

Ambulance-delivered transdermal glyceryl trinitrate versus sham for ultra-acute stroke: rationale, design and protocol for the Rapid Intervention with Glyceryl trinitrate in Hypertensive stroke Trial-2 (RIGHT-2) trial (ISRCTN26986053)

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Keywords

Acute stroke, ambulance, antihypertensive therapy, cerebrovascular disorders, glyceryl trinitrate, nitroglycerin, paramedic, randomised controlled trial

Word count

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Abbreviations

BI: Barthel Index; BP: blood pressure; FAST: face, arm, speech, time; GTN: glyceryl trinitrate; ICH: intracerebral haemorrhage; IS: ischaemic stroke; mRS: modified Rankin scale; NIHSS: National Institutes of Health Stroke Scale

ABSTRACT

Rationale Vascular nitric oxide levels are low in acute stroke and donors such as glyceryl trinitrate (GTN) have shown promise when administered very early after stroke. Potential mechanisms of action include augmentation of cerebral reperfusion, thrombolysis and thrombectomy, lowering blood pressure (BP), and cytoprotection.

Aim To test the safety and efficacy of 4 days of transdermal GTN (5 mg/day) versus sham in patients with ultra-acute presumed stroke who are recruited by paramedics prior to hospital presentation.

Sample size estimates The sample size of 850 patients will allow a shift in the modified Rankin Scale (mRS) with odds ratio 0.70 (GTN versus sham, ordinal logistic regression) to be detected with 90% power at 5% significance (2-sided).

Design The Rapid Intervention with Glyceryl trinitrate in Hypertensive stroke Trial-2 (RIGHT-2) is a multicentre UK prospective randomised sham-controlled outcome-blinded parallel-group trial in 850 patients with ultra-acute (≤ 4 hours of onset) FAST-positive presumed stroke and systolic BP ≥ 120 mmHg who present to the ambulance service following a 999 emergency call. Data collection is performed via a secure internet site with real-time data validation.

Study outcomes The primary outcome is the mRS measured centrally by telephone at 90 days and masked to treatment. Secondary outcomes include: BP, impairment, recurrence, dysphagia, neuroimaging markers of the acute lesion including vessel patency, discharge disposition, length of stay, death, cognition, quality of life and mood. Neuroimaging and serious adverse events are adjudicated blinded to treatment.

Discussion RIGHT-2 has recruited more than 500 participants from 7 UK ambulance services.

Status: Trial is ongoing.

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INTRODUCTION AND RATIONALE

Treatment options for patients with acute stroke are few and may be categorised as those with high efficacy and high or medium cost but of limited utility, such as intravenous thrombolysis, thrombectomy, and hemicraniectomy;(1-4) those with intermediate efficacy and very wide utility, specifically stroke unit care;(5) and those with limited efficacy but wide utility and low cost, e.g. aspirin.(6) There are no definitive drug treatments for patients with spontaneous intracerebral haemorrhage (ICH) although blood pressure (BP) lowering in the hyperacute period may be effective (7) and is recommended in guidelines.(8-10) Hence, there is an urgent need for affordable, widely available interventions that will improve outcome after either ischaemic or haemorrhagic stroke.

Nitric oxide (NO) donors are candidate treatments for acute stroke.(11, 12) NO is a cerebral and systemic vasodilator, modulates vascular and neuronal function, and inhibits apoptosis. Preclinical stroke studies show that NO donors improve regional cerebral blood flow, and reduce stroke lesion size if administered rapidly.(13) Five small clinical studies of NO donors have been performed in acute stroke. Intravenous sodium nitroprusside reduced BP without altering cerebral blood flow, and exhibited antiplatelet effects,(14) thereby precluding its use in ICH. Four small trials of transdermal glyceryl trinitrate (GTN, nitroglycerin) found that it lowered peripheral and central BP, 24 hour BP, and pulse pressure.(15-18) GTN had no effect on middle cerebral artery blood flow velocity, cerebral blood flow (hence, no evidence of cerebral steal), intracranial pressure, or platelet activity (so it can be given in ICH).(15, 17) Further, GTN improved vascular compliance (reduced augmentation index) and had no apparent safety concerns.(15-18) In the last of these pilot studies (Rapid Intervention with Glyceryl trinitrate in Hypertensive stroke Trial, RIGHT), GTN was administered within 4 hours of stroke onset and significantly, albeit unexpectedly, improved functional outcome in just 41 patients.(18) The large Efficacy of Nitric Oxide in Stroke trial (ENOS, 4,011 patients recruited from 173 sites in 23 countries) found that GTN was safe to administer but did not modify

outcome when given within 48 hours, except in the prespecified subgroup of 273 patients randomised within 6 hours who had an improvement in functional outcome.(19, 20)

Most trials in acute stroke have been delivered in hospital but a number of pilot studies have found that it is feasible to test new interventions and diagnostics before hospital in the community (pre-hospital) in small trials, including magnesium, insulin, brain scanning, preconditioning, and BP lowering.(18, 21-28) Recently, the large FAST-Mag trial showed that it was feasible to deliver a large phase III trial in the pre-hospital setting, at least in the US emergency care system.(29) While paramedics are uniquely placed to deliver early treatments, their experience of participating in randomised controlled trials (RCTs) to evaluate interventions is limited, in part because pre-hospital research infrastructure is still in development and because the logistics of RCTs are more challenging in this environment.(30)

On the basis of apparent positive effects of GTN on outcome in patients treated very early,(18-20) meta-analyses of these data (Figure 1),(31) and the apparent feasibility of performing large stroke trials in the pre-hospital ambulance environment,(29) the Rapid Intervention with Glyceryl trinitrate in Hypertensive stroke Trial-2 (RIGHT-2, ISRCTN26986053) is testing the safety and efficacy of transdermal GTN in patients with ultra-acute presumed ischaemic or haemorrhagic stroke who are recruited by paramedics, and the feasibility of performing a large prehospital stroke trial in the United Kingdom. We describe here the rationale, design and protocol for RIGHT-2.

OBJECTIVES

The RIGHT-2 trial is testing two primary questions in patients with ultra-acute presumed stroke:

1. What is the safety and efficacy of GTN, a nitric oxide donor, when administered within 4 hours of stroke onset?
2. What is the feasibility of performing a multicentre stroke trial where randomisation and treatment is delivered by paramedics in the pre-hospital UK National Health Service ambulance service environment?

A further two secondary questions ask:

1. What are the mechanisms by which GTN might work if it shows efficacy in patients with ultra-acute stroke? These include potential effects on reperfusion, accelerated resolution of arterial clot, cytoprotection, facilitation and amplification of thrombolysis and other reperfusion strategies, ambulatory BP, and vascular compliance.
2. Is GTN safe in patients who are found in hospital to have had a mimic rather than stroke or transient ischaemic attack (TIA)?

METHODS

Trial design

RIGHT-2 is a UK-based multicentre prospective randomised sham-controlled outcome-blinded parallel-group trial.

Ethics and regulatory approvals

The study is run according to the principles of the Declaration of Helsinki and Good Clinical Practice. Study approvals have been obtained from: UK Medicines competent authority (MHRA 03057/0064/001-0001, date 1 March 2015; EudraCT 2015-000115-40) and Research Ethics Committee (Nottingham 2, 15/EM/0055, date 24 Feb 2015), and local research & development/innovation departments in

each participating Ambulance Service and Hospital (e.g. Nottingham University Hospitals NHS Trust, date 8 Sept 2015). The trial was registered (ISRCTN26986053) and adopted by the National Institute for Health Research Clinical Research Network (date 26 Feb 2015). Trial funding from the British Heart Foundation followed external peer review (grant: CS/14/4/30972, awarded 11 Sept 2014), started on 1 May 2015, and will last three years. The Nottingham Stroke Research Partnership Group (comprising stroke survivors and carers) support the study question, design and delivery plan. Management of personal data adheres to the UK Data Protection Act 1998. The overall flow of patients through the trial, and schedule of procedures and evaluations, are summarised in Figure 2 and Table 1, respectively.

Patient population

Study-trained paramedics consider all patients they attend in the context of an emergency telephone call (999) for 'stroke'. The inclusion and exclusion criteria are deliberately limited in number to simplify enrolment in a time-limited emergency environment, and to minimise any possible increase in time at scene whilst doing trial-related procedures. Additionally, the criteria attempt to maximise the chances that a participating patient will have a stroke or TIA rather than a mimic, and to minimise recruitment of patients that are already severely dependent before their suspected stroke. No log of excluded patients is kept by either ambulance services or hospitals. Unlike in FAST-Mag,(29) doctors are not involved in recruitment or consent, with paramedics solely responsible for selection, consent and enrolment.

Inclusion criteria

- Patients presenting to paramedics in context of 999 ambulance call with suspected stroke.
- Age 18 years or more (with no maximum age).
- 'Face Arm Speech Time' (FAST) score 2 or 3.
- Time ≤ 4 hours from symptom onset.
- Systolic BP ≥ 120 mmHg.
- Informed consent from patient, or proxy consent from a relative/paramedic.

- Paramedic is from a participating ambulance station and trained in RIGHT-2 procedures, and will take patient to a participating hospital with comprehensive/primary stroke centre.

Exclusion criteria

- Patient at a Nursing Home.
- Glucose (BM stix) <2.5 mmol/l.
- Glasgow Coma Scale <8.
- Witnessed seizure/fit at presentation.
- Known life expectancy <6 months.
- Known to have taken a phosphodiesterase-5 inhibitor, such as sildenafil, within 24 hours of randomisation.
- Known sensitivity to Transiderm Nitro patch.
- Known sensitivity to DuoDERM hydrocolloid dressing.
- Known previous enrolment into RIGHT-2.

If it becomes apparent at a later point that the participant did not have a stroke, they continue in the trial and are followed-up as per protocol. Patients already taking a nitrate such as glyceryl trinitrate may be recruited into the trial.

A comparison of the inclusion/exclusion criteria with the Face Arm Speech and Time test, and modified Los Angeles Prehospital Stroke Screen (mLAPSS, as used in FAST-MAG) is given in Table 2. Overall, the RIGHT-2 inclusion/exclusion criteria lead to a similar population of patients being recruited to those identified by mLAPSS.

Consent

Consent or proxy consent is obtained at the stroke scene or in the ambulance; if patients lack capacity initially, then further consent or proxy consent is obtained at hospital. The algorithm for consent is shown in Figure 3 and comprises three stages:

- At scene/in ambulance, with supervision by paramedic, in all patients, for: Ambulance activities; four days of GTN/sham administration; day 2 repeat

brain scan (for research purposes); assessment of primary and secondary outcomes at days 90 and 365 by telephone or mail; and access to the participant's data (for trial purposes). Patient capacity is assessed using structured questions – see below.(27) Patients with capacity give written or witnessed oral consent to the paramedic otherwise proxy consent is obtained from a relative/carer/friend, or by the paramedic as witnessed by another person.

- In hospital at any time, with supervision by research team (research nurse/coordinator/doctor), in patients who lacked capacity in the ambulance, for: Follow-up procedures in hospital. Patients with capacity (assessed as above) give written or witnessed oral consent to the research team member otherwise proxy consent is obtained from a relative/carer/friend. Patients who regain capacity will be re-consented for these procedures.
- In hospital prior to additional research, with supervision by research team for: Additional research, including one or more of blood and genetic biomarkers, transcranial Doppler, pulse wave analysis, and/or ambulatory blood pressure monitoring. Patients with capacity (assessed as above) give written or witnessed oral consent to the research team member otherwise proxy consent is obtained from a relative/carer/friend.

Brief assessment of capacity by the paramedic is performed in the ambulance by explaining to the patient that they have had a suspected stroke, their BP may need lowering, and that a patch will be applied that might lower their BP.(27) They are then asked what the suspected diagnosis is ('stroke'), what might need to be done to their BP ('lower'), and how this will be done ('patch'). Lack of capacity is assumed if one or more answers are missing or incorrect, and is likely to reflect that the patient is semi-conscious, dysphasic or confused.

This overall consent approach builds on processes used in the PIL-FAST and RIGHT pilot stroke ambulance trials.(18, 26-28) It is designed to reflect that stroke is a severe and often fatal or disabling condition; that the treatment has to be given very early (and ideally in the diamond half-hour or golden hour (32)) in view of the apparent time-dependency of GTN;(18, 20) that paramedics are

independent healthcare professionals who routinely have to consent patients for treatments with potential risks given for serious conditions (such as thrombolysis for myocardial infarction); and that many patients will have capacity (22% in RIGHT (18)) so that waiver of consent would be inappropriate.(33)

Randomisation

Patients are randomised (1:1) to receive either GTN patches or sham patches. A randomisation sequence was generated by the trial programmer at the Nottingham Stroke Trials Unit (STU) using random-permuted fixed-size blocks stratified by ambulance station. Identical looking numbered treatment packs are sent in blocks (four treatment packs per block) to each ambulance station. Trial-trained paramedics only carry one treatment pack at any time. In order to minimise selection bias, a participant must be enrolled in the study before the treatment pack is opened. Patients and outcome assessors are masked to treatment allocation.

Interventions

The investigational medicinal product is transdermal GTN (5mg daily for 4 days, given as Transiderm Nitro '5', Novartis Pharmaceuticals Ltd UK). Although there is no matching placebo patch, patients randomised to the control group receive a sham patch of similar size to the GTN patch (DuoDERM – a hydrocolloid dressing of size 4.4cm x 3.8cm, ConvaTec Ltd UK). The Nottingham University Hospitals NHS Trust pharmacy prepare opaque sealed sachets containing either a GTN or sham patch, and a gauze dressing. Four of these sachets are contained within a larger plastic box (treatment pack) containing a Patient Information Sheet, Consent Form and ambulance Case Report Form. Although not identical in appearance, both the GTN and DuoDERM patches are unmarked.

The GTN/sham patches are placed on the participant's shoulders or back and the position rotated daily for four days; on days 2 to 4, patches are placed between 08.00 and 09.00 hours in hospital. The doses of patches are not adjusted during treatment. Patches are covered with a gauze dressing to further mask the patient to treatment.(17-19) In order to minimise bias that could be introduced

through knowledge of what the participant has received, the number of unmasked staff is kept to a minimum (paramedic and nursing staff administering the patch) and they are asked not to reveal the treatment to colleagues.

If there is an emergency situation where further treatment of the participant is dependent on knowledge of the administered treatment, unblinding can be performed by review of the patch on the participant's shoulder or back. Study agents are stopped if an alternative diagnosis to stroke is made (e.g. TIA or mimic), the patient withdraws consent, for safety reasons, or if unacceptable adverse events develop. New prescriptions of non-trial nitrates are avoided, where possible, during the treatment period.

Background care

Treatment is given on top of standard best medical care, including management in an acute stroke unit, and treatment with thrombolysis, mechanical thrombectomy, hemicraniectomy or other necessary surgery, intensive care, any other licensed treatment for acute stroke, and aspirin, as relevant. Clinicians are encouraged to comply with national guidelines and actively lower raised BP in hospital within the first 6 hours of ICH.(10) In addition, pre-stroke anti-hypertensive medication is re-started at the discretion of the treating doctor, e.g. once the patient is medically stable and can swallow safely or has enteral access. Systematic use of long-term oral antithrombotic and lipid lowering agents are recommended for secondary prevention in patients with ischaemic stroke, and antihypertensive therapy is encouraged, where appropriate, once the 4-day treatment period is over.

Data collection and follow-up

All patients are followed-up daily during the four days of treatment, and then at discharge from hospital, and days 90 and 365, unless death occurs earlier (Table 1, Figure 1). Patients who are unable to complete the four days of treatment as per the protocol are still followed at days 90 and 365. Baseline details are collected by the recruiting paramedic using data recorded as part of routine clinical care; these include information on demography (age, sex), stroke (onset

date/time), and physiology (blood pressure, heart rate, oxygen saturation, glucose).

On arrival at hospital, further routine clinical details are collected, including stroke syndrome,(34), stroke severity (National Institutes of Health Stroke Scale, NIHSS (35)), haemodynamics (blood pressure, heart rate). Neuroimaging (plain CT or MR scanning, ideally with CT or MR angiography) is performed on admission to diagnose stroke type (ischaemic, ICH) or non-stroke lesions. CT angiography (if performed as part of routine practice) is used to determine if GTN improves collateral blood supply and influences reperfusion. Importantly, these hospital admission measures are made on treatment following application of the first GTN/sham patch at the stroke scene; hence, it is possible that treatment will have altered the diagnosis (e.g. conversion of mild stroke to TIA), stroke syndrome and severity, and neuroimaging findings, in hospital.

Blood pressure and heart rate are collected daily, one hour after placement of the second, third and fourth treatment patches. A research plain CT (or MR) scan is performed on day 2 to allow assessment of the evolution of the infarct and symptomatic artery, or haematoma. Intermediate outcome data (e.g. NIHSS, adverse events such as headache, hypotension) are collected at day 4 by hospital research staff, and the final diagnosis is made at discharge from hospital. The primary outcome, modified Rankin Scale (mRS), is determined at day 90 with central telephone follow-up by trained staff blinded to treatment assignment; additional measures of disability, cognition, mood and quality of life are similarly recorded. The same measures are also recorded at day 365 to ensure that any findings are maintained long term.

Routine carotid ultrasound examination is performed to identify severe carotid stenosis and need for early carotid endarterectomy; such scanning may be performed at any time during the hospital admission and is not necessary prior to randomisation. Blood pressure is measured using calibrated standard semiautomatic equipment available in ambulances and hospitals.

All information is entered online over a secure encrypted password/PIN protected internet connection (HTTPS) with data validated in real-time.(19) Neuroimaging (CT or MR) are interpreted by the local investigator, and then centrally over the internet by independent assessors blinded to treatment and using a validated structured classification system.(19, 36, 37) Similarly, serious adverse events (SAEs) are adjudicated centrally over the internet by independent assessors blinded to treatment.(19)

Primary Outcome

The primary outcome is the modified Rankin Scale (mRS).(19, 38-40) mRS is determined centrally by telephone by a trained assessor at day 90 (± 7 days) according to an algorithm.(41) Assessors are masked to treatment assignment and baseline clinical characteristics. The primary analysis will compare GTN versus sham using ordinal logistic regression, both overall and in pre-specified sub-groups.

Secondary outcomes

Outcomes are assessed at hospital admission; during the four days of intervention; then at day 4, discharge from hospital, and days 90 and 365.

Hospital admission:

- Neurological impairment (NIHSS, /42)
- Systolic and diastolic blood pressure (mmHg)
- Systolic blood pressure <185 mmHg, ischaemic stroke (%)
- Systolic blood pressure <140 mmHg, ICH (%)
- Heart rate (bpm)
- Stroke lesion (infarct or haemorrhage) extent on brain scan (CT or MR)
- Stroke lesion (infarct or haemorrhage) mass effect and swelling
- Presence of intravascular thrombus (hyperattenuated artery on CT or absent flow void/high signal on FLAIR MR) and cerebral arterial patency (CT or MR angiography) on relevant brain scanning

Use and timing of hyperacute and acute treatments in hospital:

- Open-label blood pressure lowering (%)
- Intravenous thrombolysis (%)
- Intra-arterial therapy / mechanical thrombectomy (%)
- Hemicraniectomy (%)
- Surgery for ICH (%)
- Admission to intensive/critical care unit (%)
- Required artificial ventilation (%)

At day 2-4:

- Systolic and diastolic blood pressure, heart rate
- Neuroimaging: Infarct/haematoma extent, presence of hyper-attenuated artery sign (CT) or absent flow void (MR), infarct swelling, secondary haemorrhagic transformation of infarct, haematoma expansion

At day 4 (or discharge if sooner):

- Stroke recurrence (%)
- Neurological impairment – NIHSS (/42)
- Neurological deterioration from baseline (NIHSS ≥ 4 points, or ≥ 2 point increase in any domain) (%)
- Infection (pneumonia/chest, urinary tract, other) (%)
- Feeding status, non-oral (%)
- Required physiotherapy (%)
- Required occupational therapy (%)
- Required speech & language therapy (%)

At discharge/death:

- Length of stay in hospital (days)
- Patient disposition (died, institution/in hospital, home)

At days 90 and 365 by telephone (or post):

- Dependency – mRS > 2 (%)
- Disability/Activities of Daily Living - Barthel Index (BI, /100)

- Quality of life - Health Utility Status (HUS, derived from EuroQoL-5D, /1) (42)
- Quality of life - EQ-Visual Analogue Scale (EQ-VAS) (/100)
- Cognition - telephone-MMSE (/22)
- Cognition - Telephone Interview Cognition Scale (TICS, /39)
- Cognition - verbal fluency (animal naming) (∞)
- Mood - Zung Depression Scale (ZDS, /102.5) (43)
- Patient disposition (died, institution/in hospital, home)
- Stroke recurrence (%)

Safety outcomes

- Death (%)
- Serious adverse events, by day 4 (%)
- Serious adverse events, by day 4 (fatal, non-fatal, none)
- Headache, by day 4 (%)
- Infection (pneumonia, chest, urinary tract, other) by day 4 (%)
- Hypotension, requiring clinical intervention, by day 4 (%)
- Hypertension, requiring clinical intervention, by day 4 (%)

Investigator-reported serious adverse events are grouped by time of onset (during or after treatment), and validated and categorised blindly by independent adjudicators. Only fatal SAEs are collected beyond end of treatment in view of the existing large safety information available for GTN and other organic nitrates, including in acute myocardial infarction and stroke.(19, 44)

Explanatory and mechanistic additional research

RIGHT-2 aims to study potential mechanisms by which GTN versus sham might be beneficial if the primary analysis is statistically positive. These comprise:

Hospital admission (day 1)

- Plain CT brain – at all sites:
 - Identify stroke type (IS, ICH, non-stroke).
 - Location/length of hyperattenuated artery (CT) or absent flow void/increased signal on FLAIR MRI, and visibility and extent of acute

ischaemic change or haematoma.

- Pre-stroke brain health assessed by presence of leukoaraiosis, brain atrophy, prior infarct or haemorrhage
- CT angiography – at some sites:
 - IS – collateral status, and location, length, luminal reduction of any occlusion.
 - ICH – active bleeding (spot sign, larger haematoma).(45, 46)

Day 2

- Plain CT brain – at all sites:
 - IS – Infarct size, mass effect/presence of oedema, location/length of hyperdense artery, secondary bleeding.(47)
 - ICH – Haematoma size, presence of intraventricular haemorrhage, haematoma expansion, and presence of oedema.
- Pulse wave analysis - Vascular compliance, some sites: Augmentation index (AI), central BP.(16, 17)
- Transcranial Doppler - Some sites: Middle cerebral artery blood flow velocity, cerebral perfusion pressure, zero filling pressure.(16, 17)
- Blood biomarkers: NO-donation [nitrite/nitrate],(48) tissue damage (S-100).(49, 50)
- Genetic blood biomarkers.
- Hydration status - On BP response: calculated osmolarity (2Na+2K+glucose+urea); urea : creatinine ratio; packed cell volume.(51, 52)

Images are curated and adjudicated using a derivative of the MRC IST-3/ENOS system, part of the Systematic Image Review System (SIRS-2) web-based imaging system from University of Edinburgh (Wardlaw, www.neuroimage.co.uk/sirs).(19, 53, 54)

Data Monitoring Committee

The DMC review unblinded data twice yearly in respect of safety and efficacy, and consider the study in the context of other trials of altering BP in stroke. Stopping rules are based on the Haybittle-Peto rule as a guide for proof beyond

reasonable doubt for:

Hazard

- Poor outcome (mRS >2) is less frequent in the sham/control group, $P < 0.01$ (nominal, 2-sided); OR
- Death is less frequent in the sham/control group, $P < 0.01$ (nominal, 2-sided)

Efficacy

- Poor outcome (mRS >2) is less frequent in the GTN/active group, $P < 0.01$ (nominal, 2-sided); AND
- Death is less frequent in the GTN/active group, $P < 0.01$ (nominal, 2-sided); AND
- Poor outcome (mRS >2) is less frequent in the GTN/active group in ischaemic stroke, $P < 0.01$ (nominal, 2-sided); AND
- Poor outcome (mRS >2) is less frequent in the GTN/active group in intracerebral haemorrhage, $P < 0.05$ (nominal, 2-sided).

The stopping rules for efficacy are designed to persuade practitioners that such a simple and inexpensive treatment can significantly reduce poor functional outcome, including separately in patients with ischaemic stroke and ICH, and reduce death.

The DMC also monitor SAEs and neurological deterioration, and outcomes in particular subgroups of patients including those with severe stroke (TACS (34)), BP <140 mmHg, those with significant carotid disease (ipsilateral stenosis of the internal carotid artery >50%), (55) and those with a final diagnosis of non-stroke.

Sample size

The null hypothesis (H_0) is that GTN will not shift the mRS in participants with ultra-acute stroke. The alternative hypothesis (H_1) is that mRS will shift between those stroke participants randomised to GTN versus sham. A total sample size of 850 participants (425 in each arm) is required to detect an ordinal shift in mRS with an odds ratio of 0.70 (equivalent to a binary odds ratio of 0.66). (56) This

assumes an overall significance level of 5%, and 90% power, and a distribution of mRS scores as shown in Table 3 (although the sample size calculation is relatively insensitive to variations in the mRS distribution). The calculation also assumes 3% loss to follow-up, a non-stroke diagnosis (stroke mimic and TIA) of 20%, (18) and reduction for baseline co-variate adjustment of 20%. (57) If only 650 participants are recruited, there will be 82% power to detect this odds ratio using the same assumptions above. Whilst one formal interim analysis is planned, no adjustment has been made to the sample size given the pre-specified high level of significance required to stop the trial.

Analysis populations

RIGHT-2 will follow the intention-to-treat principle, i.e. analysis is based on the initial treatment assignment and not on the treatment received. Additionally, the primary efficacy analysis will be performed in patients with a stroke, or a stroke and TIA, since there is no expectation that GTN will alter outcomes in patients presenting with a mimic. Finally, a *per protocol* analysis may be performed for hypothesis-generation if the primary analysis shows a significant treatment effect.

Statistical analyses

The distribution of mRS at day 90 between GTN and sham groups will be analysed using ordinal logistic regression with adjustment for age, sex, pre-morbid mRS, FAST, pre-treatment systolic BP, index event (ICH, ischaemic stroke, TIA, mimic) and time to treatment. The effect of GTN on mRS will be studied in pre-specified subgroups comprising those based on premorbid or ambulance/pre-randomisation data, and on post-treatment data collected at admission to hospital. This distinction is necessary since GTN may alter information collected at admission to hospital such as diagnosis (e.g. stroke to TIA), stroke syndrome and severity, and neuroimaging findings.

Other outcomes will be analysed using binary logistic regression for dichotomised measures (e.g. serious adverse events), Cox proportional hazards regression

(e.g. time to death), or multiple linear regression (e.g. NIHSS, MMSE, HUS, ZDS), with presentation of 95% confidence intervals and two-sided p-values.

Study organisation and funding

RIGHT-2 is an independent academic trial performed by a UK collaborative group. The study is supervised by a Trial Steering Committee and receives advice from an International Advisory Committee. The trial is run day-to-day by a Trial Management Committee based at the Coordinating Centre in Nottingham. Approximately 7 ambulance services across the UK will recruit, initiate treatment and then transport patients to ~40 acute hospitals with a stroke service. The image data are managed by researchers at the University of Edinburgh, via databases for image management and transfer of results of image reads linked to the University of Nottingham. Independent and blinded Adjudicators classify serious adverse events for a separate and independent Data Monitoring Committee. The trial is funded by the British Heart Foundation; the University of Nottingham is the sponsor.

Publication and data sharing

In addition to this description of the RIGHT-2 protocol, subsequent publications will report the statistical analysis plan (prior to data-lock at trial conclusion), a complete description of baseline data, and the main results. This approach follows that used in the ENOS trial.(58-60) Subsequent publications will focus on the effect of GTN on potential mechanisms of action, and factors involved in delivering a large multicentre paramedic-delivered ambulance-based ultra-acute stroke trial.

In the future, data from RIGHT-2 will be added to the Cochrane Collaboration review of NO donors in stroke,(61) and integrated into individual patient data meta-analyses of nitric oxide donors,(31) and blood pressure lowering, for acute stroke (the latter through the 'Blood pressure in Acute Stroke Collaboration', BASC), and the 'Virtual International Stroke Trials Archive' (VISTA).(62) Ultimately, a subset of the data will be made available over the web, as with the

International Stroke Trial.(63) Similarly, anonymised neuroimaging data will be published.(54)

Summary and conclusions

RIGHT-2 is addressing the safety and efficacy of ultra-acute administration of GTN in patients with stroke. The trial is also examining the feasibility of performing a large multicentre stroke trial embedded within the ambulance service of the UK. The large sample size of 850 patients (exceeding the size of hemicraniectomy and thrombectomy trials, and comparable in size to alteplase trials such as ECASS-3 (64)) means that a moderate-high clinical effect can be detectable with high statistical power (90%). A positive trial could be implemented easily because of the pragmatic inclusion criteria and collection of safety data from non-stroke participants. As such, transdermal GTN could be introduced rapidly into pre-hospital clinical practice around the world since it is readily available, easy to administer, and inexpensive (£2 per patient, equivalent to €2.5/\$3). Successful delivery of the trial, whatever the result for GTN, would also support FAST-Mag (29) in demonstrating that large multicentre pre-hospital based trials are feasible in ultra-acute stroke.

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TABLE 1. Schedule of procedures and evaluations

Timing	Baseline Day 1	+15 min	Day 1	Day 2	Day 3	Day 4±1	Discharge	Day 90±7	Day 365±7
Environment	Amb	Amb	Hosp	Hosp	Hosp	Hosp	Hosp	Tel/post	Tel/post
Paramedic: Consent/proxy-consent for main trial	✓								
Enrolment	✓								
<i>Baseline assessment: FAST, BP/HR, ECG</i>	✓								
Administer GTN/sham + gauze dressing	✓			✓	✓	✓			
<i>Blood pressure & heart rate</i>		✓	✓	✓*	✓*	✓*			
<i>Paramedic-hospital handover</i>			✓						
Consent if patient lacks capacity in ambulance ~			<	=	=	=	>		
Impairment / deterioration (NIHSS/GCS)			✓	✓	✓	✓			
<i>CT brain, plain †</i>			✓	✓					
<i>CT angiography - if routine †</i>			✓						
<i>Haematology/chemistry/ECG - routine</i>			✓						
All SAEs including fatal SAEs			✓	✓	✓	✓	✓**		
Fatal SAEs							✓	✓	✓
Recurrent stroke			✓	✓	✓	✓	✓	✓	✓
Tolerability/side effects			✓	✓	✓	✓			
Plain CT scan - <i>routine if post rt-PA †</i>				✓					
<i>Additional clinical neuroimaging</i>			✓	✓	✓	✓	✓		
Consent for additional research (where appropriate)			✓	✓	✓				
24 hour ambulatory blood pressure monitoring ‡			<	=	>				
Blood biomarkers (e.g. S-100, NOx, P-selectin) ‡			<	=	>				
Genetics: EDTA sample ‡			<	=	>				
Pulse wave analysis ‡			<	=*	>				

Transcranial Doppler †			<	=*	>				
Consent if patient regains capacity			<	=	=	=	>		
Disposition (home/institution/home)							✓	✓	✓
Dependency: modified Rankin Scale								✓	✓
Disability: Barthel Index								✓	✓
Cognition: MMSE, TICS								✓	✓
Quality of Life: EQ-5D, EQ-VAS								✓	✓
Mood: Zung Depression Scale								✓	✓

*1-2 hours after administration of GTN/sham patch; **If discharge before Day 4; *Routine care in italics*. Amb: Ambulance; BP: Blood pressure; FAST: Face, Arm, Speech, Time test; GCS: Glasgow Coma Scale; Hosp: Hospital; HR: heart rate; IMP: Investigational Medicinal Product; MMSE: Mini-Mental State Examination; NIHSS: National Institutes of Health Stroke Scale; Tel: Telephone (done by central telephone questionnaire masked to treatment assignment); TICS: Telephone Interview of Cognition Status. † Imaging sent as 'volume data' ‡ Separate consent to be obtained in-hospital. 08.00 Time (+/- 2 hour) for patch administration or measurement ~Only for participants whose baseline consent was by proxy (can be anytime during hospital stay).

TABLE 2. Comparison and contrast of inclusion and exclusion criteria for RIGHT-2 (uses Face, Arm, Speech, Time test, FAST) versus FAST-MAG (used modified Los Angeles Prehospital Stroke Screen, mLAPSS)

Criteria	RIGHT-2	mLAPSS/FAST-MAG (29, 65)
Age	18-	40-95
History of seizure absent	Witnessed at/after stroke	Ever
Symptom duration	<4 hours	<2 hours
Disability/dependency	Not in nursing home	Not wheelchair bound or bedridden
Blood glucose (mmol/L)	2.5-	3.3-22.2
Face weak/droop	Yes	Yes
Arm weak/drift	Yes	Yes
Speech	Yes	-
FAST †	2 or 3	-
Grip weak/none	-	Yes
Glasgow coma scale	8-15	-

† FAST (66) = facial weakness + arm weakness + speech abnormality

TABLE 3. Distribution of modified Rankin Scale (mRS) in pre-hospital stroke trials with aggregation of rates across treatment groups. The sample size calculation for RIGHT-2 assumes the mRS distribution seen in the RIGHT pilot trial (highlighted in bold).(18) However, the calculation is relatively immune to the actual distribution; the final column shows the estimated sample size based on the different mRS distributions (and assuming power = 90%, significance = 5% and odds ratio = 0.70).

Modified Rankin Scale (%)	N	0	1	2	3	4	5	Dead	N
RIGHT (18)	41	2	17	20	15	10	12	24	821
ENOS-early (20)	273	10	16	18	19	18	6	14	815
FAST-Mag pilot (21)	20	25	15	20	5	0	0	35	853
FAST-Mag (29)	1700	21	15	16	12	10	10	15	813
Mobile stroke unit (22, 23)	100	16	17	8	12	10	11	8	805

FIGURE 1. Meta-analysis of randomised controlled trials of glyceryl trinitrate involving patients treated within 6 hours of randomisation.(18-20)

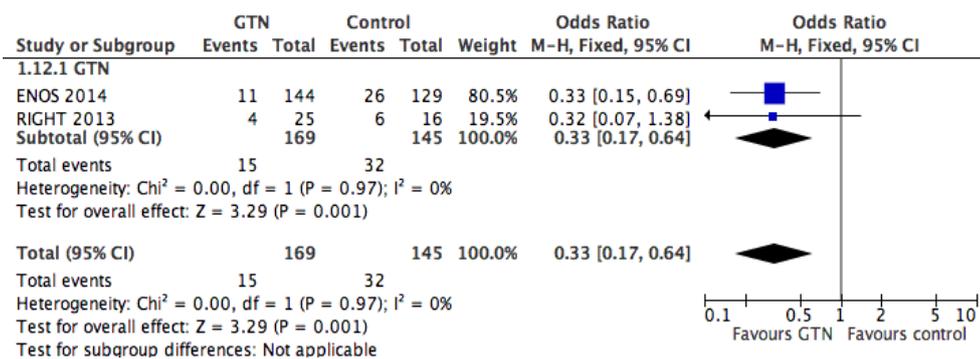


FIGURE 2. Flow of patients through trial.

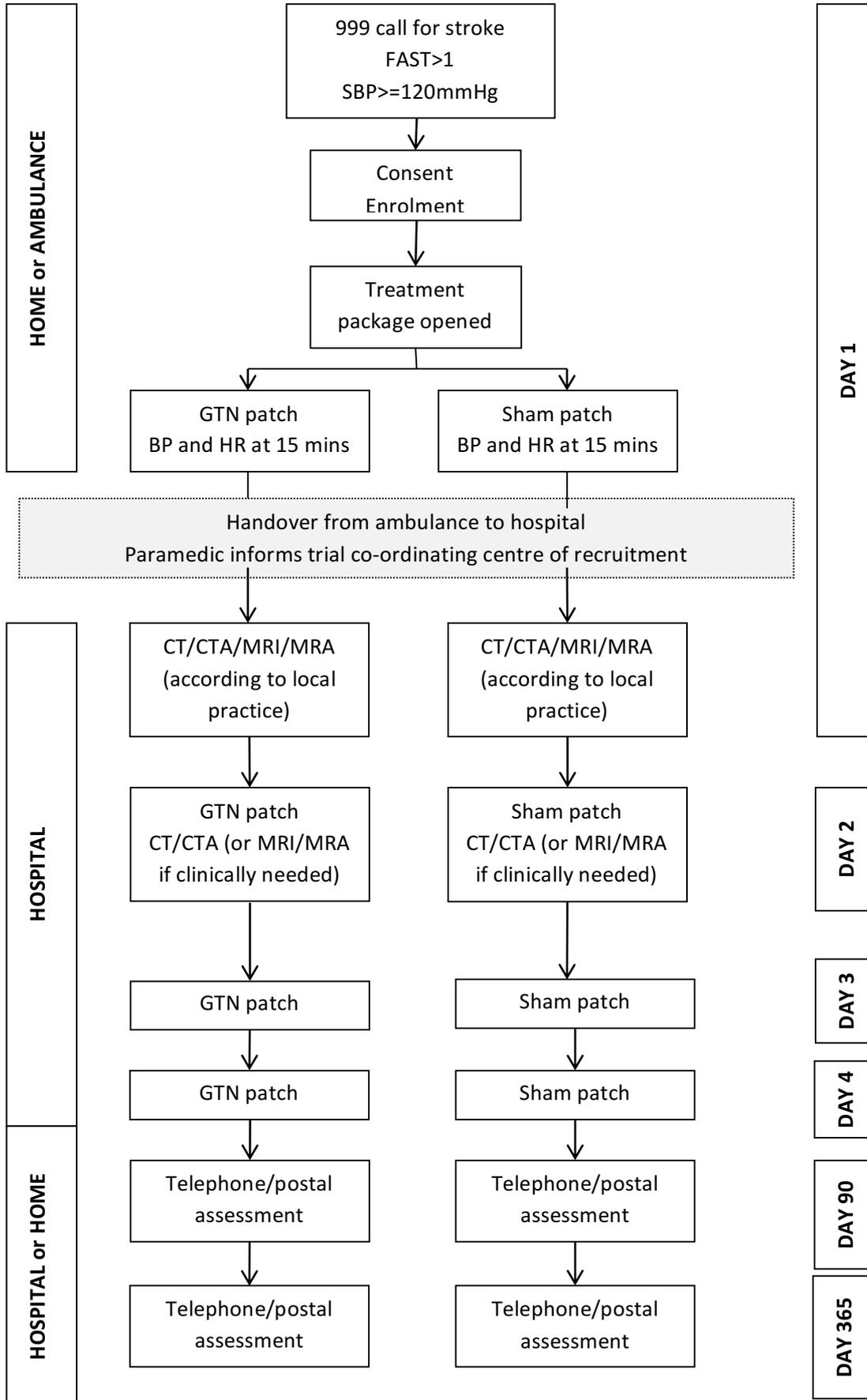
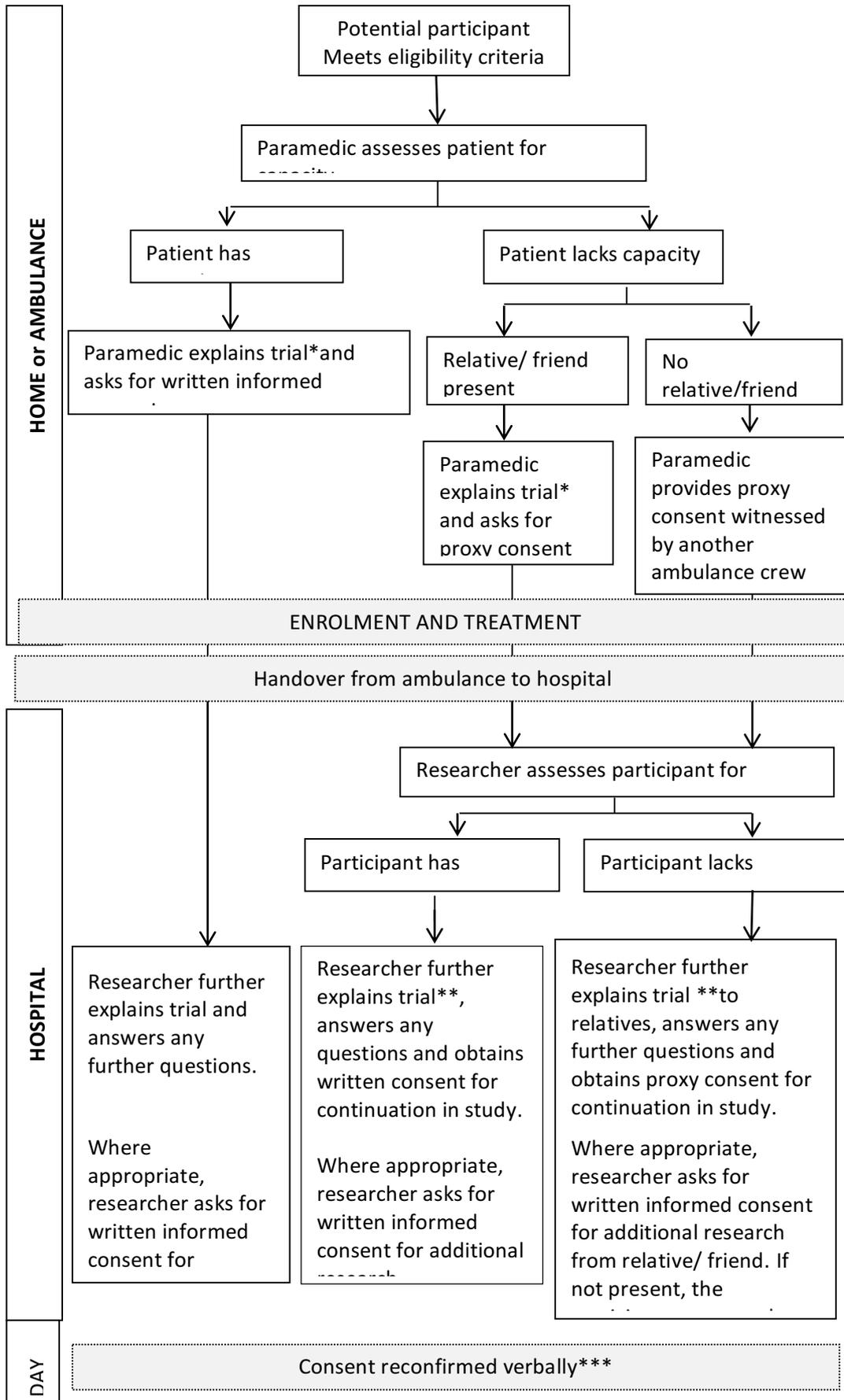


FIGURE 3. Consent algorithm



* Using short information sheet/consent form

** Using long information sheet and consent to continue form

*** If participant contacted by post, return of questionnaires will represent consent to continue participation in the study