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Pre-hospital transdermal glyceryl trinitrate in patients with ultra-acute presumed stroke (RIGHT-2): a randomised, sham-controlled, blinded, phase III, superiority ambulance-based trial

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Short title: Glyceryl trinitrate for pre-hospital stroke

A complete list of the Rapid Intervention with Glyceryl trinitrate in Hypertensive stroke Trial-2 (RAPID-2) Investigators is provided in the Appendix

RESEARCH IN CONTEXT

Evidence before this study

We searched PubMed, Embase and Web of Science for relevant articles on 12 September 2018 with search terms “stroke”, “cerebrovascular accident”, “nitric oxide donor”, and “randomised controlled trial”. We also manually searched original articles and reviews in our own references library. Searches were restricted to completed trials in human beings with abstracts or full texts published and relating to administration of glyceryl trinitrate (GTN, nitroglycerin) within 6 hours of stroke onset, and where information on functional outcome and death was available. When combining results from two randomised controlled patient-masked trials with blinded-outcome assessment, one a pilot ambulance-based study and the other a pre-specified subgroup of a large hospital-based trial, treatment with GTN within the first 6 hours of stroke onset was associated with less death, and reduced death or dependency, both overall and separately in ischaemic stroke and intracerebral haemorrhage.

Added value of this study

Ultra-acute administration of GTN in the ambulance within 4 hours of stroke onset did not alter functional outcome in patients suspected to have a stroke. It was feasible for UK paramedics to recruit, obtain consent and treat stroke patients in the pre-hospital environment.

Implications of all the evidence

We did not find evidence that ultra-early administration of transdermal glyceryl trinitrate improves functional outcome or reduces death in patients with suspected ultra-acute stroke. Large paramedic-delivered trials are possible in the UK.

SUMMARY

Background

Transdermal glyceryl trinitrate (GTN, nitroglycerin), a nitric oxide donor, may improve outcome when administered very early after stroke onset.

Methods

We undertook a multicentre paramedic-delivered ambulance-based prospective randomised sham-controlled blinded-endpoint trial in adults with presumed stroke within 4 hours of onset. Participants were randomised 1:1 to receive transdermal GTN (5 mg daily) or a similar sham dressing in the ambulance, and continued this for three days in hospital. The primary outcome was the 7-level modified Rankin Scale (a measure of functional outcome) at 90 days assessed by central telephone follow-up with blinding to treatment. Analysis was hierarchical, first in participants with a confirmed stroke or transient ischaemic attack (TIA), and then in all who were randomised (intention-to-treat) according to the statistical analysis plan. This trial is registered, ISRCTN26986053.

Findings

1149 participants (GTN 568, sham 581) were recruited by 516 paramedics from 8 UK ambulance services (October 2015 - May 2018). The median time to randomisation was 71 minutes [interquartile range 45, 116] and the final diagnosis of the index event: ischaemic stroke 597 (52%), intracerebral haemorrhage 145 (13%), TIA 109 (9%), and stroke/TIA mimic 281 (26%). GTN lowered blood pressure by 5·8/2·6 mmHg ($p<0·001$) at hospital admission compared with sham. There was no difference in the modified Rankin Scale in participants with a final diagnosis of stroke or TIA: GTN 3 [2, 5; n=420] vs sham 3 [2, 5; n=408], adjusted common odds ratio for poor outcome, acOR 1·25 (95% confidence interval, CI 0·97–1·60; p=0·083), or in all patients (GTN 3 [2, 5] vs sham 3 [2, 5]), acOR 1·04 (95% CI 0·84–1·29; p=0·69). There was no difference for secondary outcomes, death or serious adverse events.

Interpretation

Pre-hospital treatment with transdermal GTN does not appear to improve functional outcome in patients with presumed stroke. It is feasible for UK paramedics to obtain consent and treat stroke patients in the ultra-acute pre-hospital setting.

FUNDING

Funded by British Heart Foundation

BACKGROUND

High blood pressure (BP) is common in acute stroke and a predictor of poor outcome; however, large trials of BP-lowering have given variable results, and the management of high BP in ultra-acute stroke remains unclear [1] although lowering BP in intracerebral haemorrhage (ICH) is recommended in hospital.[2] Nitric oxide (NO) donors are candidate treatments for acute stroke because of their cerebral and systemic vasodilatory properties, the latter leading to a reduction in BP. Preclinical stroke studies found that NO donors improved regional cerebral blood flow and reduced stroke lesion size if administered rapidly.[3,4] Further, vascular NO levels are low in acute stroke and are associated with a poor outcome [5,6] raising the possibility that supplementing NO would be beneficial.

Five randomised trials of transdermal glyceryl trinitrate (GTN, a NO donor) in acute stroke showed that GTN lowered peripheral and central BP, 24 hour BP, pulse pressure and augmentation index.[7-11] In contrast, GTN had no effect on middle cerebral artery blood flow velocity, cerebral blood flow, intracranial pressure, or platelet function.[7-9] Although four of the trials were neutral for functional outcome,[7-9,11] GTN improved functional outcome in the phase II Rapid Intervention with Glyceryl trinitrate in Hypertensive stroke Trial (RIGHT, with randomisation by paramedics within four hours of stroke),[10] and a pre-specified subgroup analysis of the phase III hospital-based Efficacy of Nitric Oxide in Stroke trial (ENOS, with randomisation within six hours of stroke).[11,12] Summary and individual patient data meta-analyses of these five trials suggested that very early administration of GTN (312 patients within 6 hours of onset) was beneficial in both ischaemic stroke (IS) and ICH, and reduced death, disability, cognitive impairment, mood disturbance and poor quality of life.[13,14] Beyond 6 hours, treatment effects were neutral.

For stroke interventions that do not require prior neuroimaging and that may have benefit in both IS and ICH, treatment prior to hospital admission will reduce time to initiation of treatment. The Field Administration of Stroke Therapy – Magnesium (FAST-MAG) ambulance-based stroke trial successfully recruited 1700 patients in the USA,[15] but no prior large prehospital stroke trials have been completed in the UK.

We performed the phase III Rapid Intervention with Glyceryl trinitrate in Hypertensive stroke Trial-2 (RIGHT-2) trial to assess two questions: first, to determine the safety and efficacy of GTN when given very early after presumed stroke onset by paramedics prior to hospital admission; and second, to assess feasibility of performing a large multicentre ambulance-based paramedic-delivered trial in patients with presumed stroke in the UK.

METHODS

Study design

RIGHT-2 was a pragmatic multicentre paramedic-delivered ambulance-based prospective randomised sham-controlled participant- and outcome-blinded trial in adult participants with ultra-acute presumed stroke within 4 hours of onset in the UK. Details of the design, statistical analysis plan and baseline data, have been published,[16-18] and the design and protocol are summarised in the Appendix. The protocol is available at: http://right-2.ac.uk/docs/protocol50

Study population

Adult patients were eligible for inclusion following an emergency '999' telephone call for presumed stroke if they presented within 4 hours of onset of their symptoms to a trial-trained paramedic from a participating ambulance service and could be taken to a participating hospital. Patients had to have a Face-Arm-Speech-Time (FAST) score of two or three (thus ensuring the presence of a motor weakness), and a systolic blood pressure (SBP) $\geq$120 mmHg. Patients from a nursing home, or with reduced consciousness (Glasgow Coma Scale score, GCS <8/15), hypoglycaemia (capillary glucose <2.5 mmol/l), or a witnessed seizure, were excluded (see Appendix for a full listing of the inclusion and exclusion criteria).

Paramedics managed the primary consent process, and patients with capacity gave written informed consent that covered the whole trial. If capacity was absent, proxy consent was obtained from an accompanying relative, carer or friend if present, or from the paramedic if no accompanying person was present (as done in RIGHT).[10] Confirmatory consent was obtained from the patient, or their relative, carer or friend (if available) in hospital when the patient lacked capacity in the ambulance.

The final diagnosis was made after arrival at hospital by the principal investigator based on clinical and neuroimaging findings and was categorised as ICH, IS, transient ischaemic attack (TIA), or non-stroke/TIA mimic.

Randomisation and masking

Patients were randomly assigned, in 1:1 ratio, to receive transdermal glyceryl trinitrate (also known as nitroglycerin; 5 mg as Transiderm-Nitro® 5, Novartis,

Frimley UK) or a similar-appearing sham skin dressing (DuoDERM® hydrocolloid dressing, Convatec, Flintshire UK). Randomisation was stratified by ambulance station with blocks of 4 packs (2 active, 2 control) in random permuted order. Each treatment pack was sealed to maintain blinding of paramedics. Ambulances carried only one pack at a time - paramedics signed-out the treatment pack with the lowest randomisation number from their ambulance station at the start of shift and returned it if unused at end of shift. Opened but unused packs were returned to the Coordinating Centre. GTN patches/sham dressings came in marked sealed sachets so paramedics and nurses doing medication rounds in hospital knew treatment assignment. However, participants were effectively masked since the patches/dressings themselves were unlabelled, and a gauze dressing was taped over the top of the patch/dressing to provide additional blinding.

Treatment and follow-up in hospital

The first treatment (GTN or sham) was administered by the paramedic immediately after randomisation in the ambulance, and further treatments were given for up to three days whilst in hospital. Patches/dressings were placed on the shoulder or back, and the site changed daily.

Ambulance data (pre- and post-first-treatment), and hospital-collected clinical and neuroimaging data at admission (post-first-treatment), day 4 (end-of-treatment) and on death or discharge, were entered on-line into a secure web-based database system (https://nottingham.ac.uk/~nszwww/right-2/live/right-2_login.php). These data were validated and used to confirm the patient's eligibility.

Outcome measures

The primary outcome was functional outcome assessed with the 7-level modified Rankin Scale (mRS) measured at 90 days after randomisation.[19] mRS scores range from 0 to 6, with a score of 0 and 1 indicating no or some symptoms, respectively, 2-5 indicating increasing levels of disability/dependency, and 6 death. Outcomes were recorded centrally by telephone by a trained assessor masked to treatment allocation; to ensure reliable scoring, raters used a structured questionnaire.[20] If the participant could not be contacted by telephone (after multiple attempts), a questionnaire covering the same outcome measures was sent by post. The primary analysis involved a comparison of the distribution of all 7 levels of the mRS (shift) between the treatment groups.[21]

Participants were seen at day 4 (or at discharge if earlier) to determine adherence to treatment and assess neurological deterioration (increase in the National Institutes of Health stroke scale, NIHSS, by at least 4 points from hospital admission to day 4 and/or worsening conscious level in the NIHSS consciousness domain item Ia). At discharge from hospital, duration of stay, and discharge destination (to institution or home), were recorded. Pre-specified secondary outcomes at day 90 included activities of daily living (Barthel Index, BI); cognition (modified telephone Mini-Mental State Examination, t-MMSE; Telephone Interview for Cognition Scale-modified, TICS-M; categorical verbal fluency (using animal naming); health-related quality of life (European Quality of Life-5 dimensions-3 level, EQ-5D-3L, from which Health Status Utility Value, HSUV, was calculated; and EQ-visual analogue scale, EQ-VAS); and mood (abbreviated Zung depression score, ZDS), all as used in ENOS.[11]

Safety outcomes included all-cause and cause-specific case fatality; hypotension or hypertension occurring during the first 4 days (as reported by investigators); and serious adverse events (SAEs, all up to day 5, and fatal from day 5). SAEs were validated and categorised by expert adjudicators who were blinded to treatment assignment.

Plain brain scans (computerised tomography [CT] or magnetic resonance [MR] imaging) performed on arrival at hospital, were collected for central adjudication by expert neuroradiologists using assessments updated from ENOS.[11] Depending on local practice, CT/MR angiography was also performed and adjudicated centrally (see Appendix for more information). Imaging outcomes on admission to hospital included infarct extent (IST-3 score, Alberta Stroke Program Early CT Score, ASPECTS), presence of hyperdense artery, haemorrhagic transformation, and mass effect including midline shift for participants with IS; haematoma location, size, volume, extension (to subarachnoid spaces or ventricles) and mass effect including midline shift for ICH, and type and location of mimics. On the next day, a research CT or MR scan was performed to assess safety; the same factors were assessed as above for IS and ICH.[22]

Study oversight

The trial was conceived and designed by the grant applicants, and they wrote the protocol. The study was approved by the UK regulator (Medicines & Healthcare

products Regulatory Agency, ref: 03057/0064/001-0001; Eudract 2015-000115-40) and national research ethics committee (IRAS: 167115), and was adopted by the National Institute for Health Research Clinical Research Network. The trial was overseen by a Trial Steering Committee (which included three independent members and a patient-public representative), and advice given by an International Advisory Committee. The day-to-day conduct of the trial was run by a Trial Management Committee which was based at the Stroke Trials Unit in Nottingham. An independent Data Monitoring Committee reviewed unblinded data every 6 months and performed a formal interim analysis midway through the trial (see Supplement for description of the stopping rules); this analysis was performed after 714 patients had been recruited and followed-up at 90 days and the DMC recommended that the trial should continue. Study data were collected and quality-assured by the RIGHT-2 Coordinating Centre in Nottingham. Analysis, interpretation, and report writing were performed independently of the funder and sponsor. The corresponding author wrote the first draft of the manuscript, and this was edited and commented on by the writing committee, all of whom approved the decision to submit the manuscript for publication.

Statistical analysis

A total sample size of 850 participants (425 in each arm) was required to detect a shift in mRS with a common odds ratio of 0·70.[17] This assumed an overall significance level of 5%, 90% power, distribution of mRS scores as shown in the Appendix,[23] 3% loss to follow-up, mimic and TIA rate of 20%, and reduction for baseline co-variate adjustment of 20%.[24] During the trial, the non-stroke diagnosis rate was noted to exceed 30%. Since this would reduce the number of participants recruited with a stroke diagnosis, and therefore the statistical power in this group, the overall sample size was increased from 850 to 1050 to maintain the overall effect size and statistical power. Further, a decision was made by the Trial Steering Committee to perform a hierarchical analysis, this comprising a sequential analysis performed in two progressively inclusive cohorts based on the final in-hospital diagnosis: participants with confirmed stroke or TIA (cohort 1/target disease population); and stroke, TIA, or non-stroke/TIA (mimic), i.e. all patients (cohort 2/intention-to-treat, ITT). Further information is given in the Appendix.

The primary outcome was assessed using ordinal logistic regression with adjustment for age, sex, premorbid mRS, FAST score, baseline SBP, index event (ICH, IS, TIA,

mimic), time to randomisation, and reperfusion treatment (thrombectomy, alteplase, none).[17] The assumption of proportional odds was tested using the likelihood ratio test. Heterogeneity of the treatment effect on the primary outcome was assessed in pre-specified subgroups by adding an interaction term to an adjusted ordinal logistic regression model. An unadjusted and *per protocol* (as defined in the appendix) analysis is shown for completeness. Death was analysed using Kaplan-Meier and adjusted Cox regression models. Other outcomes were assessed using adjusted binary logistic regression (neurological deterioration, headache, hypotension, hypertension, feeding status, disposition, death in hospital), Cox regression (death), ordinal logistic regression (mRS, disposition), multiple linear regression (NIHSS, length of stay in hospital, t-MMSE, TICS-M, animal naming, ZDS, EQ-5D HSUV EQ-VAS) and analysis of covariance (blood pressure). A global outcome (comprising ordered categorical or continuous data for mRS, BI, ZDS, TICS-M and EQ-5D-HSUV) was analysed using the Wei-Lachin test.[25] Participants who did not receive their assigned treatment, who did not adhere to the protocol, or who had a stroke mimic were all still followed up in full at day 90 and are included in the main analyses. No adjustments were made for multiplicity of testing since all secondary analyses were hypothesis-generating and designed to support the primary analysis. Data are shown as number (%), median [interquartile range], mean (standard deviation), and odds ratio, hazard ratio, difference in means or Mann-Whitney difference (global) with 95% confidence intervals. Primary analyses were done as randomised (cohort 2) using observed outcome data only, and performed with SAS software (version 9.4). In sensitivity analyses, we performed a per-protocol analysis, and missing mRS data were imputed using multiple regression-based imputation.

Role of the funding source

The trial was funded by the British Heart Foundation (CS/14/4/30972) and sponsored by the University of Nottingham. There was no commercial support for the trial, and GTN patches and sham dressings were sourced by the Pharmacy at Nottingham University Hospitals NHS Trust. The funder did not contribute to data collection, analysis, interpretation, manuscript writing or decision to submit. The corresponding author and two statisticians (PS, LJW) had full access to all the data in the study; additionally, the corresponding author had final responsibility for the decision to submit for publication and is the guarantor for the trial.

RESULTS

Study population

Between 22 October 2015 and 23 May 2018 (appendix Table 1), a total of 1149 participants (cohort 2) were enrolled (568 randomised to GTN patch and 581 to sham dressing, CONSORT diagram, Figure 1) by 516 (of 1492, 35%) trial-trained paramedics based at 184 ambulance stations in 8 (of 13, 62%) ambulance services in England and Wales; these participants were taken to 54 hospitals. For logistical reasons, screening logs were not kept. All patients had consent in the ambulance, this being obtained from 603 (53%) patients; 429 (37%) relatives, carers or friends; and 117 (10%) paramedics. The CONSORT diagram for all stroke/TIA (cohort 1) is shown in appendix Figure 1. Demographic and clinical characteristics were similar in the two treatment groups across the whole trial population and in patients with stroke or TIA (Table 1). The mean age was 72·5 (standard deviation 14·6) years, women comprised 48% of the participants, 60% of participants had a maximum FAST score=3, and 26% a GCS <14. The final diagnosis of the qualifying event was: IS 52%, ICH 13%, TIA 9% and a stroke/TIA-mimicking condition 26%. Common causes of stroke mimics included seizure (18%), migraine (17%) and functional symptoms (15%).

Time intervals

The median time from the onset of symptoms to randomisation was 71 minutes [interquartile range, 45, 116] and to start of study drug 73 [48, 118] minutes. Overall, study drug was received within 30, 60 and 120 minutes of symptom onset in 59 (5%), 439 (38%) and 865 (75%) of participants respectively.

Adherence and protocol violations

Adherence to the first randomised treatment was excellent in both the confirmed stroke/TIA group (cohort 1/target disease population, 849, >99%) and in all participants (cohort 2/ITT, 1144, >99%) (appendix Table 2, appendix Figure 1). In the *per protocol* definition of adherence, which required that at least the first two doses of treatment were received, only 571 (67%) of cohort 1 and 631 (55%) of cohort 2 were adherent; common reasons for non-adherence were a diagnosis of a non-stroke, early discharge, a medical decision to stop randomised treatment, a procedural error, or missing trial medication (appendix Table 2). Just 382 (45%) of participants with a stroke or TIA (cohort 1), and 408 (36%) of participants overall

(cohort 2), received all four days of treatment.

There were 38 protocol violations in the ambulance and these mainly comprised inclusion of patients beyond 4 hours, FAST score <2, SBP <120 mmHg, or from a nursing home (appendix Table 8). The most common protocol violations in hospital involved not administering the second day's treatment, or failure to obtain secondary consent.

Blood pressure treatment and achieved blood pressure levels

In the target population of stroke or TIA, BP at baseline was 163·2 (24·7)/91·9 (18·5) mmHg (Table 1) and fell in both GTN and sham groups over the 4 days following randomisation (appendix Figure 2). Following the treatment, systolic and diastolic BP were lower in the GTN group by 5·8/2·6 mmHg (p<0·0001/p=0·0026) at hospital admission, and 5·3/2·6 mmHg (p =0·0002/p=0·0054 at day 2, as compared with sham. The difference in BP between the GTN and sham groups then diminished with no difference at days 3 and 4. Similar findings were seen for the effect of GTN on BP in all patients (cohort 2, appendix Figure 3). In all patients, symptomatic hypotension was more common with GTN (21 [4%] vs 9 [2%], adjusted odds ratio, aOR 2·49, 95% CI 1·11, 5·57; p=0·026) (Table 2). Heart rate did not differ between the treatment groups (data not shown).

Primary outcome

Vital status and mRS were available in 1122 (98%) and 1102 (96%) participants respectively (Figure 1); there was no differential loss to follow-up or withdrawals between the treatment groups. Blinding was maintained with participants unable to identify which medication they had received (appendix Table 3).

In the cohort of confirmed stroke and TIA (cohort 1/target disease population), there was no strong evidence of an effect of GTN on functional outcome at 90 days in comparison with sham (GTN 3 [2, 5] vs sham 3 [2, 5], acOR 1·25, 95% CI 0·97–1·60, p=0·083; Table 2, Figure 2); the acOR 1·25 suggests a tendency in favour of sham treatment. In sensitivity analyses, there was no difference in mRS when compared as mean difference, proportions with poor outcome (mRS>2), mRS in the *per protocol* population, or when data were imputed for participants without a recorded mRS at day 90 (Table 2). A significant interaction of the effect of GTN on mRS was present for time to randomisation with a negative effect of GTN apparent in those participants

recruited within one hour of symptom onset (Figure 3); no other significant effect modification by subgroups was detected. *Post hoc* assessment of the treatment effect on mRS in clinically relevant subgroups defined on or after admission to hospital (i.e. potentially affected by treatment) showed a significant interaction with a worse outcome in patients with a more severe stroke on admission to hospital (post-treatment NIHSS >12) (appendix Figure 4).

When assessed in components of the target population (cohort 1/target disease population), mRS did not differ between GTN and sham in participants with stroke (GTN 3 [2, 6] vs sham 3 [2, 5], acOR 1·26, 95% CI 0·96, 1·64; p=0·095; N=722), ischaemic stroke (GTN and Sham median score 3 IQR [2, 5], acOR 1·15, 95% CI 0·85, 1·54; p=0·36; N=580), or TIA (GTN median 3 IQR [1, 3] versus Sham 2 [1, 3], acOR 1·57, 95% CI 0·74, 3·35; p=0·24; N=105). However, GTN was associated with a non-significantly worse outcome in patients with a final diagnosis of ICH (GTN median 5 IQR [4, 6] versus Sham 5 [3, 5], acOR 1·87, 95% CI 0·98, 3·57; p=0·057; N=142; appendix Figure 6).

The analysis of all patients in the trial (cohort 2/ITT) also showed that mRS did not differ between GTN and sham in the primary analysis (median for both groups 3, IQR [2, 5], acOR 1·04, 95% CI 0·84–1·29, p=0·69; Table 2, appendix Figure 5) or in any sensitivity analysis (data not shown). In pre-defined subgroups, there was a significant interaction by final diagnosis (appendix Figure 6); in contrast to the effect of GTN in stroke or TIA (see above), GTN appeared to be associated with an improved mRS in patients with a mimic (non-stroke/TIA) (GTN and Sham median score 3 IQR [1, 4], acOR 0·54, 95%CI 0·34–0·85, p=0·008); in a *post hoc* analysis, this positive finding was not localised to any particular type of mimic (data not shown).

Secondary outcomes

GCS at admission to hospital was 0·4 point lower in patients randomised to GTN. Because of this difference, we performed a *post hoc* sensitivity analysis adding baseline GCS to the statistical model for the primary outcome in cohort 1; this had minimal effect on the result, OR 1·22 (95% CI 0·95, 1·56). Otherwise, there was no evidence of any other differences between GTN and sham in secondary outcomes in the cohort of participants with confirmed stroke/TIA (Table 2, appendix Table 4). A global analysis encompassing the primary outcome and pre-specified secondary outcomes showed no difference (Table 2, appendix Figure 7).

As compared to sham, patients with an ischaemic stroke who were randomised to GTN were less likely to have thrombectomy (appendix Table 5); conversely, patients receiving GTN were more likely to be ventilated on an intensive care unit. Use of other standard stroke treatments did not differ between the randomised treatment groups.

There were no differences between GTN and sham in secondary outcomes at day 90 (Table 2).

Safety

The proportion of deaths at day 4 did not differ between GTN and sham either in the target confirmed stroke/TIA population (cohort 1) or in the full ITT population (Table 2). Similarly, the proportion of deaths by day 90 did not differ in the target confirmed stroke/TIA population (cohort 1; aHR 1·24, 95% CI 0·91–1·68, p=0·17; Table 2, appendix Figure 8) and all population (cohort 2; Table 2, appendix Figure 9). The commonest causes of death were progression or recurrence of the index stroke, and pneumonia. A slight excess of headaches by day 4 was apparent for GTN. The number of participants experiencing one or more SAEs did not differ between the GTN and sham groups (188 [33%] vs 170 [29%), p=0·16; appendix Table 6) although cardiovascular SAEs were more common with GTN. No suspected unexpected serious adverse reactions (SUSAR) occurred.

Imaging outcomes

The on-treatment hospital-based imaging findings for participants with a final diagnosis of stroke or TIA are shown in appendix Table 7. In ischaemic stroke, scanning was performed on admission and day 2 at 2·2 hours and 27·7 hours, respectively, after the onset after stroke or TIA. There were no differences between GTN and sham in respect of infarct size, swelling or mass effect on plain brain CT. Patients receiving intravenous thrombolysis were non-significantly less likely to have haemorrhagic transformation with GTN as compared with sham: 5 (3%) vs 11 (8%), OR 0·38 (95% CI 0·13, 1·13; p=0·082). For participants with ICH, scanning was performed on admission at an average of 2·3 hours and 28·9 hours after the onset after stroke or TIA (appendix Table 7). In comparison with sham, GTN was associated with larger haematoma (OR 1·95, 95% CI 1·07, 3·58; p=0·030) and more mass effect (OR 2·42, 95% CI 1·26, 4·68; p=0·0083) at hospital admission.

Meta-analysis of completed trials

Addition of the results for participants with confirmed stroke/TIA (cohort 1/target disease population) in RIGHT-2 to the positive published Cochrane review for hyper-acute administration of GTN [14] resulted in neutral effects for end-of-trial death or dependency (mRS>2), OR 0·80 (95% CI 0·59–1·10; p=0·17; heterogeneity I^2 16%, p=0·30) and death, OR 0·52 (95% CI 0·16–1·72; p=0·28; heterogeneity I^2 86%, p=0·0007) (appendix Figure 10). Heterogeneity between trial results was apparent for death emphasising the difference between the results for RIGHT-2 versus the earlier RIGHT and ENOS-early trials.

DISCUSSION

RIGHT-2 recruited 1149 patients from 8 UK ambulance services and these were taken to 54 hospitals; 516 paramedics from 184 ambulance stations performed screening and consent, and delivered treatment and early follow-up measurement. Consent or proxy consent was obtained from patients; relatives, carers or friends of the patient; or by the recruiting paramedic. Treatment was commenced very early, and faster than in hospital-based trials, with 38% of patients treated in the first 60 minutes after stroke onset ('golden hour' [26]). Hence, we have confirmed that it is feasible to perform a large multicentre paramedic-delivered ambulance-based trial in patients with suspected stroke in the UK in patients with suspected stroke. Having confirmed feasibility, we compared the effect of GTN with sham and found that treatment with GTN did not influence functional outcome in patients with the target diagnosis of confirmed stroke or TIA, or in the overall recruited population.

The results for GTN differ from those reported in a previous small phase II ambulance-based trial (RIGHT, with recruitment within 4 hours of onset) and a subgroup of a large phase III trial (ENOS, recruitment <6 hours of onset).[10,12] There are several potential explanations for these discrepant results. First, GTN may simply be ineffective in very early stroke, as suggested by the absence of any effect of GTN on multiple secondary outcomes and a global outcome, and neutral meta-analyses when combining ENOS-early, RIGHT and RIGHT-2. Second, the discrepant findings may be due to chance rather than any true positive or negative effect of GTN. Chance may also account for the observation that GTN appeared to be beneficial in participants with a final diagnosis of non-stroke/TIA irrespective of the underlying mimic diagnosis. Third, the difference between RIGHT-2 and ENOS-early/RIGHT may be real due to intrinsic differences in their design: RIGHT-2 randomised patients far earlier (median 71 minutes) than in RIGHT and ENOS-early combined (median 257 minutes) and so will have recruited a different cohort of patients; as compared with these earlier trials, participants in RIGHT-2 were older, and more likely to have pre-morbid dependency, diabetes, previous stroke, and ischaemic heart disease; and, among the ICH patients, more likely to still be in a period of hematoma expansion. All these factors may have contributed to different effects of GTN on functional outcome, as was apparent for reductions in systolic BP (6·2 mmHg in RIGHT-2 vs. 9·4 mmHg in ENOS-early). Last, studies showing a positive effect of GTN within 6 hours used a 7-

day treatment period and had higher rates of adherence so it is conceivable that GTN was not given for long enough in RIGHT-2.

Although RIGHT-2 was a neutral trial, GTN was associated with a tendency for a worse functional outcome in confirmed stroke/TIA patients (cohort 1), with a 95% confidence interval covering a range from a clinically insignificant benefit (OR 0·97) to a clinically significant hazard (OR 1·60). This tendency towards harm was particularly seen in patients with ICH, very early stroke (<1 hour) and severe stroke (GCS<12, NIHSS>12). Further, the imaging findings support a negative effect of GTN in ultra-acute ICH with larger haematoma, and more haematoma expansion, perihaematoma oedema, mass effect, and mid-line shift. A number of explanations might explain potential hazard in ICH, which has a higher base rate of ultra-early neurological deterioration than IS,[27] and these are given in decreasing order of likelihood. First, the earliest stage in haemostasis is vasoconstriction and GTN may prevent this protective response and so lead to very early haematoma expansion. Second, although we did not identify antiplatelet effects with GTN in a previous study of patients with stroke,[7] others have reported this in laboratory experiments [28] and GTN could therefore have amplified haematoma expansion in ICH thereby countering any effects of lowering BP. Third, venodilators such as sodium nitroprusside and GTN have been shown experimentally and clinically to raise intracerebral pressure (ICP) and reduce cerebral blood flow, particularly if ICP is already elevated.[29,30] Reduced blood flow might then induce peri-haematoma ischaemia. Although pilot work did not find a negative effect of GTN on CBF or cerebral perfusion pressure in hospitalised patients with recent stroke,[8,9,31] these studies were not in the ultra-acute period after stroke and were mainly in ischaemic stroke and so may not be directly relevant to the RIGHT-2 population. Last, GTN can stimulate the formation of reactive oxygen species such as superoxide (O_2^-) and peroxynitrite ($OONO^-$) and these can attenuate vasodilation and increase the potential for cellular damage.[32]

Preclinical studies of neuroprotective and collateral enhancement therapy in ischaemic stroke suggest that treatment is most effective when administered rapidly after symptom onset. Ideally treatment would be started prior to hospital admission to reduce "stroke-to-needle" time.[33] The US FAST-MAG trial successfully randomised 1700 participants in ambulances to receive intravenous magnesium or placebo within 2 hours of symptom start, and took them to 36 hospitals.[15] RIGHT-2 extends these observations demonstrating that a large stroke ambulance trial can be performed

embedded in the UK national health service involving multiple ambulance services and hospitals. Hence, other interventions that do not require prior CT scanning could be tested in this environment in the future. By extrapolation, paramedics will also be able to administer such interventions routinely in the ambulance once they have been shown to be effective in one or more types of stroke, and safe in mimics.

The present trial has several strengths, including the large sample size; generalisability due to wide inclusion criteria; central concealment of treatment assignment; excellent adherence to the first dose of allocated treatment; prospective collection of multiple functional outcomes as well as safety measures such as hypotension and hypertension; near-complete follow-up (96% of patients had their primary outcome recorded); and central, blinded assessment of outcomes at day 90. Patients received modern care including stroke unit admission, thrombolysis, thrombectomy, and hemicraniectomy.

Several limitations are also present. First, GTN was administered in a single-blind design since there are no commercial sources of placebo patches. In spite of this, patient-blinding at day 90 was successful through use of a near identical sham patch, and both GTN and sham patches were unmarked; placement of a gauze dressing over the patch [8,9 10] gave additional blinding. Further, outcomes measured at day 90 were assessed centrally by trained staff masked to treatment assignment who were not involved in hospital care of enrolled patients. Second, many patients did not receive randomised treatment for the intended minimum period of two days, and even fewer for the full four days; hence, participants may have received inadequate treatment. Third, the difference in blood pressure between GTN and sham was small and less than that seen in the large ENOS trial.[11,12] Whilst this might reflect inaccuracies in BP measurement in the emergency environment of ambulance and hospital admission, it might also explain the lack of benefit in ischaemic stroke. Fourth, we had to increase the sample size, an unplanned change necessary because of the higher-than-planned mimic rate. Last, the trial's wide inclusion criteria recruited a population of stroke patients that would not normally enter hospital-based trials. In this respect, a group of participants with very severe ICH were enrolled who deteriorated rapidly and then died; this may have neutralised any treatment effect.

So far as we are aware, this is the first acute stroke trial to use a hierarchical approach to analysis in which the first analysis in the primary family was performed in

the target population, with the potential for a subsequent primary analysis across the entire ITT population. We followed this pre-defined plan [17] since the high mimic rate had the potential to dilute out any treatment effect. Although the nonpositive result in the target population precluded testing the ITT population with multiplicity control, this approach had the advantage that the primary analysis of the study directly addressed the core question of biologic benefit of agent administration in patients with the disease of interest.

In summary, treatment with transdermal GTN administered before hospital did not alter functional outcome in participants with ultra-acute stroke. The signals of potential adverse effect of GTN in ICH are not definitive, but suggest the advisability of close safety monitoring in ongoing trials of pre-hospital GTN in ultra-acute ICH (ISRCTN99503308). Nevertheless, earlier findings in the large ENOS trial, including in ICH, suggest that transdermal GTN is safe when administered later in hospital [11] and may continue to be used for lowering BP, for example prior to thrombolysis. Finally, the study shows that large ambulance-based studies are feasible in the UK and, by extrapolation and taking account of the FAST-MAG trial,[15] to most developed countries.

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Other authors do not report potential conflict of interests relevant to this article.

Contributors

PMB was chief investigator, a grant applicant, participated in the steering committee, collected, verified, and analysed data and drafted this report, and is project guarantor. PS was trial statistician, involved in the design of the trial, participated in the steering committee, wrote the first draft of the statistical analysis plan, and verified and analysed data. CSA was an international advisor who provided guidance on trial delivery and interpretation. SA adjudicated serious adverse events. JPA was the trial physician supporting the chief investigator and trial delivery. EB was an international advisor who provided guidance on trial delivery and interpretation. LC adjudicated brain scans. MD was the national paramedic lead coordinating ambulance service trial delivery. TE was a grant applicant, participated in the steering committee, and advised on trial delivery. PJG was statistician to the data monitoring committee. DH was senior trial manager and chaired the management committee. LH

programmed and supported the web interface and trial databases. TH wrote the approval documents and information sheets, and provided statistical advice. KK performed brain scan measurements in participants with intracerebral haemorrhage. GM adjudicated brain scans. AAM was statistician, grant applicant and participated in the steering committee. KM was an independent member of the steering committee. SJP was an international advisor who provided guidance on trial delivery and interpretation. SP was statistician, grant applicant and participated in the steering committee. JP was a grant applicant, participated in the steering committee, and advised on trial delivery. CP was a grant applicant, participated in the steering committee, and advised on trial delivery by ambulance services. MR adjudicated serious adverse events. TGR was a grant applicant, participated in the steering committee, and advised on trial delivery. CR was a grant applicant, participated in the steering committee, and advised on trial delivery. PR was a national advisor who provided guidance on trial delivery and interpretation. ECS was an international advisor who provided guidance on trial delivery and interpretation. NS was an international advisor who provided guidance on ambulance trial delivery and interpretation. JLS was an international advisor who provided guidance on ambulance trial delivery and interpretation. AS was sponsor. ANS was a grant applicant, participated in the steering committee, and advised on trial delivery by ambulance services. JMW was a grant applicant, participated in the steering committee, and led adjudication of brain scans. LJW was trial statistician, involved in the design of the trial, participated in the steering committee, and verified and analysed data. GV was independent chair of the steering committee. NS was deputy chief investigator, a grant applicant, and participated in the steering committee. All members of the writing committee commented on the analyses and drafts of this report and have seen and approved the final version of the report.

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A complete list of Investigators in the Rapid Intervention with Glyceryl trinitrate in Hypertensive stroke Trial-2 (RIGHT-2) is provided in the appendix.

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Table 1. Baseline ambulance and hospital admission characteristics of patients enrolled in the RIGHT-2 trial, all stroke and TIA (cohort 1/target disease population) and all (cohort 2, intention-to-treat) patients. Data are number (%), median [IQR], or mean (standard deviation).

Characteristic	**Confirmed stroke/TIA**		**All**	
	GTN	Sham	GTN	Sham
Ambulance data (pre-randomisation)				
Number of patients	434	418	568	581
Consent by (%)				
Participant	220 (51)	206 (49)	296 (52)	307 (53)
Relative, carer or friend	169 (39)	172 (41)	213 (38)	216 (37)
Paramedic	45 (10)	40 (10)	59 (10)	58 (10)
Age (years)	73·7 (12·8)	75·3 (12·3)	72·3 (14·6)	72·7 (14·6)
Sex, male (%)	234 (54)	220 (53)	294 (52)	300 (52)
Time from onset to randomisation (minutes)	70 [45, 107]	70 [45, 110]	70 [45, 115]	72 [45, 118]
ECG, AF/flutter (%)	81 (24)	77 (22)	92 (21)	95 (20)
Systolic blood pressure (mmHg)	163·4 (24·5)	163·0 (24·9)	161·5 (24·7)	162·8 (25·5)
Diastolic blood pressure (mmHg)	92·2 (19·1)	91·5 (17·8)	91·5 (18·5)	91·6 (17·2)
Heart rate (bpm)	81·6 (18·7)	82·2 (18·6)	81·7 (18·0)	82·6 (19·2)
Glasgow coma scale <14 (%)	123 (28)	106 (25)	162 (29)	140 (24)
FAST score =3 (%)	276 (64)	270 (65)	343 (60)	347 (60)
Hospital admission data (post randomisation)				

Number of patients	434	418	568	581
Ethnic group, non-white (%)	35 (8)	43 (10)	50 (9)	63 (11)
Pre-morbid mRS >2 (%)	76 (18)	68 (16)	115 (20)	108 (19)
Medical history (%)				
Hypertension	252 (58)	249 (60)	313 (56)	330 (58)
Diabetes mellitus	82 (19)	86 (21)	109 (20)	118 (21)
Previous stroke	100 (23)	87 (21)	137 (25)	135 (24)
Ischaemic heart disease	66 (15)	72 (17)	95 (17)	101 (18)
Smoking, current	63 (18)	51 (15)	89 (19)	79 (17)
Qualifying event (%)				
Ischaemic stroke	302 (70)	295 (71)	302 (53)	295 (51)
Intracerebral haemorrhage	74 (17)	71 (17)	74 (13)	71 (12)
Stroke type unknown	1 (0)	0 (0)	1 (<1)	0 (0)
TIA	57 (13)	52 (12)	57 (10)	52 (9)
Non-stroke/TIA mimic	-	-	134 (24)	163 (28)

IQR: interquartile range; GTN: glyceryl trinitrate; AF: atrial fibrillation; bpm: beats per minute; FAST: face/arm/speech/time test; mRS: modified Rankin Scale; TIA: transient ischaemic attack

Table 2. Primary and secondary outcomes at days 4 and 90 in all stroke and TIA (cohort 1, modified intention-to-treat) and all (cohort 2, intention-to-treat) patients. Data are number (%), median [interquartile quartile range] or mean (standard deviation). Comparison by adjusted binary logistic regression (BLR), Cox proportional hazards regression (Cox), ordinal logistic regression (OLR), or multiple linear regression (MLR), with adjustment for age, sex, pre-morbid mRS, FAST score, pre-treatment systolic BP, index event (intracerebral haemorrhage, ischaemic stroke, transient ischaemic attack, mimic), time to randomisation, and reperfusion therapy (mechanical thrombectomy, alteplase, none) (unless stated). The effect of treatment for GTN versus sham are shown as odds ratio (OR), hazard ratio (HR), or difference in means (DIM), with 95% confidence intervals.

		Cohort 1	**mITT**	**Confirmed stroke/TIA**			**Cohort 2**	**ITT**	**All**	
Outcome	N	GTN	Sham	acOR/aOR/aDIM	p	N	GTN	Sham	acOR/aOR/aDIM	p
	852	N=434	N=418	(95% CI)		1149	N=568	N=581	(95% CI)	
Day 90 mRS *(/6)*										
Primary outcome	828	3 [2, 5]	3 [2, 5]	1·25 (0·97, 1·60)	0·083	1102	3 [2, 5]	3 [2, 5]	1·04 (0·84, 1·29)	0·69
Sensitivity analyses										
Unadjusted	828	3 [2, 5]	3 [2, 5]	1·05 (0·83, 1·33)	0·70	1102	3 [2, 5]	3 [2, 5]	0·99 (0·81, 1·22)	0·96
Mean	828	3·4 (2·0)	3·4 (1·9)	0·14 (-0·07, 0·36)	0·19	1102	3·2 (2·0)	3·2 (1·9)	0·01 (-0·17, 0·19)	0·92
mRS>2 (%)	828	286 (68)	282 (69)	1·11 (0·79, 1·57)	0·55	1102	358 (66)	373 (67)	1·02 (0·76, 1·38)	0·88
Per protocol	714	3 [2, 5]	3 [2, 5]	1·22 (0·93, 1·60)	0·14	959	3 [2, 5]	3 [2, 5]	1·05 (0·84, 1·33)	0·65
Imputed	852	3 [2, 5]	3 [2, 5]	1·23 (0·96, 1·57)	0·10	1149	3 [2, 5]	3 [2, 5]	1·05 (0·85, 1·30)	0·65
Hospital admission										
NIHSS (/42)	755	10·5 (7·6)	10·4 (7·7)	0·34 (-0·51, 1·19)	0·43	931	9·7 (7·6)	9·4 (7·5)	0·14 (-0·61, 0·89)	0·72

GCS (/15)	835	13·5 (2·3)	13·8 (2·0)	-0·37 (-0·64, -0·10)	0·007	1076	13·7 (2·2)	13·9 (1·9)	-0·19 (-0·42, 0·04)	0·10
FAST [/3]	799	2·3 (0·9)	2·2 (1·0)	0·09 (-0·02, 0·19)	0·10	985	2·2 (1·0)	2·1 (1·0)	0·03 (-0·07, 0·13)	0·51
OCSP, TACS (%)	822	161 (38)	149 (37)	1·13 (0·82, 1·55)	0·45	1046	176 (34)	174 (33)	1·03 (0·78, 1·37)	0·83
Day 4 (discharge)										
Death (%)	849	20 (5)	18 (4)	1·17 (0·57, 2·39)	0·68	1128	22 (4)	19 (3)	1·19 (0·60, 2·35)	0·63
ND (%) [a]	534	60 (23)	56 (21)	1·14 (0·74, 1·77)	0·56	586	62 (21)	59 (20)	1·08 (0·70, 1·65)	0·73
Headache (%) [b]	843	41 (10)	28 (7)	1·41 (0·84, 2·37)	0·19	1117	49 (9)	36 (6)	1·43 (0·90, 2·27)	0·13
Hypotension (%) [b]	844	18 (4)	9 (2)	2·07 (0·90, 4·75)	0·085	1118	21 (4)	9 (2)	2·49 (1·11, 5·57)	0·026
Hypertension (%) [b]	844	89 (21)	93 (22)	0·82 (0·57, 1·18)	0·28	1118	106 (19)	108 (19)	0·96 (0·69, 1·33)	0·81
Feeding: non-oral (%)	806	123 (30)	132 (33)	0·89 (0·63, 1·26)	0·51	1049	130 (25)	139 (26)	0·89 (0·65, 1·24)	0·50
In hospital events										
Length of stay	847	17·4 (29·7)	19·1 (28·9)	-1·35 (-5·16, 2·46)	0·49	1126	14·6 (27·0)	15·3 (25·7)	-1·09 (-4·00, 1·81)	0·46
Died (%)	847	72 (17)	60 (14)	1·28 (0·84, 1·96)	0·26	1126	78 (14)	63 (11)	1·35 (0·90, 2·02)	0·15
Died/institution (%)	831	180 (42)	167 (41)	1·17 (0·84, 1·61)	0·35	1102	193 (35)	186 (33)	1·08 (0·81, 1·46)	0·60
Day 90										
Death (%)	841	97 (23)	79 (19)	1·24 (0·91, 1·68)	0·17	1122	105 (19)	98 (17)	1·11 (0·84, 1·47)	0·47
Disposition (%) [c]	809	1 [1,2]	1 [1,2]	1·32 (0·96, 1·82)	0·086	1069	1 [1,2]	1 [1,2]	1·11 (0·83, 1·47)	0·48
EQ-5D HSUV (/1) [d]	798	0·4 (0·4)	0·4 (0·4)	-0·02 (-0·07, 0·03)	0·42	1055	0·4 (0·4)	0·4 (0·4)	0·00 (-0·04, 0·05)	0·95
BI (/100) [d]	795	56·2 (45·0)	57·5 (43·9)	-2·74 (-7·82, 2·33)	0·29	1048	60·3 (43·7)	61·3 (43·1)	-0·24 (-4·54, 4·06)	0·91
TICS-M [d e]	439	12·4 (12·3)	13·2 (12·1)	-0·87 (-2·63, 0·90)	0·34	551	13·5 (12·3)	13·7 (11·8)	0·06 (-1·50, 1·63)	0·94
ZDS (/100) [d e]	499	67·3 (29·7)	66·0 (29·1)	1·38 (-2·87, 5·63)	0·52	638	66·5 (28·8)	65·1 (28·6)	0·53 (-3·22, 4·28)	0·78
Global outcome (MWD)	828	-	-	-0·02 (-0·10, 0·06)	0·62	1102	-	-	0·00 (-0·07, 0·06)	0·92

Home time (days) ‡	682	55·8 (49·2)	55·5 (46·8)	-0·30 (-6·14, 5·54)	0·92	903	63·5 (48·9)	63·7 (46·9)	2·18 (-2·81, 7·16)	0·39

BI: Barthel index; DIM: difference in means; FAST: face/arm/speech/time test (calculated from NIHSS); EQ-5D: Euro-Quality of life-5 Dimensions; EQ-VAS: Euro-Quality of life-Visual Analogue Scale; HSUV: health status utility value (calculated from EQ-5D); mRS: modified Rankin scale; ND: neurological deterioration; NIHSS: National Institutes of Health Stroke Scale; OCSP: Oxford Community Stroke Project; TACS: total anterior circulation syndrome (in IS and ICH); TIA: transient ischaemic attack; TICS-M: modified telephone interview cognition scale; t-MMSE: telephone mini-mental state examination; ZDS: Zung depression scale; MWD: Mann-Whitney difference

[a] Neurological deterioration from hospital admission: NIHSS >=4 points or >=2 point increase in any domain

[b] Clinical

[c] Disposition: home, institution/in hospital, died.

[d] Death scored as: BI -5, verbal fluency (animal naming) -1, EQ-VAS -1, home time -1, t-MMSE -1, TICS-M -1, EQ-5D HSUV 0, GCS 2, mRS 6, NIHSS 43, ZDS 102·5

[e] Incomplete TICS-M and ZDS due to inability by participants with severe stroke to respond to questions

The *per protocol* population is defined in the Appendix.

Figure 1. Consort flow diagram of patient randomisation, outcome, and losses to follow-up for all patients (cohort 2, intention-to-treat). Data are number (%)

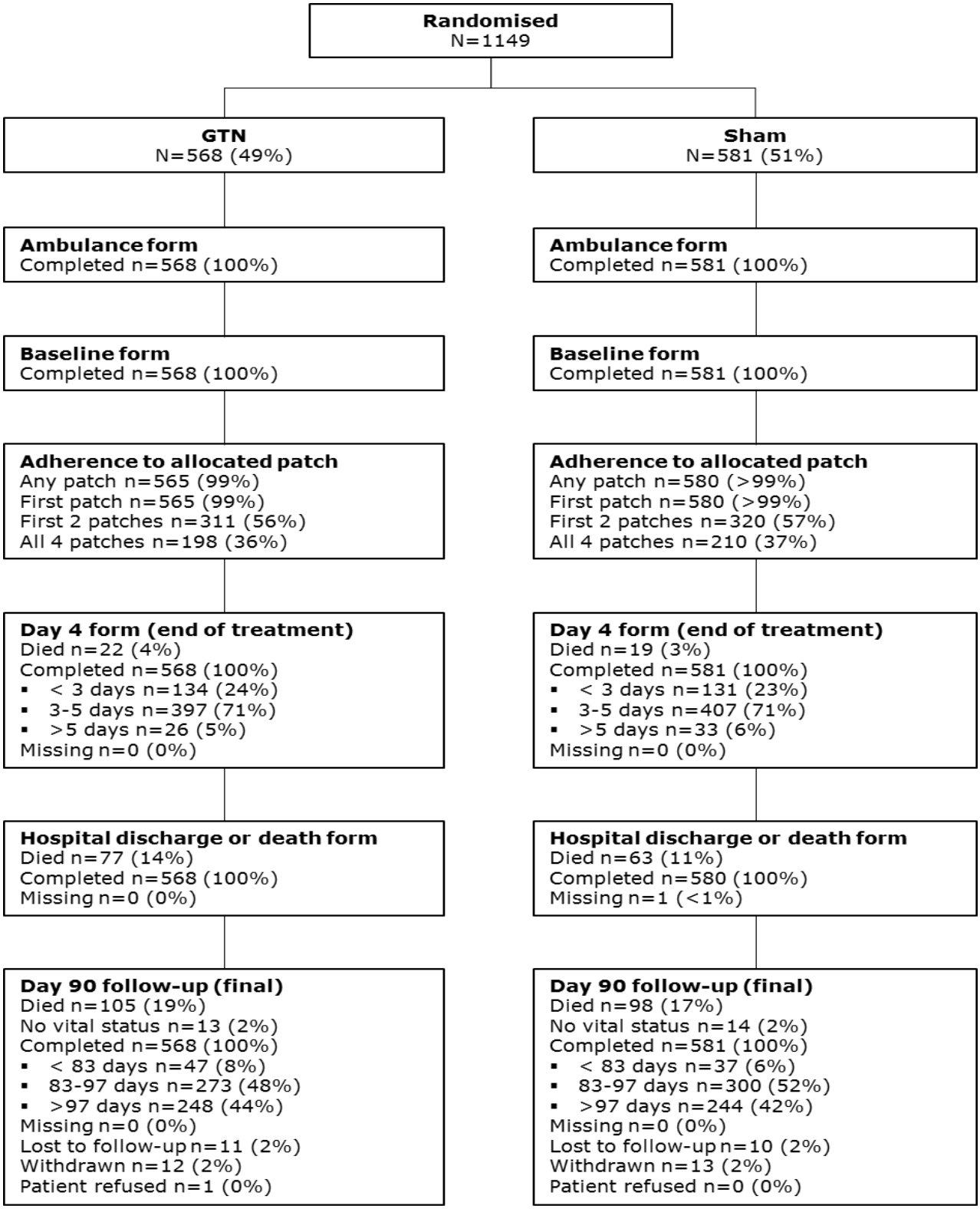


Figure 2. Distribution of modified Rankin Scale (mRS) at day 90 for glyceryl trinitrate (GTN) versus sham, in stroke/TIA patients (cohort 1, modified intention-to-treat). Comparison by ordinal logistic regression adjusted for age, sex, pre-morbid mRS, FAST score, pre-treatment systolic BP, index event (intracerebral haemorrhage, ischaemic stroke, transient ischaemic attack, mimic) and time to randomisation.

Adjusted common odds ratio 1·25 (95% confidence intervals 0·97, 1·60), p=0·083

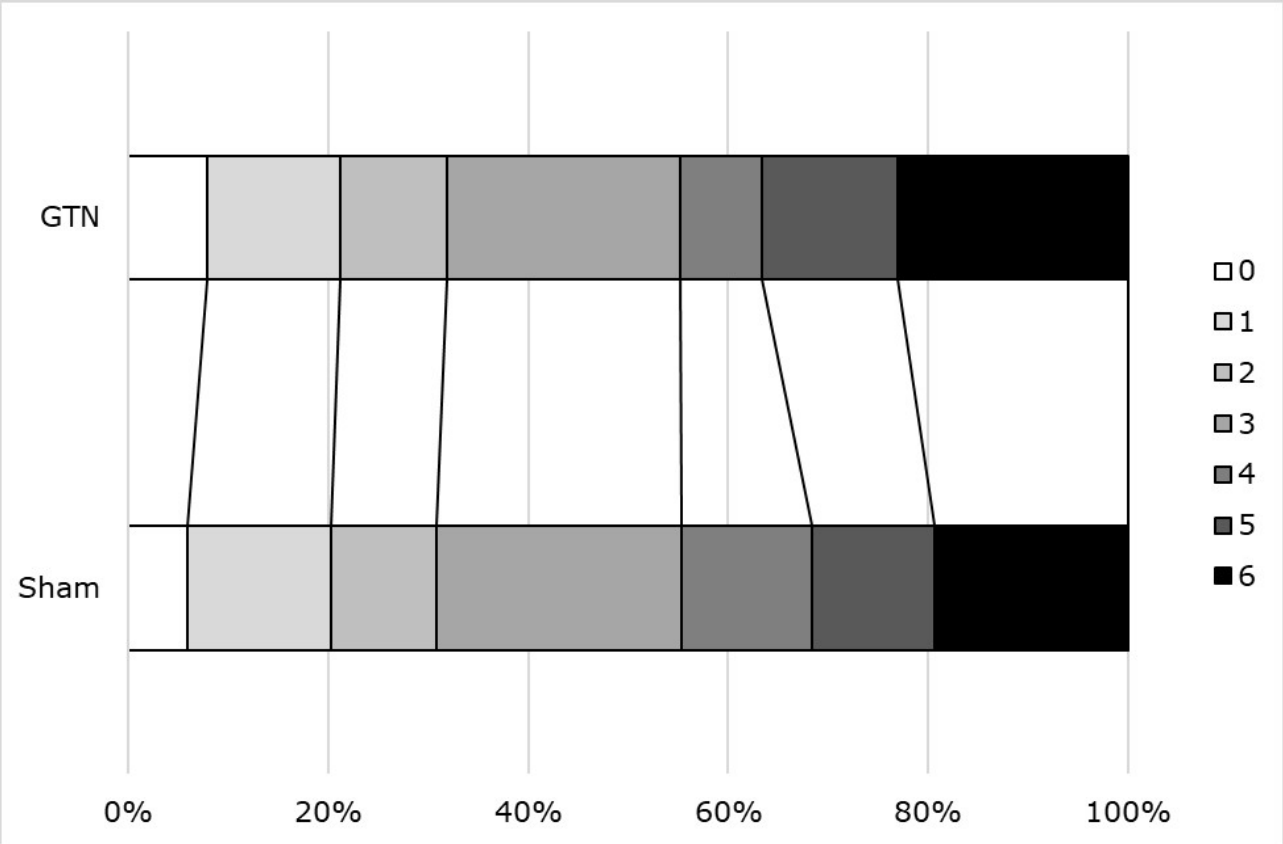
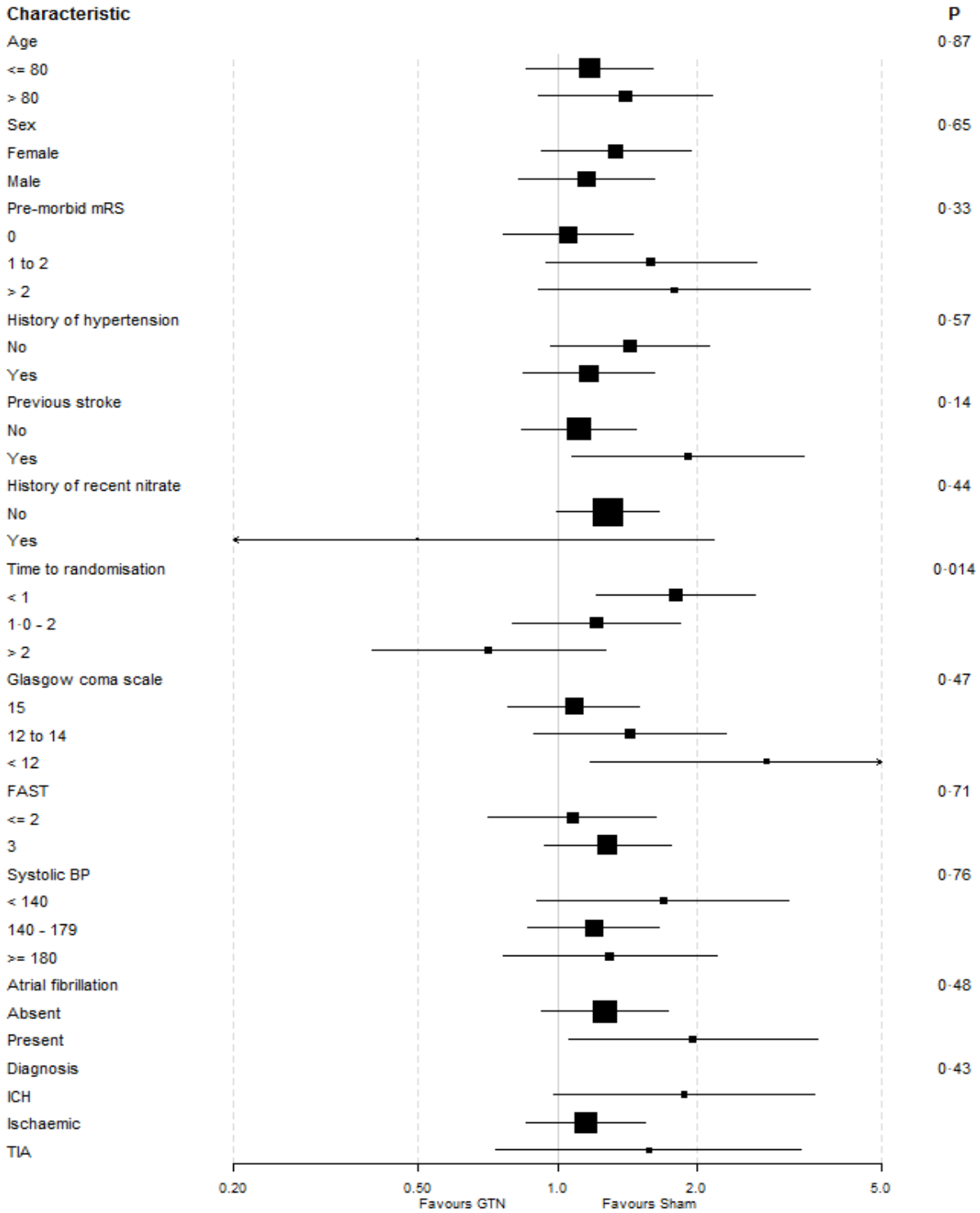


Figure 3. Forest plot of effect of GTN versus Sham on mRS at day 90 in stroke and TIA patients in pre-specified subgroups defined before treatment and admission to hospital. Data are odds ratio (95% confidence intervals) and interaction test. Comparison by ordinal logistic regression adjusted for age, sex, pre-morbid mRS, FAST, pre-treatment systolic BP, index event (intracerebral haemorrhage, ischaemic stroke, transient ischaemic attack, mimic), time to randomisation and reperfusion therapy (alteplase, intra-arterial therapy, none).


Characteristic
P
Age
0·87
<= 80
> 80
Sex
0·65
Female
Male
Pre-morbid mRS
0·33
0
1 to 2
> 2
History of hypertension
0·57
No
Yes
Previous stroke
0·14
No
Yes
History of recent nitrate
0·44
No
Yes
Time to randomisation
0·014
< 1
1·0 - 2
> 2
Glasgow coma scale
0·47
15
12 to 14
< 12
FAST
0·71
<= 2
3
Systolic BP
0·76
< 140
140 - 179
>= 180
Atrial fibrillation
0·48
Absent
Present
Diagnosis
0·43
ICH
Ischaemic
TIA
0.20
0.50
1.0
2.0
5.0
Favours GTN
Favours Sham