral autoregulation is lost after stroke, hence, perfusion may increase as blood pressure is elevated.</td>
</tr>
<tr>
<td>----------</td>
<td>---------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>Induced hypertension (part of triple ‘H’ therapy) may improve outcome in subarachnoid haemorrhage.</td>
</tr>
<tr>
<td></td>
<td>Case series found that levarterenol or phenylephrine appeared to improve outcome.</td>
</tr>
<tr>
<td></td>
<td>Phenylephrine improved cerebral blood flow.</td>
</tr>
<tr>
<td></td>
<td>Hypertension is associated with forced vasodilatation, cerebral oedema, capillary vasoconstriction, and therefore hypoperfusion, and possibly reinfarction or bleeding. Vasoconstrictors could exacerbate this.</td>
</tr>
<tr>
<td></td>
<td>Acute stroke is associated with a raised cardiac output. Inotropes could exacerbate this, and contribute to cardiac ischaemia and failure.</td>
</tr>
<tr>
<td></td>
<td>Diaspirin cross-linked haemoglobin raised blood pressure and worsened outcome in ischaemic stroke.</td>
</tr>
<tr>
<td></td>
<td>Development of aptiganel, a NMDA ion channel blocker which raised blood pressure, abandoned.</td>
</tr>
<tr>
<td></td>
<td>Most sympathomimetics have pro-platelet effects. Such agents should probably be avoided in acute ischaemic stroke.</td>
</tr>
</tbody>
</table>

It is commonly held that blood pressure should not be lowered during the acute phase of stroke. First, acute stroke is associated with a loss of cerebral autoregulation such that lowering blood pressure might reduce cerebral perfusion. Hence, it is important to assess the effects of any hypotensive agent on cerebral blood flow. Second, the results of trials of two classes of antihypertensive agents, calcium channel blockers and β-receptor antagonists, suggest that these may worsen outcome (table 1).

However, it is inappropriate to extrapolate data from one class of antihypertensive agents to any other. Both calcium channel blockers and β-receptor antagonists have properties which may be detrimental, e.g. they are both negatively inotropic while calcium channel blockers may induce cerebral steal. In contrast, small studies involving other antihypertensive agents suggest that cerebral perfusion can be maintained in spite of moderate blood pressure lowering. For example, perindopril and losartan (angiotensin converting enzyme inhibitors) lowered blood pressure without altering global cerebral blood flow or middle cerebral artery blood velocity. Similarly, nitric oxide donors such as sodium nitroprusside and glyceryl trinitrate maintained regional cerebral blood flow whilst reducing mean arterial blood pressure (e.g. by ~10 mmHg) in studies of patients with acute ischaemic stroke. This contrasts with studies of calcium channel blockers which found that they can reduce cerebral blood flow. Third, the ACCESS trial, a small trial of candesartan in acute ischaemic stroke, was positive on a secondary outcome (combined cerebrovascular and cardiovascular events at 12 months). However, neither the primary outcome (death or disability) nor blood pressure were altered by treatment. Fourth, bendrofluazide, a thiazide diuretic used commonly for treating hypertension, does not lower blood pressure or alter cerebral blood flow in the acute stroke setting.
In a related question, one small trial examined how long antihypertensive medication should be continued once it has been started. Unfortunately, the study only involved 112 patients and older antihypertensive medications were used, including acetazolamide, clonidine, frusemide, and nifedipine. Continuing or stopping medication did not alter functional outcome although the trial was grossly underpowered to assess this measure. In the TONE trial, which assessed the effectiveness of non-drug therapy in controlling blood pressure in 975 elderly patients, secondary analysis examined the safety of stopping their antihypertensive therapy. Though patients with recent stroke or myocardial infarction were excluded, the results suggest that antihypertensive medication can be safely withdrawn without increasing cardiovascular events. In contrast to this, discontinuing antihypertensives could be detrimental; in a small study of just 40 patients with essential hypertension, post-ischaemic skin blood flow was decreased in those who had had their antihypertensive medication recently withdrawn.

Around 50% of patients admitted with acute stroke have a previous history of hypertension of whom more than two thirds are admitted taking one or more antihypertensive drugs (unpublished data from the stroke register, 1994-1998, at King’s College Hospital, London). In view of the uncertainties over whether to alter blood pressure, it is unsurprising that similar uncertainties exist on whether to continue or stop pre-stroke antihypertensive medication during the acute phase immediately post stroke. No randomised controlled trials addressing this question have been completed.

1.3 Current management of blood pressure immediately post stroke

In the absence of randomised evidence, current management of blood pressure immediately post stroke is largely derived from expert opinion. Arguments in favour of both treating, and not treating, hypertension have been promulgated over many years. Expert consensus recommends that patients with serious co-morbid conditions such as hypertensive encephalopathy, heart failure or ischaemia, aortic dissection, or continued intracerebral bleeding, should have their blood pressure lowered. Otherwise, guidelines have varied considerably and are often contradictory:

a) Do not lower blood pressure.

b) Do not lower blood pressure below certain levels.

c) Do not lower blood pressure by more than a given percentage.

d) Control blood pressure if it exceeds certain thresholds.

e) Lower blood pressure to below certain levels.

f) Treatment should start when post-stroke clinical conditions are stable.

More recent guidelines confirm that there is at present no evidence that lowering blood pressure has a beneficial effect in acute stroke. Other guidelines avoid specific advice all together. Until more definitive data are available, it is generally agreed that a cautious approach to the treatment of arterial hypertension in acute stroke is recommended.

We assessed typical practice by asking more than 100 UK physicians with an interest in stroke (comprising general physicians, geriatricians and neurologists) and attendees at the 8th European Stroke Conference (Venice, April 1999) to answer a questionnaire. The following points were revealed:

a) Physicians would intervene ‘routinely’ at extreme BP levels – high ≥ 220/120 mmHg, low ≤ 90/60 mmHg.

b) Decisions on whether to intervene would be tempered by other factors – a history of previous hypertension, known carotid stenosis, and the type of stroke (ischaemic or haemorrhagic).
c) Physicians varied in their opinion on whether to continue (48%) or stop (18%) pre-stroke antihypertensive medication.

d) Considerable uncertainty existed for all these questions and most respondents supported the need for a trial to help answer these questions.

1.4 Which hypotensive agent?

There are no definitive answers to this question. Nevertheless, existing information allows us to make an informed decision.

*Effect of candidate agents on blood pressure*

A number of vasoactive drugs lower blood pressure during the acute phase of stroke, including angiotensin converting enzyme inhibitors, angiotensin receptor antagonists, calcium channel blockers, magnesium and prostacyclin (table 2). In contrast, other vasoactive agents do not appear to alter BP, at least during the acute phase of stroke, e.g. naftidrofuryl and bendroflumethiazide.

*Effect of candidate agents on cerebral blood flow (CBF)*

Very limited, and inconsistent, work exists studying the effect of vasoactive drugs on cerebral blood flow. Haemodilution, naftidrofuryl, and piracetam may each improve CBF whilst nitrates, angiotensin converting enzyme inhibitors and angiotensin receptor antagonists appear to have no effect on it; conflicting data exists for calcium antagonists.

*Effect of candidate drug classes on functional outcome*

Definitive data only exist for calcium channel blockers (flunarizine, lifarizine, nimodipine, nicardipine) which had no overall beneficial or detrimental effect on outcome. Nevertheless, several of these studies found that these drugs worsened outcome, in some cases in parallel with their blood pressure lowering effect, e.g. INWEST. Evidence also suggests that β-receptor antagonists also worsened outcome after acute stroke. Several antihypertensive classes have no data relevant to acute stroke, e.g. alpha receptor antagonists and centrally acting drugs.

**Table 2. Drug classes for lowering blood pressure in acute stroke (updated from 25,47)**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Comments</th>
<th>Effect on death/dependency outcome in large stroke trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha receptor antagonists</td>
<td></td>
<td>No trial data</td>
</tr>
<tr>
<td>Angiotensin II receptor</td>
<td>May or may not alter blood</td>
<td>Neutral 42</td>
</tr>
<tr>
<td>antagonists</td>
<td>pressure 42,83</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maintain global cerebral blood</td>
<td></td>
</tr>
<tr>
<td></td>
<td>flow 83</td>
<td></td>
</tr>
<tr>
<td>Angiotensin converting enzyme</td>
<td>Maintain global cerebral blood</td>
<td>Not known</td>
</tr>
<tr>
<td>inhibitor</td>
<td>flow 61,89</td>
<td></td>
</tr>
<tr>
<td>Beta receptor antagonists</td>
<td>Negative inotrope. Effect on</td>
<td>Death increased 46</td>
</tr>
<tr>
<td></td>
<td>CBF unknown</td>
<td></td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>May reduce regional cerebral</td>
<td>Neutral 90</td>
</tr>
<tr>
<td></td>
<td>blood flow 50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blood pressure lowering associated with a worse outcome 48,49,91,92 Mildly</td>
<td></td>
</tr>
</tbody>
</table>
negatively inotropic

<table>
<thead>
<tr>
<th>Centrally acting drugs</th>
<th>Induces adverse central events, e.g. stupor, apnoea. Development in stroke abandoned</th>
<th>No trial data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrorphan</td>
<td>No known 93</td>
<td>51</td>
</tr>
<tr>
<td>Diuretics</td>
<td>Can cause haemoconcentration &amp; dehydration. No acute effect on BP 65</td>
<td>Not known</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>Maintains regional cerebral blood flow. 63,64 Donors may (sodium nitroprusside 63), or may not (glyceryl trinitrate 93), have antiplatelet effects. Used in alteplase trials 6,40,41</td>
<td>Not known 94</td>
</tr>
<tr>
<td>Prostacyclin</td>
<td>Intravenous</td>
<td>Not known 95</td>
</tr>
</tbody>
</table>

The biology of nitric oxide makes it an appealing candidate agent for lowering blood pressure and improving outcome after stroke. 96

**1.5 Physiology of nitric oxide**

Nitric oxide is synthesised from the amino acid, L-arginine, by nitric oxide synthase (NOS), a family of related enzymes:

*Endothelial NOS (eNOS)*

eNOS is present in vascular endothelium and produces nitric oxide constitutively. Endothelial nitric oxide regulates vascular tone causing vasodilatation, and inhibits platelet aggregation and phagocyte adhesion and migration. Nitric oxide production within endothelium may protect brain tissue from ischaemia since experimental studies have shown that:

a) 'Knockout' animals deficient in eNOS (which manifests itself as hypertension 97) have larger strokes than wild-type animals with the enzyme. 98-100

b) In mice, eNOS is a mediator for vascular endothelial growth factor (VEGF) and angiogenesis, regulates brain derived neutotrophic factor (BDNF) expression in the ischaemic brain and influences progenitor cell proliferation and neuronal migration and in turn affects functional recovery after stroke. 100

*Neuronal NOS (nNOS)*

nNOS is found in 2% of neurones and produces nitric oxide constitutively. Neuronal-derived nitric oxide is a neurotransmitter and appears to exacerbate acute ischaemic injury since experimental studies have shown that:

a) 'Knockout' animals deficient in nNOS have smaller strokes than wild type animals with the enzyme. 101,102 and demonstrate increased ischaemia induced neurogenesis. 102

b) Selective inhibitors of nNOS (7-nitroindazole, ARL 17477, PPBP) reduce stroke lesion size 99,103-105 and increase neurogenesis. 102
Inducible NOS (iNOS)
iNOS is an inducible enzyme present in immune cells (phagocytes) where it produces large quantities of nitric oxide used for killing bacteria, parasites and tumour cells. Nitric oxide derived from iNOS appears to worsen ischaemic stroke since experimental studies have shown that:

a) 'Knockout' animals deficient in iNOS have smaller strokes than wild type animals with the enzyme.\(^9\)

b) The selective iNOS inhibitor, aminoguanidine, reduces stroke lesion size.\(^{105-107}\) Inhibiting iNOS activity indirectly (with blockade of tetrahydrobiopterin, a co-factor for NOS activity) also significantly reduces infarct size.\(^{108}\)

1.6 Nitric oxide production in acute stroke

Acute stroke is associated with altered nitric oxide synthesis, measured as the stable metabolites nitrate and nitrite (NOx):

a) Intrathecal nitrate levels were reduced in acute stroke and negatively associated with final infarct size in 12 stroke patients.\(^\text{109}\)

b) Plasma nitrate concentrations were lower in patients with acute ischaemic stroke and primary intracerebral haemorrhage than controls in two studies.\(^\text{110,111}\) It is likely that this reflects reduced endothelial production of nitric oxide. Reduced endothelial nitric oxide synthesis could contribute to the cause of hypertension immediately post-stroke although this requires further study.

c) Plasma nitrate concentrations were positively correlated with baseline Glasgow Coma Scale, i.e. patients with severe stroke had lower nitrate levels.\(^\text{111}\)

d) Plasma nitrate concentrations were lower in patients who had a poor outcome resulting in in-hospital death or institutionalisation as compared with those who were discharged home.\(^\text{111}\)

e) In patients with ischaemic stroke, plasma NO concentrations are negatively correlated with lesion size.\(^\text{112}\)

f) CSF and plasma L-arginine concentrations (the NO precursor) are negatively correlated with infarct volume and were significantly lower in patients with early neurological deterioration and poor outcome.\(^\text{113}\)

1.7 Nitric oxide, a candidate treatment for acute stroke

Current treatments for acute stroke are characterised by having selective modes of action - alteplase is a thrombolytic drug whilst aspirin irreversibly blocks platelet cyclooxygenase. In contrast, nitric oxide is a multimodal agent that may modulate a number of detrimental pathophysiological changes associated with acute stroke. In particular, exogenous nitric oxide:

a) Will supplement low endogenous levels.

b) Lowers blood pressure in acute stroke,\(^\text{63,64,93,114}\) thereby potentially reducing the risk of stroke complications such as recurrence. Lowering blood pressure in haemorrhagic stroke may reduce the incidence of haematoma expansion and secondary infarction.

c) Is a cerebral vasodilator which improves or maintains regional cerebral blood flow in experimental\(^\text{115}\) and human acute ischaemic stroke.\(^\text{63,64}\)

d) Is an antileucocyte agent.\(^\text{116,117}\) Phagocytes, especially neutrophils and monocytes, constitute the main part of the inflammatory phase of stroke and may contribute to the loss of penumbra.
e) Antagonises the NMDA ion channel thereby potentially attenuating the glutamate cascade.

f) Can attenuate neuronal apoptosis, thereby potentially reducing neuronal loss in the penumbra.

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g) Enhances neurogenesis and angiogenesis (via synthesis of VEGF) in the ischaemic rat brain, hence could improve blood supply and help re-establish neuronal circuitry.

The relevance of these actions will depend, in part, on when nitric oxide is given after stroke onset. For example, putative neuroprotective effects would require administration within a few hours. Other mechanisms might be relevant in patients treated hours or even a few days later, e.g. preventing recurrent stroke. This multimodal nature of nitric oxide increases the likelihood of a trial involving it being positive. Since primary intracerebral haemorrhage could be complicated by surrounding ischaemia (perhaps due to local pressure effects), nitric oxide may also benefit patients with haemorrhagic stroke, providing it is given in a form that does not significantly inhibit haemostasis.

Other properties make nitric oxide, when administered as glyceryl trinitrate, attractive as a potential treatment for acute stroke. Glyceryl trinitrate:

a) Can be administered transdermally which is advantageous in dysphagic patients (where enteral access cannot always be guaranteed) and allows for a visual check on compliance.

b) Is effective and safe in acute coronary syndromes and heart failure (which commonly co-exist with stroke) and can increase cardiac output in such conditions.

c) Is readily available and inexpensive.

1.8 Pre-clinical studies of nitric oxide in stroke

Experimental studies involving cultured neurones and induced stroke in animals support the potentially beneficial role for nitric oxide in treating acute stroke. Nitric oxide or its donors:

a) Block NMDA-induced NMDA receptor-channel activity when given as S-nitrosocysteine, SIN-1, 1-nitrosopiperidline, or sodium nitrite.

b) Increase GABA levels thereby down-regulating neuronal excitability when given as nitric oxide or SNAP.

c) Increase cerebral blood flow when given as sodium nitroprusside, SIN-1, CAS 754, or spermine NO.

d) Reduce infarct size when given as sodium nitroprusside, SIN-1, or spermine NO. SNAP, another nitric oxide donor, has also conferred neuroprotection in ischaemic experimental stroke.

e) Reverse vasoconstriction secondary to experimental subarachnoid haemorrhage when given as S-nitrosothiolione.

f) Modulate neuronal plasticity (e.g. through learning, long-term potentiation, long-term depression) and could, therefore, improve recovery post-stroke.

g) Have potential to enhance neurogenesis after focal cerebral ischaemia and hence promote functional recovery.

Nevertheless, it appears that nitric oxide can be neurotoxic in certain circumstances:

a) Nitric oxide donors (sodium nitroprusside) can mediate glutamate neurotoxicity in a concentration dependent manner.
b) Low doses of nitric oxide donors (sodium nitroprusside) are neuroprotective and high doses neurotoxic (table 3) or ineffective.128,134

c) High doses of nitric oxide donors (sodium nitroprusside) which significantly reduce blood pressure (mean pressure < 50 mmHg) induce arterial boundary zone infarction in experimental subarachnoid haemorrhage.135

d) Nitric oxide in the form [NO+] is neuroprotective whereas [NO dot] is neurotoxic.136

Table 3. Effect of dose of sodium nitroprusside relative to control on outcome in rats exposed to transient middle cerebral artery occlusion (controls n=13) 134

<table>
<thead>
<tr>
<th>Sodium nitroprusside, iv dose (mg/kg/hour)</th>
<th>0.25</th>
<th>0.50</th>
<th>2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diastolic blood pressure (%)</td>
<td>-4</td>
<td>-9</td>
<td>-30</td>
</tr>
<tr>
<td>Lesion size, relative to control (n=16) (%)</td>
<td>68.6</td>
<td>75.1</td>
<td>∞</td>
</tr>
<tr>
<td></td>
<td>(n=7)</td>
<td>(n=9)</td>
<td>(n=5)</td>
</tr>
</tbody>
</table>

1.9 Pilot studies of nitric oxide in acute stroke

Four studies have examined nitric oxide donors in patients with acute stroke:

**Sodium nitroprusside**

Intravenous sodium nitroprusside, a spontaneous nitric oxide donor, was infused into 22 patients within 24 hours of the onset of ischaemic stroke, and 12 matched control subjects, in an open uncontrolled study.63 This study investigated the effects of sodium nitroprusside, given at a dose which reduced mean arterial blood pressure by 10 mmHg (<10%), on platelet function (aggregation and adhesion molecule expression) and cerebral blood flow (assessed using SPECT). Sodium nitroprusside significantly reduced platelet aggregation, and P-selectin and glycoprotein GP IIIa expression. In a sub-set of patients, sodium nitroprusside appeared to increase relative penumbral blood flow.65 Interestingly, patients required a lower dose of sodium nitroprusside to achieve the 10 mmHg fall in mean arterial blood pressure than did age-matched controls (0.19 versus 0.26 μg/kg/min, 2p=0.048) suggesting that patients with acute ischaemic stroke have a relative deficiency of vascular nitric oxide, as shown in studies of CSF and blood NOx and L-arginine levels,109-111,113 and are therefore supersensitive to exogenous nitric oxide.

However, sodium nitroprusside is unlikely to have wide utility in acute stroke since its effects on blood pressure require close monitoring and it produces cyanide if given continuously for several days.

**Glyceryl trinitrate**

Transdermal glyceryl trinitrate or matching placebo was administered daily to 37 patients within 5 days of stroke onset in a double-blind randomised placebo controlled trial.93 Glyceryl trinitrate significantly reduced 24 hour ambulatory systolic (7.9 & 5.6%) and diastolic (5.7 & 5.3%) blood pressure at days 1 and 7 respectively; there was evidence for tolerance, a known property of organic nitrates, at day 7 (figure 2). Glyceryl trinitrate had no effect on the expression of platelet adhesion molecules (GPIa, GPIb, GPIIIa, P-selectin).93 Patients treated with glyceryl trinitrate did not have more headache than controls.
In a second trial with GTN, 90 patients were randomised to transdermal glyceryl trinitrate (n=60) or open control (n=30) within five days (most < 3 days) of stroke onset. GTN was administered daily in three schedules: 5 mg for 10 days (n=20), 5 mg for 4 days then 10 mg for 6 days (n=20), and 10 mg for 10 days (n=20). 24 hour ambulatory blood pressure monitoring was performed on days 0, 1, 4, 5 and 10 (table 4).

Table 4. Effect of transdermal glyceryl trinitrate (by dose) on mean 24-hour arterial blood pressure (mmHg) in 90 patients with acute stroke.

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>5mg</th>
<th>5/10mg</th>
<th>10mg</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>109.4 (14.4)</td>
<td>109.0 (15.8)</td>
<td>111.3 (13.8)</td>
<td>109.4 (12.1)</td>
<td>109.9 (13.8)</td>
</tr>
<tr>
<td>Day 1*</td>
<td>108.8 (15.1)</td>
<td>102.5 (13.9)</td>
<td>103.4 (14.9)</td>
<td>101.5 (12.6)</td>
<td>102.5 (13.6)</td>
</tr>
<tr>
<td>Day 4</td>
<td>104.9 (16.8)</td>
<td>106.2 (12.5)</td>
<td>100.3 (14.4)</td>
<td>103.8 (11.9)</td>
<td>103.4 (13.0)</td>
</tr>
<tr>
<td>Day 5</td>
<td>103.0 (15.9)</td>
<td>106.4 (14.8)</td>
<td>96.1 (11.4)</td>
<td>102.9 (10.9)</td>
<td>101.9 (13.0)</td>
</tr>
<tr>
<td>Day 10</td>
<td>100.1 (15.7)</td>
<td>101.7 (12.4)</td>
<td>96.8 (10.1)</td>
<td>101.5 (13.6)</td>
<td>99.9 (12.1)</td>
</tr>
</tbody>
</table>

Mean (SD); comparison with one-way ANOVA adjusted for baseline; multiple comparison vs. control using Dunn’s test; *Primary outcome: comparison of GTN at each dose with control; †P<0.05; ‡P<0.01.

GTN significantly lowered mean arterial blood pressure on day 1 by 6.2% as compared with control subjects. When analysed by allocated group, a reduction in mean blood pressure was seen on day 1: 5 mg, 5.3%; 5/10 mg, 7.0%; and 10 mg, 6.7%, as compared with control (ANOVA P = 0.05). Increasing the dose from 5 to 10 mg on day 5 resulted in an overall reduction in blood pressure of 11.4% as compared with leaving the dose at 5 mg (P = .006). GTN at 10 mg non-significantly lowered blood pressure. Headache occurred more commonly in patients taking GTN: 9/60 (15%) versus 0/30 (0%) (p=0.027). One patient stopped GTN prematurely due to headache. Although the study was too small to reliably assess functional outcome, no difference was seen for death and dependency at 3 months between GTN and control.

A third trial of GTN (5 mg) investigated its parallel effects on CBF and BP at one hour post placement of the first patch. Whilst BP reduced by 23mmHg (14%), CBF did not change (table 5 and figure 3). Additionally, GTN did not alter cerebral perfusion pressure, suggesting that any tendency to increase intracerebral pressure through venodilatation was offset by increased blood efflux.
Table 5. The effects of GTN on peripheral and central blood pressure

<table>
<thead>
<tr>
<th>Blood Pressure</th>
<th>Reduction in BP mmHg (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>23 (14)</td>
<td>0.034*</td>
</tr>
<tr>
<td>Diastolic</td>
<td>4 (3)</td>
<td>0.47</td>
</tr>
<tr>
<td>Central</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>22 (13)</td>
<td>0.048*</td>
</tr>
<tr>
<td>Diastolic</td>
<td>4 (3)</td>
<td>0.55</td>
</tr>
</tbody>
</table>

*Significant values compared to control (p<0.05)

Figure 3: Quantitative cerebral blood flow (xenon CT) in (A) left middle cerebral artery acute ischaemic stroke; and (B) left PICH. Each panel: bottom left, CT scan; top left, CBF before GTN; top right, CBF with GTN; bottom right, difference in CBF. 64

A recent systematic review analysed the effect of GTN on systemic haemodynamics (figure 3). 137 GTN reduces haemodynamic measures known to be related to poor prognosis (blood pressure, pulse pressure and rate pressure product) in patients with acute/subacute stroke, and as such is a suitable candidate agent for testing whether BP should be lowered in patients with acute stroke.

Figure 3: Forest plot of the effect of NO donors on systolic blood pressure 137

Despite the lack of data available about handling BP immediately post stroke, thrombolysis trials have used various agents to lower acutely raised blood pressure. 41,138,139 In particular, once patients were enrolled into the NINDS rtPA trial, a blood pressure in excess of 180/105 in the first 24 hours...
post randomisation was treated. Agents used safely included both intravenous sodium nitroprusside and topical nitroglycerine.

1.10 Which nitric oxide donor and what route of administration

A number of nitric oxide donors with varying characteristics and administration routes are currently licensed (table 6). The route of administration is important when choosing a drug for the treatment of acute stroke (table 7). Glycerol trinitrate, given transdermally, appears to offer a straightforward approach to delivering nitric oxide in acute stroke. The absence of significant effects on platelet function suggests it can be given safely to patients with primary intracerebral haemorrhage.

Table 6. Nitric oxide donors

<table>
<thead>
<tr>
<th>Nitric oxide donor</th>
<th>Characteristics</th>
<th>Disadvantages</th>
<th>Route(s) of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyceryl trinitrate</td>
<td>Vasodilator</td>
<td>Nitrate tolerance</td>
<td>Intravenous</td>
</tr>
<tr>
<td></td>
<td>Produces [NO+]</td>
<td>No significant effect on platelets and phagocytes, so can be given in PICH.</td>
<td>Sublingual Transdermal</td>
</tr>
<tr>
<td>Isosorbide mono/dinitrate</td>
<td>Vasodilator</td>
<td>Nitrate tolerance</td>
<td>Intravenous</td>
</tr>
<tr>
<td></td>
<td>Produces [NO+]</td>
<td>Little, if no, effect on platelets and phagocytes</td>
<td>Oral Sublingual</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>Vasodilator Antiplatelet</td>
<td>Needs intra-arterial blood pressure monitoring. Forms cyanide if given for</td>
<td>Intravenous</td>
</tr>
<tr>
<td></td>
<td>63 Antileucocyte</td>
<td>several days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Produces [NO+]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7. Routes by which nitric oxide can be delivered immediately post stroke

<table>
<thead>
<tr>
<th>Route</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous, e.g. GTN, ISDN, SNP</td>
<td>Rapid onset</td>
<td>Requires close monitoring of blood pressure to titrate dose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time-consuming for staff to prepare</td>
</tr>
<tr>
<td>Oral, e.g. ISDN, ISMN</td>
<td>Easy to administer</td>
<td>Cannot be given safely to dysphagic patients (which complicates 33% of acute stroke) necessitating enteral access</td>
</tr>
<tr>
<td>Sublingual, e.g. GTN, ISDN</td>
<td>Rapid onset</td>
<td>Rapid onset and offset of effects thereby producing transient haemodynamic</td>
</tr>
<tr>
<td></td>
<td>Easy to administer</td>
<td></td>
</tr>
</tbody>
</table>
ENOS PROTOCOL, version 1.5, 10th July 2008
Philip Bath, telephone: +44 115 823 1768; fax: +44 115 823 1771; e-mail philip.bath@nottingham.ac.uk

| Transdermal, e.g. GTN | Easy to administer  
|                       | Steady state plasma levels achieved within 1 hour\textsuperscript{140}  
|                       | Effect can be reversed quickly by removing patch  
|                       | Dose can be adjusted by dividing the patch\textsuperscript{140}  
|                       | Compliance can be monitored visually  

\textit{Effects}
2. ENOS PROTOCOL

2.1 Aims of trial

1) Assess the balance of risk and benefit of lowering blood pressure with GTN immediately after ischaemic and haemorrhagic stroke.
2) Assess whether pre-stroke antihypertensive therapy should be continued or stopped temporarily after stroke.

2.2 Trial design

ENOS is a prospective, collaborative, international, multicentre, randomised, parallel-group, single blinded, controlled, factorial trial.

a) Intervention 1: routine care plus transdermal glyceryl trinitrate, or routine care and avoid transdermal glyceryl trinitrate.
b) Intervention 2: in patients on antihypertensive therapy immediately prior to the index stroke – routine care plus continue pre-stroke antihypertensive therapy, or routine care plus stop pre-stroke antihypertensive therapy (in addition to intervention 1).

2.3 Subjects

Patients presenting with an acute stroke syndrome with residual motor weakness within 48 hours of onset.

2.4 Inclusion criteria

a) Adult (age ≥ 18 years).
b) Clinical stroke syndrome with limb weakness lasting at least 1 hour (i.e. not likely to be a transient ischaemic attack).
c) Residual limb weakness at the time of enrolment (SSS Arm <6 and/or Leg <6, appendix C).
d) Onset ≤ 48 hours. If the time of onset is unknown, apply the time when the patient was last known to be well. [This timeframe covers the period of maximum uncertainty over altering blood pressure and should permit the vast majority of otherwise eligible patients to be recruited]
e) Conscious (Glasgow Coma Scale > 8).
f) Systolic blood pressure in range 140 mmHg to 220 mmHg inclusive on the basis of at least one of the three baseline pre-randomisation measures.
g) Independent prior to stroke (pre-morbid modified Rankin Scale ≤ 2).
h) Meaningful consent, or assent from a relative or carer if the patient is unable to give meaningful consent (e.g. in cases of dysphasia, confusion, or reduced conscious level).

2.5 Exclusion criteria

a) Definite need for nitrate therapy: e.g. concurrent myocardial infarction, unstable angina, left ventricular failure. Patients admitted on nitrates for the management of stable angina may stop these for the 7 day trial treatment period.
b) Contraindication to nitrates therapy: e.g. hypersensitivity to nitrates, dehydration, hypovolaemia, hypertrophic obstructive cardiomyopathy, aortic stenosis, cardiac tamponade, constrictive pericarditis, mitral stenosis, marked anaemia, closed-angle glaucoma, sildenafil (Viagra) or related drug, within 24 hours.

c) Definite need for pre-stroke antihypertensive, anti-anginal or anti-heart failure medication: e.g. concurrent angina, heart failure.

d) Definite need for new antihypertensive, anti-anginal or anti-heart failure medication during acute stroke: e.g. concurrent angina, heart failure, hypertensive encephalopathy, aortic dissection.

e) Patients expected, on the basis of existing investigations, to require surgical intervention (e.g. clot evacuation, carotid endarterectomy) during the treatment or follow-up period.

f) Known intracerebral pathology other than stroke, e.g. subarachnoid haemorrhage, brain tumour, cerebral abscess.

g) Other serious condition which is likely to prevent outcome assessment at 90 days, e.g. advanced cancer.

h) Previous enrolment in ENOS.

i) Current involvement in another trial of an experimental drug. [Patients may be randomised into observational studies or non-drug trials.]

j) Not available for follow-up, e.g. no fixed address, overseas visitor.

k) Females of childbearing potential where pregnancy cannot be excluded by a negative pregnancy test, pregnancy, or breastfeeding.

l) Need for new antihypertensive therapy to lower systolic blood pressure to achieve the enrolment range of 140-220mmHg

m) New (not prescribed pre-stroke) antihypertensive medication commenced after stroke onset.

### 2.6 Consent/assent

The informed consent of each patient will be obtained, in writing, by a clinical investigator. If the patient is unable to write (e.g. where there is dominant hand weakness, ataxia or dyspraxia), witnessed verbal consent as witnessed may be recorded on the consent form.

Alternatively, a relative or carer may give their assent for the patient to participate in the trial if the patient is unable to give informed consent (e.g. in cases of dysphasia, confusion, or reduced conscious level). If the patient regains capacity during the trial period they will be asked to sign a consent after assent form.

In the absence of relatives or carers able to give assent on behalf of an incapacitated patient, an independent physician may give assent where local rules allow.

The clinical investigator taking consent may be the doctor, nurse or therapist (physio, occupational or speech) depending on local practice.

These approaches are standard practice in acute stroke trials.

### 2.7 Co-enrolment into other trials

Concurrent uncoordinated co-enrolment of patients into two or more trials has the potential for introducing bias,\(^{141}\) e.g. when the treatments have a similar mechanism of action, when
interventions might interact, or when it would be difficult to ascribe adverse events to one or other intervention.

Patients should not be enrolled into ENOS if they are already in another drug trial. They may be enrolled into concurrent non-drug rehabilitation trials. A patient may enter another drug trial 90 days after the treatment phase of ENOS has finished.

2.8 Randomisation

All patients eligible for inclusion and for whom consent or assent has been obtained will be randomised centrally over the Internet. Randomisation will be performed using:

- Stratification on use of antihypertensive medication within 48 hours of stroke onset, admission blood pressure level (systolic blood pressure 140-160 mmHg, >160 mmHg), country and use of tPA.
- Minimisation on age, stroke type, stroke severity (SSS), history of stroke, history of hypertension, and history of nitrate use within previous two days.

Patients will have an equal chance of receiving GTN or control, and of continuing or stopping pre-stroke antihypertensive medication (if already on such medication).

In the event that the ENOS website cannot be accessed, patients may be randomised by telephoning the emergency randomisation number. These patients will be randomised without stratification or minimisation.

2.9 Treatment strategies

2.9.1 Investigational Medicinal Product

The ENOS trial compares patients randomised to receive transdermal glyceryl trinitrate (GTN, the active group) versus those randomised to receive no GTN (control group) for 7 days. Transdermal glyceryl trinitrate patches (5 mg per day, equivalent to 0.2 mg per hour) should be supplied by the local centre. No specific brand of GTN is recommended and any patch is acceptable as long as the Summary of Product Characteristics (SmPC, or its equivalent in some territories around the world) specifies a release of 5mg of GTN in 24 hours. GTN patches should be applied to the chest, upper arm or shoulders, and the area of placement rotated each day over the 7 day treatment period; a gauze dressing is placed over the GTN patch to maintain patient blinding. The GTN dose of 5 mg per day reduces 24 hour mean arterial blood pressure by approximately 8%. GTN must not be administered in another formulation (ointment, intravenous, sublingual) as part of the trial intervention. As the GTN patches are ‘ward stock’ (see section 2.9.5) there is no specific labelling used for the ENOS trial.

2.9.2 Control treatment

There is no placebo patch in the ENOS trial so the control group receive no GTN patch. However, patients are blinded to treatment (single-blind design) through the placement of a gauze dressing over the chest, upper arm or shoulders, with the area of placement rotated each day over the 7 day treatment period (see next section 2.9.4).

2.9.3 Timing of gauze dressing with or without GTN patch

The first gauze dressing, with a GTN patch if randomised to this, will be applied at the time of enrolment (day 1). Subsequent patches and coverings should be applied daily during a drug round.
(typically during the breakfast drug round) and kept on for a full 24 hours, i.e. not taken off overnight. As a result, the first gauze dressing/GTN patch may be in place for less than 24 hours depending on the time of randomisation.

Patients should only be entered into the trial if they are expected to remain in hospital for a further 7 days after randomisation. If a patient is discharged before day 7, they should, if possible, be given GTN patches to take home for daily application to complete the 7 days of treatment; we accept that a patient would lose blinding in this circumstance and that blood pressure measurements will not usually be available but this solution does ensure that a full course of treatment will be received, a key aspect of the trial.

2.9.4 Drug Supply and Handling
Hospitals/pharmacies should choose their own supplier of 5mg GTN patches, which may be usual ‘ward stock’. As is common with acute stroke trials, medication may best be dispensed and kept on the relevant ward or department ready for use as soon as the patient is randomised. It may be kept as ‘ward stock’ or as separate trial medication according to the practices of the randomising hospital. Once a patient is randomised to receive GTN, transdermal GTN patches should be prescribed, typically on the patient’s drug chart. GTN should then be recorded as having been administered to that patient using the ENOS GTN accountability log.

2.9.5 Continue versus stop pre-stroke antihypertensive medication
Patients receiving pre-stroke antihypertensive therapy will be randomised to continue or stop this for 7 days. This medication should not be given on admission if it is planned for the patient to be enrolled into the trial (see 2.9.1). If a patient is dysphagic, medication should be given via a nasogastric (NG) tube if the drugs can be crushed. Slow-release drug formulations should not be given via an enteral tube since ‘crushing’ damages the slow-release mechanism and can lead to acute hypotension and a short length of action. It is permitted to change a sustained release formulation for a non-sustained formulation of the same drug, for example ‘nifedipine SR’ could be changed to standard nifedipine given three times per day. However, the drug or class of medication should not be changed to allow administration through a NG tube for the 7 day treatment period of the trial. [This would amount to starting a new antihypertensive which is not permitted.] If medication is not received because of problems with enteral access (or any other reason) record it on the day 7 form. The dose of pre-stroke antihypertensives should not be altered during the 7 day treatment phase of the trial unless severe hypertension or hypotension occur - see sections 2.14 and 2.15. There is no specific method for blinding of the continue/stop part of the trial. After 7 days, antihypertensive therapy may be started, or re-started if stopped at admission, according to the treating doctors’ recommendations.

2.9.6 Blinding
ENOS is a single blind trial with the patient (but not investigators or clinical staff) blinded to treatment. Patient blinding is achieved by covering the GTN patch (if randomised to GTN) or an equivalent area of skin (if randomised to no GTN) with a dry gauze dressing. The gauze dressing (and GTN patch if randomised to this) should be changed and rotated as to its position daily. There is no specific method for blinding of the continue/stop part of the trial. 90 day follow up assessments (including the primary outcome) are carried out by national assessors blinded to clinical treatment.

2.10 Other Acute Interventions

Standard treatments may be given for the management of acute stroke, these including:
Hyperacute treatments
- Oxygen
- Fluids (e.g. saline) for rehydration
- Alteplase for ischaemic stroke (if used according to an approved protocol in centres where this is regarded as best medical care)

Acute treatments
- Admission to an Acute Stroke Unit
- Antibiotics for infection
- Insulin for hyperglycaemia
- Paracetamol for pyrexia
- Aspirin/antiplatelet for ischaemic stroke
- Leg compression stockings
- Nasogastric tube for administration of fluids, food and drugs
- Admission to a Stroke Rehabilitation Unit

Putative treatments which have not been shown to be effective should not be administered, these including corticosteroids, glycerol and haemodilution. Similarly, experimental drug treatments should not be co-administered.

Patients expected to need surgical intervention (e.g. clot evacuation, hemicraniectomy, carotid endarterectomy) during the treatment or follow-up period should not be enrolled into the trial.

All treatments should be noted on the day 7 form.

2.11 Outcome measures

The main follow-up is to be performed centrally at 90 days, a standard period for most acute stroke trials. Internationally, follow up will usually be carried out by the National Coordinating Centre except where countries with multiple languages require additional assessors in other centres. In the UK the ENOS International Coordinating Centre hosts the National Coordinating Centre. Central follow-up will ensure that blinding to treatment group is ensured. Events are defined in Appendix I.

Primary:
The proportion of patients who are dead or dependent at 90 days (end of follow-up), based on the modified Rankin scale (score of 3-6, where death = 6).

Secondary:
Events at or within 7 days (enter on ‘day 7’ form):
- Recurrent stroke.
- Scandinavian Stroke Scale (SSS) score.
- Symptomatic deep vein thrombosis.
- Symptomatic pulmonary embolism.
- Adverse events - headache, symptomatic hypotension.

Haemodynamic (enter on ‘day 7’ form):
- Blood pressure daily between 1 and 7 days (see section 2.13).

Hospital events (enter on ‘hospital events’ form):
- Length of stay in hospital.
b) Discharge disposition (death, institution, or home).
c) Time at home

Outcome at 90 days (to be collected centrally):
   a) Barthel Index (< 60/100, including death).
   b) Barthel Index (< 60/100, excluding death), i.e. disability.
   c) Barthel Index ≥ 95/100 at 90 days, i.e. an excellent outcome 5,142.
   d) Quality of life – EuroQol 143.
   e) Cognition - , TICS-M144 and verbal fluency145. (see appendix G)
   f) Mood – Zung Depression rating Scale 146,147.
   g) Disposition.
   h) Re-hospitalisation following discharge.

Safety measures at 7 days (enter on ‘day 7’ form):
   a) Death.
   b) Neurological deterioration. (negative change in SSS).
   c) Symptomatic intracranial haemorrhage.
   d) Major extracranial haemorrhage.
   e) Symptomatic hypotension.
   f) Symptomatic hypertension.

Safety measures at 90 days:
   a) Death.

2.12 Brain imaging

2.12.1 Neuroimaging
Neuroimaging is not required prior to enrolment in the trial. However, all patients must have a CT or MR brain scan performed for the presenting stroke episode either prior to enrolment or during the first 7 days following randomisation to:
   a) Exclude a non-stroke cause, e.g. cerebral tumour, cerebral abscess.
   b) Diagnose primary intracerebral haemorrhage.

This first scan will usually be the clinical scan as required for all stroke patients. Each local investigator should enter the result of this baseline CT or MR scan on the Day 7 case report form. Neuroimaging data should be uploaded electronically via the ENOS website to the trial’s database; data uploading and alternative methods for submitting the scan are given below in the Scan transfer section (2.12.2).

If a second CT or MR scan is performed for clinical reasons (e.g. deterioration) within the 7 day trial treatment period, the scan should be submitted to the ENOS International Coordinating Centre as in section 2.12.2 below.

If the baseline scan was performed before randomisation, centres should obtain, where possible, a second CT or MR scan on day 7±1; neuroimaging data submitted as in section 2.12.2 below. This additional scan is not a compulsory part of the trial but, if available, will be compared with the baseline scan to assess the evolution of the stroke lesion (size, development of haemorrhagic transformation if an infarct) and appearance of early recurrent lesions. An additional payment is available for each day 7 scan performed providing data from the pre-randomisation scan and the
other trial documentation (see section 2.21.1) has been submitted to the ENOS International Coordinating Centre.

<table>
<thead>
<tr>
<th>Time of Baseline Scan</th>
<th>Before Randomisation</th>
<th>After Randomisation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Additional Clinical Scans (if done) (Day 1 - 7)</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Day 7 ENOS Scan (Day 7 +/- 1 day)</td>
<td>✓</td>
<td>✗</td>
</tr>
</tbody>
</table>

2.12.2 Scan transfer and storage
CT and/or MR brain scans at baseline and day 7 should be sent electronically over the web, on a CD or DVD, or by film (the latter two mailed to the ENOS International Coordinating Centre in Nottingham). Ideally, investigators should use the secure internet upload facility provided on the ENOS website which includes automatic anonymisation of images. If films are posted, these will be digitised and the resulting data, along with that submitted on CD/DVD, will be anonymised. All digital brain image data will be stored on computer servers for analysis and archiving. The systems have been designed to ensure the highest levels of data security and patient confidentiality, and will be further enhanced if future technological advances permit it. The enhancements to the current system may include the use of e-Science and Grid technologies if they prove to be superior to current systems. The use of e-Science infrastructure within the MRC NeuroGrid project for the ENOS imaging data could: ensure more reliable, secure and confidential archiving of the imaging data; connect sites for rapid and secure flow of data; enable distributed data analysis with image analysis tools; enhance collaborative working between members of the research team; and, improve the power and applicability of studies.

2.12.3 Assessment of brain images
All brain scans (baseline and day 7) will be assessed by at least one expert reader, using either a workstation or by means of a web-based image assessment tool, both of which present anonymised images to the reader. In the case of web-based adjudication, the image data remain on the trial server with the system presenting anonymous images in Joint Photographic Experts Group (JPEG) format (with no personal or demographic or other information). Adjudication of brain images is performed via a secure internet link to the ENOS database using a structured questionnaire. The assessor, who is blinded to treatment allocation, records their interpretation of the scan by means of the on-screen questionnaire and adjudicates on the presence of stroke, haemorrhage, occluded arteries, ASPECT score, mass effect, white matter disease, atrophy, and other visible lesions. The independent image adjudicators will advise on the conduct of this work.

2.13 Blood pressure measurement
As a central aim of this study is to ascertain the effect of lowering blood pressure immediately post stroke, it is vital that blood pressure is measured in an accurate, reproducible, unbiased, and
validated manner. Measurements made using routine ward mercury or aneroid sphygmomanometers, or most semi-automatic devices, are not sufficient in these respects.

All blood pressure measurements should be performed using an Omron 705CP or 705CP II automated blood pressure monitor. This device has been validated by the British Hypertension Society, in contrast to other automated devices which have not been found to be accurate or reliable, and is the monitor used in the recent ASCOT hypertension trial involving 20,000 patients. Baseline systolic and diastolic blood pressure and heart rate data are taken in triplicate (3 measurements taken in immediate succession) and readings entered on the baseline form. Subsequent blood pressures should be measured in duplicate (2 readings taken in immediate succession) for 7 days in the non-paretic arm with the patient supine or sitting 1-2 hours after the placement of the daily gauze dressing (and GTN patch in those randomised to receive this). BP and heart rate readings should be printed out using the Omron printer and attached to the Omron ‘print-out’ sheet. The interval of 1-2 hours between gauze/patch placement and measurement of BP means the peak BP – lowering effect of treatment will be recorded. BP measurements and their timings should be entered on the day 7 form. Two Omron monitors will be supplied to each centre and should only be used for patients in the ENOS trial. These monitors will be checked by staff from the National Coordinating Centres during site visits. The ENOS International Coordinating Centre will provide new Omron machines or calibrate existing machines every 3 years.

2.14 Significant falls in blood pressure during treatment

Significant falls in blood pressure, or frank hypotension, may occur with glyceryl trinitrate in the presence of clinical or biochemical hypovolaemia or dehydration, e.g. with an elevated urea or packed cell volume. Patients with clinical evidence of dehydration should not be enrolled in the trial until they have been treated with crystalloid (saline) or colloid solutions.

If symptomatic falls in blood pressure occur, e.g. with ‘faintness’ or neurological deterioration (see appendix I), standard treatments should be given including raising the patient's legs, administering intravenous saline or colloid, or remove the glyceryl trinitrate patch if hypotension is sustained. Following adequate hydration, it should, in most cases, be possible to restart glyceryl trinitrate therapy on the next day, perhaps initially at a lower dose if available.

2.15 Severe hypertension

The natural history for blood pressure during acute stroke is to fall over the first week. However, it may fluctuate significantly and transient increases in blood pressure to levels above those at admission are common. If very severe and sustained hypertension (systolic blood pressure > 220 mmHg) develops during the 7 day treatment phase of the trial, the investigator may treat as per local guidelines, or use this suggested stepped and systematic approach to its management.

In general:
  a) Monitor closely.
  b) Treat with open label glyceryl trinitrate (patch, paste or intravenous), or labetalol (intravenous) as per the NIH NINDS rtPA trial 29 (labetalol 10mg intravenously over 1 to 2 minutes; the dose may be repeated or doubled every 10 to 20 minutes, up to 150 mg). This must be in addition to continuing the randomised treatment(s). Stop this open-label treatment once blood pressure levels settle.
Pre-stroke antihypertensive therapy:
   c) Where possible, avoid increasing (if randomised to continue this) or re-starting (if randomised to stop this) pre-stroke hypertensive therapy since most oral antihypertensive treatments take several hours or even days to start working effectively.

2.16 Headache
Headache is a common complication after stroke and usually resolves after a few days. GTN can also cause headache (‘nitrate headache’) and this too usually resolves after a few days. If headache occurs during the 7 day treatment phase, we suggest the following systematic approach to its management:
   a) Reassure the patient that headache is common after stroke, whether or not GTN has been given, and that it wears off over after a few days.
   b) Administer paracetamol (acetaminophen) orally, by nasogastric tube, or rectally.
   c) Remove the GTN patch for that day and try to re-start it the next day

2.17 Carotid duplex scanning
All patients with ischaemic stroke should have, where possible, carotid duplex scanning during the treatment or follow-up phase of the trial to identify cases of significant ipsilateral internal carotid stenosis. This will allow the interaction between blood pressure lowering, carotid stenosis, and outcome to be studied. Funding is available to support carotid studies in patients who do not warrant one on clinical grounds, e.g. due to severe stroke with poor prospect of recovery where carotid endarterectomy would not be considered. However, it is not compulsory to perform carotid duplex scanning as part of the ENOS trial if local centres cannot accommodate.

2.18 Serious Adverse Events

2.18.1 Adverse Events
Adverse events will not be recorded due to their high incidence in stroke patients and the established nature of the trial interventions.

2.18.2 Serious Adverse Events (SAEs)
SAEs will be recorded by the local investigators using the web-based SAE form. SAEs should be completed within 24 hours of investigators being aware of the event. They are recorded during the 90 day trial period but are not required once 90 day follow up is complete. SAEs are those which are:
   • Fatal
   • Cause disability
   • Are Life Threatening
   • Lead to hospital admission or prolong discharge in a hospitalised patient
   • Birth defects in children born to trial participants
If a SAE occurs this information should be entered onto the SAE webpage. Likely causality (see section 2.18.2.1) and whether the SAE fulfils the criteria for a SUSAR (see section 2.18.3) should be entered.
2.18.3 Causality

**Unrelated or improbable**: a clinical event including laboratory test abnormality with temporal relationship to trial treatment administration which makes a causal relationship incompatible or for which other drugs, chemicals or disease provide a plausible explanation. This will be counted as “unrelated” for notification purposes.

**Possible**: a clinical event, including laboratory test abnormality, with temporal relationship to trial treatment administration which makes a causal relationship a reasonable possibility, but which could also be explained by other drugs, chemicals or concurrent disease. This will be counted as “unrelated” for notification purposes.

**Probable**: a clinical event, including laboratory test abnormality, with temporal relationship to trial treatment administration which makes a causal relationship a reasonable possibility, and is unlikely to be due to other drugs, chemicals or concurrent disease. This will be counted as “related” for notification purposes.

**Definite**: a clinical event, including laboratory test abnormality, with temporal relationship to trial treatment administration which makes a causal relationship a reasonable possibility, and which can definitely not be attributed to other causes. This will be counted as “related” for notification purposes.

All SAEs will be adjudicated independently as to causality.

2.18.4 Withdrawals

The study interventions are given for 7 days. It may be necessary to withdraw GTN for medical reasons (e.g. unacceptable side effects, significant hypotension requiring intervention) or if a patient withdraws consent for continuation of treatment. If the diagnosis turns out to be non-stroke during the 7 day treatment period, investigators should decide whether it is appropriate re-instigate pre-stroke antihypertensives (if applicable) and/or continue GTN (if applicable). In these instances, all follow up assessments should be carried out according to the protocol.

If a patient withdraws consent to randomised treatment, they should be asked whether they are still willing to have follow-up information collected, e.g. at day 7, hospital discharge and day 90. The patient may withdraw from all trial activities at their request at any stage.

2.18.5 Suspected Unexpected Serious Adverse Reactions (SUSARs)

SUSARs are serious adverse reactions which are serious (as defined for SAEs), unexpected (i.e. they are not recognised reactions for the trial interventions), and are likely to relate to treatment rather than being a complication of stroke, i.e. they occur during treatment. Investigators should enter on the SAE form whether SAEs are being submitted as a SUSAR. The long established nature of the interventions in ENOS, e.g. glyceryl trinitrate and licensed antihypertensive agents, make the occurrence of SUSARs very unlikely. Assessment of SUSARs by the Independent Adjudicator will be expedited to comply with the European Union Clinical Trials Directive (as enacted in the UK in May 2004); if the Independent Adjudicator agrees that a SAE is a SUSAR, the ENOS International Coordinating Centre in Nottingham, UK will inform the trial’s sponsor (the University of Nottingham), UK regulator (MHRA), UK MREC, Chairman of the DMEC, and all Principal Investigators (worldwide) by e-mail or fax.

Known adverse reactions for glyceryl trinitrate, antihypertensive agents, and stroke complications are provided for Investigators.
2.19 Blood samples

Tertiary questions in ENOS include assessing the effects of the interventions on blood biomarkers, and whether a patient’s genotype alters response to the interventions. These blood measures are optional although the power of statistical analyses of them will depend on the number of patients who contribute blood samples. Centres who wish to participate in the blood biomarker study should have appropriate storage facilities including access to a centrifuge and freezer.

Blood samples should be taken at baseline (4 ml into EDTA, 8 ml clotted) and on day 7±1 (8 ml clotted); if it is not possible to take a blood sample at enrolment, both clotted (8 ml) and EDTA (4 ml) samples may be taken on day 7. Clotted (serum) samples should be centrifuged prior to freezing; the EDTA samples should be frozen without centrifugation. Baseline and day 7 blood samples should be anonymised (identify them with the centre number, patient number, patient initials, and date of sample) and stored locally in a freezer at -20°C (or lower if possible at -60°C to -80°C) and accounted for using the Blood Sample Freezer Log. The ENOS International Coordinating Centre will arrange transfer of blood samples to Nottingham UK, for analysis. Blood samples will be destroyed once analysis is completed, this being dependent on the trial’s completion date.

2.18.1 Soluble markers of outcome and efficacy
Several blood biomarkers are surrogate markers of outcome, such as S-100 and neurone specific enolase. However, whether they and other blood factors (to be identified during the course of the ENOS trial) are also markers of the efficacy of interventions has yet to be determined.

2.18.2 Genetic studies
The consent/assent forms allows the patient/relative to opt-into the genetic sub-study through initialising a separate box on the form. Patients may continue in the trial if they or their next-of-kin elect not to consent to the genetics sub-study. The patient or next-of-kin may request destruction of the genetic samples at any time after consent and prior to creation of an anonymised database.

An important aim of the genetic analyses is to determine whether polymorphic differences in candidate genes explain blood pressure and outcome responses to GTN (pharmacogenetic analysis). The exact genetic analyses to be performed are undefined at present and will depend on relevant scientific information available at the time of laboratory analysis and prior to sample destruction. Likely analyses will include genes related to the synthesis and metabolism of nitric oxide (e.g. endothelial nitric oxide synthase) and the mechanism of action of antihypertensive agents (e.g. polymorphisms in receptors).

2.20 Sample size

2.20.1 Glyceril trinitrate or control
The required sample size for the trial is 5,000 subjects (2,500 per group). The sample size calculation is based on the potential efficacy of glyceril trinitrate and assumes the following parameters:
  a) An overall significance (alpha) of 5%. (This is standard in efficacy trials in acute stroke.)
  b) A power (1-beta) of 90%. (This is higher than for most stroke trials but reduces the risk of a false negative result/type II error.)
  c) A control rate for day 90 Rankin score 3-6 of 50%. (This figure is reasonable in the light of recent trials in acute stroke.)
d) An event rate in the glyceryl trinitrate group of 45%.

e) The difference in control and treatment rates results in an absolute risk reduction (ARR) of 5% and relative risk reduction (RRR) of 10%. (Achieving an absolute 5% reduction in the risk of a poor outcome would make glyceryl trinitrate more effective than aspirin [ARR 1.3%, \textsuperscript{12}] but less effective than intravenous and intra-arterial thrombolysis [ARR 12-25% \textsuperscript{4-6,150,151} and hemicranieotomy [ARR 51%\textsuperscript{152}].)

The sample size calculation takes account of losses due to non-compliance (5.6% of patients could not complete 6 weeks of treatment with transdermal glyceryl trinitrate in GISSI-3 \textsuperscript{153}) or patients lost to follow-up (3% in the International Stroke Trial \textsuperscript{1}).

Sufficient patients will be enrolled in three subgroups to test the efficacy of glyceryl trinitrate versus control in:

a) Ischaemic stroke - 4000 patients to detect an ARR of 6% with 90% power.

b) Hypertensive subjects - 2000 patients recruited with systolic blood pressure > 160 mmHg to detect an ARR of 8% with 90% power.

c) Patients treated early - 2000 patients recruited within 12 hours to detect an ARR of 8% with 90% power.

2.20.2 Stopping or continuing pre-stroke antihypertensive therapy

The power of the analysis assessing the effect of continuing or temporarily stopping pre-stroke antihypertensive medication is 90%, assuming:

a) 40% of patients (~2000) will be taking antihypertensive medication immediately prior to their stroke (based on data from 1,595 patients admitted to King’s College Hospital (London, UK) stroke service \textsuperscript{154,155} between 1994 and 1998).

b) An ARR of 8%.

However, it is likely that assumption (a) is low since rates of treatment for essential hypertension are increasing; the rate in the trial after recruitment of 774 patients was 47%. Hence, the detectable absolute risk reduction is likely to be less than 8%. It is important to note that there are no completed studies investigating this question so ENOS will provide important information, whatever its findings. Additionally, the single-blind nature of the comparison of GTN with control means there would be a potential for bias whereby investigators based decisions on continuing or stopping pre-stroke antihypertensive therapy according to GTN randomisation. Randomising patients to continue or stop pre-stroke antihypertensive therapy will remove the potential for this bias.

2.21 Data management and analysis

2.21.1 Data Management

Data will be collected on digital forms submitted over the internet. The forms will relate to:

a) Baseline data form.

b) Day 7 form (end of treatment).

c) Hospital event/discharge form (to be completed at discharge form hospital, death prior to discharge or at 90 days if still an inpatient).

d) 90 day outcome form. This will be filled in at the National Coordinating Centre on the basis of central, blinded telephone, postal or clinic follow-up.

e) Serious Adverse Event form.
f) CT/MRI adjudication forms –to be completed by the independent neuroradiology adjudicator.
g) SAE adjudication form.

Where Internet access is not close to the patient, data will be entered onto paper case report forms (CRF) as an ‘aide memoire’. The baseline data must be entered immediately over the Internet (see section 2.8 Randomisation for advice on internet failure). Other assessments must be carried on time and submitted electronically within 7 days of collection. Paper CRFs should be kept as source documents at the recruiting site in the patient trial file. Data entered over the Internet will be stored directly into an electronic database managed at the University of Nottingham. It will be checked for completeness, consistency and accuracy in ‘real-time’ and errors highlighted immediately for correction over the Internet. Other data queries will be returned to the investigator by e-mail. If a patient dies after discharge from hospital and prior to the 90 day follow-up, the local investigator should, where possible, inform their National Coordinating Centre and the ENOS International Coordinating Centre using the supplied Serious Adverse Event form.

The following information should be faxed together to the ENOS International Coordinating Centre within 3 weeks of enrolment; please state the patient and centre details on the fax cover sheet:

a) Patient contact details including patients own address and telephone, those of the GP and next of kin (x 2 if possible) and NHS number if applicable
b) Consent / assent form and consent after assent form if applicable
c) Omron blood pressure print out sheet.
d) A photocopy of the part of the drug prescription chart relating to the 7 day trial treatment period
e) CT / MR report(s)
f) Carotid ultrasound reports.

2.21.2 Analysis
Analyses will be by intention-to-treat and will be performed once the trial database has been locked.

The principal analyses will assess the effect of:

a) Transdermal glyceryl trinitrate,
b) Continuing or stopping pre-stroke antihypertensive medication,

don the combined outcome of death or dependency (modified Rankin Scale of 3-6).

The effect of the two interventions on the primary outcome will be performed within the following subgroups of subjects:

a) By age - ≤ 75 years, > 75 years.
b) By gender – male, female.
c) By stroke type – ischaemic stroke, haemorrhagic stroke.
d) By stroke sub-type – lacunar, cortical, posterior fossa.
e) By stroke severity – severe (SSS ≤30), moderate/mild (SSS >30).
f) By baseline blood pressure – severe hypertension (systolic blood pressure > 160 mmHg), mild hypertension (systolic blood pressure 140-160 mmHg).
g) By blood pressure change between day 1 and baseline – change in mean arterial blood pressure < 5%, ≥ 5% to 15%, >15%
h) By cardiac rhythm – sinus rhythm, atrial fibrillation/flutter.
i) By treatment delay - ≤ 6 hours, > 6 to ≤ 12 hours, > 12 to 24 hours, >24 hours.
j) By history of prior hypertension – previous hypertension, no previous hypertension.
k) By history of recent nitrate exposure – recent use, no recent use.
l) By patients receiving open label tPA.

Logistic regression analysis will be used to assess the effect of the interventions on the primary outcome corrected for important baseline prognostic factors as listed above. Time to death will be analysed using Kaplan Meier statistics and Cox regression will be used to adjust for baseline prognostic factors. The potential for interaction between glyceryl trinitrate and pre-stroke antihypertensive medication will also be investigated. Secondary analyses will assess the interventions on outcomes related to early, end-of-trial, and safety events, as listed in section 2.11, outcome measures.

2.22 Monitoring of centres

Trial data will be checked for validity and internal consistency, and measures taken to identify any scientific misconduct. Following recruitment of each patient, the consent (assent) form, CT +/- carotid duplex results will be faxed to the ENOS International Coordinating Centre to confirm that the patient exists and that they (or their relative) have given valid consent (assent). Similarly, the drug chart (that part relating to the 7 day trial treatment period) will be faxed to the ENOS International Coordinating Centre to confirm that the randomised treatment was given.

2.22.1 Site Monitoring Visits

Centres will be visited at least once during recruitment and source data examined to:

a) Ensure each patient exists.
b) Ensure that a valid consent/assent form is present in the hospital notes.
c) Check version control
d) Confirm patient demographics, stroke type, blood pressure readings.
e) Confirm that clinical data provided matches source documentation.
f) Confirm prescription of the trial drug(s).
g) Ensure the site is meeting its responsibility for the maintenance of the site trial file.
h) To ensure compliance with Good Clinical Practice (GCP) / International Conference on Harmonisation (ICH).

2.22.2 Protocol Violations and Deviations

A Protocol Violation is a deviation from the trial protocol where a patient is included outside the inclusion/exclusion criteria for the trial, and where deviations from the protocol could affect the trial delivery or interpretation significantly.

A Protocol Deviation is a minor deviation from the protocol that affects the conduct of the trial in a minor way. This includes any deviation from the trial protocol that is not listed as a protocol violation.

A complete list of protocol violations and some examples of protocol deviations are contained in appendix L.
2.23 Data Monitoring and Ethics Committee (DMEC)

Efficacy and safety aspects of the trial will be monitored by a Data Monitoring and Ethics Committee (DMEC) which will consist of three independent members (see 2.27, Trial Administration). The DMEC will receive unblinded data from an independent statistician (Paul Silcocks of the University of Nottingham’s Clinical Trials Support Unit), the trial statistician will support both the independent statistician and queries from the DMEC but will remain blinded to treatment assignment. Data (defined in section 2.11, Outcome Measures) will be given by treatment groups: (i) GTN versus No GTN (ii) Continue versus stop pre-stroke antihypertensive medication (iii) GTN and continue antihypertensives versus GTN and stop antihypertensives versus No GTN and continue antihypertensives versus No GTN and stop antihypertensives. As well as outcome measures the DMEC will also review recruitment, baseline data and its balance areas in the treatment group, completeness of data, compliance to treatment, co administered treatments, and outcome by sub groups. They will also review and adjudicate serious adverse events, SUSARs and protocol violations (section 2.32). The DMEC will usually meet yearly by teleconference and the chairman will usually receive 6 monthly updates from the independent statistician.

The Data Monitoring and Ethics Committee may request extra data or further analyses if a safety concern arises and, if appropriate, recommend to the Trial Steering Committee to halt the study or amend the trial protocol. Such recommendations might include limiting recruitment to patients:

- a) With a baseline blood pressure in a particular range.
- b) With one or other stroke type.
- c) Presenting in a particular time period after stroke onset.
- d) Presenting with a particular range of severity.

The Trial Steering Committee, collaborating investigators and trial administrative staff will remain ignorant of the results of these analyses unless the trial protocol needs to be altered or the trial stopped.

The Data Monitoring and Ethics Committee will develop their own rules for recommending that the trial should stop or its protocol be altered but these should take account of the following factors:

- a) A difference of at least 3 standard deviations should be present in any analysis before triggering any change in trial activity.
- b) Concerns over safety are more important then efficacy, i.e. the stopping rules should be asymmetric.
- c) Consistency should be present among the safety variables if they are going to be the basis for altering the conduct of the trial, i.e. a difference in neurological deterioration between the treatment groups is likely to be paralleled by a difference in early death and late death/dependency rates.
- d) Short-term disadvantages may be associated with late advantages at the end of follow-up, e.g. an increased early rate of symptomatic haemorrhage may be associated with improved outcome at 90 days (as seen with thrombolysis).

2.24 Secondary prevention

Unless there are clear contraindications, patients with ischaemic stroke should receive optimal secondary prevention which should be reviewed once the 7 day treatment phase with glyceryl trinitrate has finished. Measures include:
a) Antithrombotic therapy – aspirin, dipyramidamole or clopidogrel. Aspirin should be started once CT scanning has excluded primary intracerebral haemorrhage. Dipyramidamole or clopidogrel should be prescribed according to local guidelines.

b) Anticoagulation therapy – patients should receive an oral anticoagulant if they have non-rheumatic atrial fibrillation and have no contraindication to it.

c) Antihypertensive therapy – pre-stroke hypertensive medicines should be re-started, if stopped at admission, once the 7 day treatment phase has finished. If patients remain hypertensive after 1 week and were not on medication prior to their stroke, then investigators should consider starting therapy, e.g. with a thiazide diuretic and/or angiotensin converting enzyme inhibitor.\(^{156,157}\)

d) Lipid lowering therapy – with an ischaemic stroke patients should usually be treated with cholesterol lowering therapy (such as statins) according to current guidelines.

e) Diabetes – good glucose control should be maintained after stroke.

Patients with primary intracerebral haemorrhage should have their blood pressure controlled as in (c) above.

2.25 Trial practice and procedures

2.25.1 Good Clinical Practice / International Committee for Harmonisation
The trial will conform to the Medical Research Council Guidelines for Good Clinical Practice in Clinical Trials, the International Committee for Harmonisation and the EU directive where applicable.

2.25.2 Internet
Randomisation and data registration will be performed over the internet using a standard ‘web’ browser. Access to the trial server will be controlled by usernames and passwords. Data exchange between local computer and server will be encrypted (128 bit) in both directions.

2.25.3 Trial Specific Documents
Trial specific documents are prepared by the ENOS International Coordinating Centre and are version controlled and dated. Approved versions must be used and should be on headed note paper from the recruiting hospital. Trial specific documents require ethical approval.

Updated documents will be distributed to the local centres by email as well as being mounted on the trial website. Centres should confirm receipt of the new version by email. Following this, the new version should be used with all patients and one copy of each of the old versions should be archived in the site trial file. All up to date documents are also available on the website.

Within individual countries participating in the ENOS trial it may be necessary to update versions of trial specific documents according to law that do not apply to other countries. The additional burden of retranslating documents is also noted. As such, National Coordinating Centres may choose not to update their versions, as long as the documents continue to fulfil legal obligations in the country of use. It is acknowledged that individual countries may have specific requirements e.g. Mauri regulations in New Zealand. Therefore, different countries may have different version numbers from one another. All centres within a single country will use the same version number for any given document.
2.26 Liability

2.26.1 UK
Insurance and indemnity for trial participants and trial staff is covered within the NHS Indemnity Arrangements for clinical negligence claims in the NHS are issued under cover of HSG (96)48. The University of Nottingham as trial sponsor maintains trials insurance for the ENOS trial and will indemnify all other parties for negligent and non-negligent harm as a result of adherence to the protocol.

2.26.2 International
The University of Nottingham as trial sponsor maintains trials insurance for the ENOS trial and will, generally speaking, indemnify all other parties for negligent harm as a result of adherence to the trial protocol. However, insurance is not uniform across countries and international centres may need to make their own provision to cover their participation in the trial, including non-negligent harm. The University of Nottingham may not take out a separate clinical trials insurance policy for a country if it is standard practice for investigators to be covered for negligent harm within clinical trials as a result of adherence to the trial protocol under their usual medical indemnity policy. Additionally, the University of Nottingham may not take out a separate insurance policy for a country if it is not usually necessary for clinical trials insurance to be available in that country. Individual countries should liaise with the ENOS International Coordinating Centre about specific arrangements for their country.

2.27 Trial administration
The trial will be managed and monitored as follows:

2.27.1 Sponsor
The University of Nottingham is the trial sponsor and assumes all legal responsibilities for this role.

The University of Nottingham
Mr Paul Cartledge
Head of Research Grants and Contracts
Research Innovation Services
King’s Meadow Campus
Lenton Lane
Nottingham, NG7 2NR
UK

2.27.2 ENOS International Coordinating Centre
The ENOS International Coordinating Centre will co-ordinate the trial. They have overall responsibility for the conduct of the trial. They are responsible for provision of trial materials, collation of data, analysis of data and reporting of the final results. They also act as the National Coordinating Centre within the UK and will act as primary point of contact for UK centres, National Coordinating Centres, and as secondary point of contact for international local centres. They will perform blinded 90 day follow up on UK patients and monitor UK centres (see section 2.22).

ENOS International Coordinating Centre
Division of Stroke Medicine
University of Nottingham  
Clinical Science Building  
City Hospital campus  
Nottingham, NG5 1PB  
UK  
Tel: +44 115 823 1770  
Fax: +44 115 823 1771  
Email: enos@nottingham.ac.uk  
Internet: http://www.enos.ac.uk  

2.27.3 National Coordinating Centres  
While overall responsibility for the trial rests with the ENOS International Coordinating Centre, the National Coordinating Centres will assist with the running of the trial internationally. Within their country they will:  
- recruit local centres,  
- act as primary point of contact for local centres  
- assume responsibility for translation and management of version control for trial specific documents  
- carry out site monitoring visits for centres in their country  
- perform 90 day follow up for patients recruited in their country  
- hold investigator meetings  
- represent ENOS at academic meetings  
- meet the responsibilities of local centres as outlined below  

2.27.4 Local ENOS centres  
Local ENOS centres are responsible for:  
- recruiting patients  
- providing accurate information on the trial participants  
- maintaining site files  
- training of local staff in trial procedures  
- otherwise fulfilling their obligations as investigators according to Good Clinical Practice.  

2.27.5 Trial Steering Committee (TSC)  
Chairman (independent, to oversee the study): Professor Graham Venables (Sheffield, UK)  
Chief Investigator: Professor Philip Bath (Nottingham, UK)  
Consultant Health Economist: Professor David Whynes (Nottingham, UK)  
Consultant Neuroradiologist: Professor Joanna Wardlaw (Edinburgh, UK)  
Consultant Statistician: Professor Stuart Pocock (London, UK)  
Consultant Stroke Trialist: Professor Kennedy Lees (Glasgow, UK)  
MRC Independent Experts: Professor Pierre Amarenco (Paris, France), Dr Keith Muir (Glasgow, UK)  
Sponsor’s Representative: Professor Hywel Williams (Nottingham, UK)  

2.27.6 Data Monitoring and Ethics Committee (DMEC)  
Neurologist/trialist: Professor Peter Sandercock (Chairman, Western General Hospital, Edinburgh)  
Stroke physician: Professor Kjell Asplund (Stockholm)  
Epidemiologist: Dr Colin Baigent (Clinical Trials Service Unit, Oxford)  
Independent Statistician: Paul Silcocks (University of Nottingham, Clinical Trials Unit)
2.27.7 Independent adjudicator
Role: to adjudicate serious adverse events.
Hungary: Dr Dániel Bereczki

2.28 Approval
Approval for the trial has been obtained from the following bodies:
   a) Trent Multicentre Research Ethics Committee.
   b) Local Research Ethics Committees at each hospital (if required).
   c) UK MHRA Clinical Trials Authorisation (with ‘roll-over’ from Doctors Dentists Exemption
      [DDX]).
   d) Licensing Authorities in countries where required (see
      https://www.nottingham.ac.uk/stroke-medicine/enos/jevpybki.htm).

2.29 Publication and data-sharing policy
Data and results will be shared as follows:

2.29.1 Presentation
The trial results will be presented to the investigators, and to funding bodies, and major
international and national scientific meetings, in the name of the Investigators.

2.29.2 Publication
The main results from the trial will be written by a ‘Writing Committee’ and published in quality
peer-reviewed journal(s) in the name of the investigators, i.e. ENOS Investigators. Secondary
publications will also be published. Abstracts will be presented as ‘ENOS Investigators, person(s)’,
where the person(s) act as a contact point for the study. Local investigators may present or publish
data relating to their centre once the main trial findings have been published and following approval
from the Trial Steering Committee.

2.29.3 Data
Group and individual patient data will be shared with the Cochrane Collaboration through the
‘Blood pressure in Acute Stroke Collaboration’ (BASC) and Cochrane systematic review of
nitric oxide in acute stroke after publication of the primary results and prespecified secondary
analyses.

2.29.4 Data Protection
All trial staff and investigators will endeavour to protect the rights of the trial’s participants to
privacy and informed consent. The ENOS trial will adhere to the UK Data Protection Act, 1998.
The CRF will only collect the minimum required information for the purposes of the trial.

Personal information (e.g. name and address of patients and secondary contacts) about trial
participants will be held at local centres and will be passed onto the International Coordinating
Centre, Nottingham, UK and to National Coordinating Centres for centres situated outside the UK.
This is necessary for the coordination and execution of the blinded 90 day follow up assessments
which are carried out centrally for each country. Patient information will be held on a database in
Nottingham but will be separated from all clinical information; the latter remain anonymous
( identifiable only by initials, trial number and age). Computer held data including the trial database
will be held securely and password protected. Computer data will be backed up regularly to an off site secure repository (to enable disaster recovery). Personal patient information will be used only for the purposes of the ENOS trial and will not be passed on to third parties. The personal patient information will be deleted at the end of the trial.

Paper CRFs will be held securely, in a locked room, or locked cupboard or cabinet. Access to the information will be limited to the trial staff and investigators and relevant regulatory authorities.

Trial paperwork will be anonymised, scanned and stored on a digital archiving system. This is with the exception of consent forms and patient details form. This will comply with the Data Protection Act and confidentiality rules, as outlined above.

Anonymised CT data may be shared with the allied MRC funded Neurogrid project and may be used for educational and research purposes.

Where permissible, the ENOS National Coordinating Centres may use central databases to obtain additional follow-up information on patients enrolled into the trial. In the UK, this will involve use of the NHS Medical Research Information Service, Office of National Statistics (ONS) database. When information will be gathered on patients in this way, it will be clearly stated in the country specific patient/relative information sheets.

Information about the trial in the participant’s medical records / hospital notes will be treated confidentially in the same way as all other confidential medical information.
### Appendix A. Timetable of study - start-up phase

**Note** Baseline (Day 0) refers to everything prior to the first patch (or no patch) being applied. Patch application marks the start of Day 1. Day 2 starts when the patch is changed. Day 1 may be less than 24 hours.

<table>
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<tr>
<th>Day</th>
<th>Screen</th>
<th>Day 0 (baseline)</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Discharge</th>
<th>Day 90</th>
</tr>
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<td></td>
</tr>
<tr>
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<td></td>
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<td>Y</td>
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</tr>
<tr>
<td>GCS</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>SSS</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>CT scan</td>
<td></td>
<td>&lt;</td>
<td>-</td>
<td>-</td>
<td>&lt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Record other treatments</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Day 7 efficacy measures</td>
<td></td>
<td></td>
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<td></td>
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<td>X</td>
<td></td>
</tr>
<tr>
<td>Day 7 safety measures</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Length of stay/time at home</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>X</td>
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</tr>
<tr>
<td>Discharge disposition</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Modified Rankin scale</td>
<td>Pre-morbid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Barthel Index</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>EuroQOL (quality of life)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Cognition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Zung Depression scale</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Blood (EDTA)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood (serum)</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Notes for Appendix A:**

<table>
<thead>
<tr>
<th>Y</th>
<th>Administer intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>Perform measure. &lt; - &gt; indicates the time range in which the measure may be made</td>
</tr>
<tr>
<td>Blood pressure, heart rate</td>
<td>Blood pressure and heart rate should be recorded twice daily during the 7 days of treatment with glyceryl trinitrate patch/control</td>
</tr>
<tr>
<td>GCS</td>
<td>Glasgow Coma Scale</td>
</tr>
<tr>
<td>SSS</td>
<td>Scandinavian Stroke Scale</td>
</tr>
<tr>
<td>CT scan</td>
<td>Should be performed between admission and day 7 of treatment</td>
</tr>
<tr>
<td>Other treatments</td>
<td>Record use of antihypertensive medication(s), aspirin, alteplase</td>
</tr>
<tr>
<td>Follow-ups</td>
<td>Up to day 7 and discharge – local; day 90 centrally</td>
</tr>
<tr>
<td>Disposition</td>
<td>Record as: own home (previous or new), institution (warden flat, nursing home, long stay hospital) or died in hospital</td>
</tr>
<tr>
<td>Cognitive Assessment</td>
<td>TICS-M and verbal fluency test</td>
</tr>
<tr>
<td>Blood - EDTA</td>
<td>EDTA blood sample (4 ml in FBC tube) to be frozen (≤ -20 °C) without separation/centrifugation – for pharmacogenetic assessment of outcome and blood pressure responses to GTN</td>
</tr>
<tr>
<td>Blood - serum</td>
<td>Centrifuge 8 mls clotted blood, separate serum, and freeze (≤-20 °C) – for serum markers of efficacy and outcome, including S-100</td>
</tr>
</tbody>
</table>
Appendix B. Glasgow Coma Scale

Eye movement

1 = None
2 = To pain
3 = To speech
4 = Spontaneous

Verbal response

1 = None
2 = Incomprehensible
3 = Inappropriate
4 = Confused
5 = Orientated

Motor response

1 = None
2 = Extension
3 = Flexor response
4 = Withdrawal
5 = Localises pain
6 = Obeys commands

Score out of 15 (range 3 – 15)
Appendix C. Scandinavian Stroke Scale

1. Consciousness

6 = fully conscious
4 = somnolent, can be awakened to full consciousness
2 = reacts to verbal command, but is not fully conscious
0 = unconscious

2. Eye movements

4 = no gaze palsy
2 = gaze palsy present
0 = conjugate eye deviation

3. Arm, motor power (on affected side)

6 = raises arm with normal strength
5 = raise arm with reduced strength
4 = raise arm with flexion in elbow
2 = can move, but not against gravity
0 = paralysis

4. Hands, motor power (on affected side)

6 = normal strength
4 = reduced strength
2 = some movement, fingertips do not reach palm
0 = paralysis

5. Leg, motor power (on affected side)

6 = normal strength
5 = raises straight leg with reduced strength
4 = raises leg with flexion of knee
2 = can move, but not against gravity
0 = paralysis

6. Orientation

6 = correct for time, place and person
4 = 2 of these
2 = 1 of these
0 = completely disorientated
7. Speech

10 = no aphasia
6 = limited vocabulary or incoherent speech
3 = more than yes/no, but not longer sentences
0 = only yes/no or less

8. Facial palsy

2 = none/dubious
0 = present

9. Gait

12 = walks 5 m without aids
9 = walks with aids
6 = walks with help of another person
3 = sits without support
0 = bedridden wheelchair

Total score =

See $^{158}$
Appendix D. Rankin Scale (modified)

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No symptoms at all</td>
</tr>
<tr>
<td>1</td>
<td>No significant disability, despite symptoms; able to carry out all usual duties and activities</td>
</tr>
<tr>
<td>2</td>
<td>Slight disability; unable to carry out all previous activities but able to look after own affairs without assistance</td>
</tr>
<tr>
<td>3</td>
<td>Moderate disability; requiring some help, but able to walk without assistance</td>
</tr>
<tr>
<td>4</td>
<td>Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance</td>
</tr>
<tr>
<td>5</td>
<td>Severe disability; bedridden, incontinent and requiring constant nursing care and attention</td>
</tr>
<tr>
<td>6</td>
<td>Dead</td>
</tr>
</tbody>
</table>

Score 0 to 6 (range 0-6)

See\textsuperscript{159,160}
Appendix E. Barthel Index

<table>
<thead>
<tr>
<th>Task</th>
<th>Criteria</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bowels</td>
<td>Incontinent</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Occasional accident (once per week)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Continent</td>
<td>10</td>
</tr>
<tr>
<td>Bladder</td>
<td>Incontinent, or catheterised and unable to manage alone</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Occasional accident (maximum once per 24 hours)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Continent</td>
<td>10</td>
</tr>
<tr>
<td>Grooming</td>
<td>Needs help with personal care</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Independent face/hair/teeth/shaving (implements provided)</td>
<td>5</td>
</tr>
<tr>
<td>Toilet use</td>
<td>Dependent</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Needs some help, but can do something alone</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Independent (on and off, dressing, wiping)</td>
<td>10</td>
</tr>
<tr>
<td>Feeding</td>
<td>Unable</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Needs help cutting, spreading butter, etc.</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Independent</td>
<td>10</td>
</tr>
<tr>
<td>Transfer (bed to chair and back)</td>
<td>Unable, no sitting balance</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Major help (one or two people, physical), cab sit</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Minor help (verbal or physical)</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Independent</td>
<td>15</td>
</tr>
<tr>
<td>Mobility</td>
<td>Immobile</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Wheelchair independent, including corners</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Walks with help of one person (verbal or physical)</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Independent (but may use any aid: for example stick)</td>
<td>15</td>
</tr>
<tr>
<td>Dressing</td>
<td>Dependent</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Needs help but can do about half unaided</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Independent (including buttons, zips, laces, etc.)</td>
<td>10</td>
</tr>
<tr>
<td>Stairs</td>
<td>Unable</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Needs help (verbal, physical, carrying aid)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Independent</td>
<td>10</td>
</tr>
<tr>
<td>Bathing</td>
<td>Dependent</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Independent (or in shower)</td>
<td>5</td>
</tr>
</tbody>
</table>

Score out of 100 (range 0-100)

See^{160,161}
Appendix F. EuroQOL

Group 1
I have no problems in walking about
I have some problems in walking about
I am confined to bed

Group 2
I have no problems with self care
I have some problems with washing or dressing
I am unable to wash or dress myself

Group 3
I have no problems performing my usual activities (e.g. work, study, housework, family or leisure activities
I have some problems performing usual activities
I am unable to perform my usual activities

Group 4
I have no pain or discomfort
I have moderate pain or discomfort
I have extreme pain or discomfort

Group 5
I am not anxious or depressed
I am moderately anxious or depressed
I am extremely anxious or depressed

Health state today by visual analogue scale (best imaginable to worst imaginable)
See\textsuperscript{143}
Appendix G. Cognitive Testing

TICS-M – Adjusted for the ENOS Trial

Please note that this test is designed for telephone use. In the event follow up is done in person the entire test must be completed verbally, i.e. the memory words must not be shown to the patient.

Orientation:
1(a). What day of the week is it? Day [ ]
   (b). What is today’s date? Date [ ]
   (c). What season are we in? Season [ ]
2. What is your age? Age [ ]

Registration/ Free Recall:
3. I am going to read you a list of 10 words. Please listen carefully and try to remember them. When I am done, tell me as many as you can in any order. Ready?

   Cabin [ ]
   Pipe [ ]
   Elephant [ ]
   Chest [ ]
   Silk [ ]
   Theatre [ ]
   Watch [ ]
   Whip [ ]
   Pillow [ ]
   Giant [ ]

Now tell me the words you can remember

Attention/Calculation:
4. Please take away 7 from 100 93 [ ]
   Now continue to take 7 away from what you have left over until I ask you to stop 86 [ ]
   79 [ ]
   72 [ ]
   65 [ ]

5. Please count backwards from 20 to 1 No mistakes [ ]

Comprehension, Semantic and Recent Memory:
6. What do people usually use to cut paper? Scissors [ ]
7. What is the prickly green plant found in the desert? Cactus [ ]
8. Who is the head of state now? Correct Name [ ]
9. What is the opposite direction to east? West [ ]

Language/Repetition:
10. Please listen carefully and repeat this: Exactly right [ ]
   “No ifs, ands or buts
Delayed Recall:

11. Please repeat as many of the 10 words I asked you to remember earlier

<table>
<thead>
<tr>
<th>Animal</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cabin</td>
<td></td>
</tr>
<tr>
<td>Pipe</td>
<td></td>
</tr>
<tr>
<td>Elephant</td>
<td></td>
</tr>
<tr>
<td>Chest</td>
<td></td>
</tr>
<tr>
<td>Silk</td>
<td></td>
</tr>
<tr>
<td>Theatre</td>
<td></td>
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<tr>
<td>Watch</td>
<td></td>
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<tr>
<td>Whip</td>
<td></td>
</tr>
<tr>
<td>Pillow</td>
<td></td>
</tr>
<tr>
<td>Giant</td>
<td></td>
</tr>
</tbody>
</table>

Score 1 point for each correct answer. Maximum score = 39

Score ________

Concentration (from MMSE)   Spell WORLD backwards (or language specific equivalent)

Score out of 5

Verbal Fluency

Now you have 1 minute to name as many animals as you can think of. Ready? Start now!

Write down each word and score 1 mark for each animal named. Do not score repetitions.

Total score ___________

See^{144}
Appendix H. Zung Depression rating Scale (short)

<table>
<thead>
<tr>
<th>With scores:</th>
<th>Seldom or never</th>
<th>Some of the time</th>
<th>Good part of time</th>
<th>Most of time</th>
</tr>
</thead>
<tbody>
<tr>
<td>I feel down-hearted and blue</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I have trouble sleeping at night</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Morning is when I feel best</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>I can eat as much as I used to</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>I get tired for no reason</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I find it difficult to make decisions</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I feel hopeful about the future</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>I feel that I am useful and needed</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>My life is somewhat empty</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I still enjoy the things I used to do</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Short Zung IDS Index = \( 100 \times \frac{\text{Total}}{40} \)

Depression \( \Rightarrow 70 \)

See \(^{146,147,160} \)
Appendix I. Definitions

**Acute Stroke Unit**
A high-dependency nursing unit (or area) caring only/mainly for patients with acute stroke and providing close monitoring of neurological and vascular signs.

**Disposition**
Home, institution (e.g. warden controlled, nursing home), dead

**Major extracranial bleeding**
Clinically overt bleeding associated with one or more of:
- Transfusion of > 2 red cell units of blood
- A fall in haemoglobin of 20 g/l (= 2 g/dl, = 1.24 mmol/l)
- Bleeding into retroperitoneum, intraocular space or major joint
- Bleeding leading to permanent treatment cessation

**Neurological deterioration**
A reduction in SSS of $\geq 5$ points, or decrease in consciousness level by $\geq 3$ points, as compared with baseline.

**Recurrent stroke**
Classified as haemorrhagic or ischaemic (if documented by CT scan or autopsy), or of unknown type. The time from stroke onset and side will be noted. (This definition deliberately does not attempt to differentiate true recurrence from extension of the presenting lesion since this is clinically and radiologically difficult unless recurrence occurs in a new arterial territory.)

**Significant hypotension**
A symptomatic fall in blood pressure of > 20% as compared with baseline necessitating intervention with intravenous colloid or crystalloid (saline).

**Stroke Rehabilitation Unit**
A dedicated rehabilitation unit (or area) caring only/mainly for patients with recent stroke and providing multi-disciplinary therapy (e.g. physiotherapy, occupational therapy, speech & language therapy).

**Symptomatic deep vein thrombosis (DVT)**
The clinical suspicion of DVT will need confirmation by either venography or ultrasound examination.

**Symptomatic intracranial haemorrhage**
Neurological deterioration, or death, associated with intracranial haemorrhage found on CT scan or autopsy.

**Symptomatic pulmonary embolism (PE)**
The clinical suspicion of PE will need confirmation by either high-probability ventilation-perfusion lung scintigraphy, pulmonary angiography, inconclusive V/Q scan and DVT, or autopsy.

See $^{162}$
Appendix J. ENOS Trial Protocol Violations

The following constitute a ‘protocol violation’:

- Patient under 18 years of age
- Randomisations >48 hours from onset of symptoms
- GCS <8 at randomisation
- No limb weakness evident at any point in stroke history
- Limb weakness not present at randomisation
- Limb weakness present for less than 1 hour in total
- Failure to obtain consent or assent of patient
- Patient BP outside range 140-220 systolic at inclusion
- Patient with mRS >2 prior to stroke
- Failure to identify pre-stroke antihypertensive medications at baseline
- Patient pregnant or breastfeeding at inclusion
- Patient enrolled with known severe concomitant illness
- Patient enrolled with known intracranial pathology other than stroke
- Patient involved at time of randomisation or within 90 day in another medicinal clinical trial
- Patient continues to receive usual antihypertensive when randomised to ‘stop’ usual treatments
- Failure to stop pre-existing regular nitrate treatments for the 7 day trial period
- Administration of new post-stroke antihypertensive prior to randomisation
- Patient does not receive GTN patch for first 4 days of trial if randomised to do so
- Patient has no cranial imaging or autopsy
- Failure to complete SAEs where appropriate
- Follow up assessments are performed outside the specified time as shown below:
  - 7 day follow up: >7 days past the due date,
  - 90 day follow up and hospital discharge: >30 days past the due date
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