Tranexamic acid for hyperacute primary IntraCerebral Haemorrhage (TICH 2)

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Short title: **Tranexamic acid for IntraCerebral Haemorrhage TICH-2**

Acronym: **TICH-2**

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SYNOPSIS

Title	Tranexamic acid for hyperacute primary IntraCerebral Haemorrhage (TICH-2)
Acronym	TICH-2
Short title	Tranexamic acid for intracerebral haemorrhage (TICH-2)
Chief Investigator	Dr. Nikola Sprigg
Objectives	To assess whether tranexamic acid is safe and reduces death and dependency after hyperacute (within 8 hours of onset) spontaneous intracerebral haemorrhage. To assess the effects of tranexamic acid on haemostasis and inflammatory factors after spontaneous intracerebral haemorrhage.
Trial Configuration	A phase III prospective pragmatic double blind randomised placebo controlled trial
Setting	Secondary care
Sample size estimate	With alpha=0.05, power=90%, assuming losses to follow up=5% and covariate adjustment reduces sample size by 20%, 2,000 participants will need to be recruited to detect a treatment effect of OR 0.74 by shift analysis of mRS outcome
Number of participants	2000
Eligibility criteria	Patients within 8 hours of acute spontaneous intracerebral haemorrhagic stroke. Exclusion criteria will be one or more of: patients with intracerebral haemorrhage secondary to anticoagulation, thrombolysis or known underlying structural abnormality such as arterial venous malformation, aneurysm, tumour,venous thrombosis as cause for the intracerebral haemorrhage. Note it is not necessary for investigators to exclude underlying structural abnormality prior to enrolment, but where an underlying structural abnormality is already known, these patients should not be recruited, contra-indication to tranexamic acid, severe pre-morbid disability (modified rankin scale >4), Glasgow coma scale <5, or life expectancy < 3 months due to other disease (e.g. advanced metastatic cancer), randomising event was secondary to trauma, female patient of childbearing potential, pregnant or breastfeeding at randomisation, geographical or other factors that prohibit follow up at 90 days, participation in another drug or devices trial concurrently, with the exception of the secondary prevention trial RESTART. Participants enrolled in TICH-2 may be enrolled in RESTART after 21 days.
Description of interventions	Intravenous tranexamic acid: 1g loading dose given as 100 mls infusion over 10 minutes, followed by another 1g in 250 mls infused over 8 hours. Comparator – matching placebo (normal saline 0.9%)

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	administered by identical regimen.
Duration of study	48 months. Participants will be followed up for one year.
Randomisation and blinding	Patients will be randomised (1:1) to receive either tranexamic acid or placebo (0.9 % saline). Randomisation will be performed by the Stroke Trials Unit (STU) and involve computerised minimisation on key prognostic factors: age; stroke severity; systolic blood pressure. Patients randomised to placebo will receive intravenous normal saline. Patients and outcome assessors will be blind to treatment allocation.
Outcome measures	**Primary outcome**: Death or dependency (modified Rankin Scale, mRS) day 90. **Secondary clinical outcomes:** At day 7 (or discharge if sooner), neurological impairment (NIHSS). At day 90, disability (Barthel index), Quality of Life (EuroQol), cognition, cognition and mood (TICS and ZDS). At day 365, mRS, disability (Barthel index, Quality of Life (EuroQol), cognition, cognition and mood (TICS and ZDS). Safety: death, serious adverse events, thromboembolic events, seizures. Costs: length of stay in hospital, re-admission, institutionalisation. Radiological efficacy/safety (CT scan): change in haematoma volume from baseline to 24 hours, haematoma location, and new infarction.
Statistical methods	Death or dependency (ordinal shift on mRS) at day 90 will be analysed by intention-to-treat using ordinal logistic regression (OLR), with adjustment for minimisation factors. The assumption of proportional odds will be tested using the likelihood ratio test. Comparison of tranexamic acid versus control.

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ABBREVIATIONS

AE	Adverse Event
ARSAC	The Administration of Radioactive Substances Advisory Committee
CI	Chief Investigator
CRF	Case Report Forms
CT	Computer Axial Tomographic
DSMC	Data Safety Monitoring Committee
DWI	Diffusion weighted imaging
DWIHL	Diffusion weighted imaging hyperintense lesions
HTA	Health Technology Assessment
ICH	Intracerebral haemorrhage
ICRP	International Commission on Radiological Protection
IMP	Investigational Medicinal Product
INTERACT	Intensive Blood Pressure Reduction in Acute Cerebral Haemorrhage-Trial
MHRA	Medicines and Health Care Products Regulatory Agency
MI	Myocardial Infarction
MRI	Magnetic Resonance Imaging
mRS	Modified Rankin Scale
NHS	National Health Service
NIHR	National Institute of Health Research
NIHSS	National Institute of Health Research Stroke Scale
OR	Odds Ratio
PAD	Peripheral artery disease
SICH	Spontaneous Intracerebral Haemorrhage
R&D	Research and Development
REC	Research ethics Committee
SAE	Serious Adverse Event
STU	Stroke Trials Unit
SUSAR	Suspected Unexpected Serious Adverse Reaction
TIA	Transient Ischaemic Attach
TSC	Trial Steering Committee
VTE	Venous Thromboembolism

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TRIAL / STUDY BACKGROUND INFORMATION AND RATIONALE

Haemorrhagic stroke

Haemorrhagic stroke, or intracerebral haemorrhage caused by bleeding in the brain, can be devastating and is a common cause of death and disability, both in the UK and worldwide. Despite development of effective treatments for ischaemic stroke (thrombolysis, aspirin, hemicraniectomy) there is no proven effective treatment for spontaneous intracerebral haemorrhage (SICH).

Haematoma expansion

Outcome after intracerebral haemorrhage is closely related to whether brain bleeding expands after onset, so called haematoma expansion, or whether re-bleeding occurs; both are associated with a bad outcome (death and disability).[1] Contrast extravasation during contrast-enhanced CT, and CT angiography, have been shown to predict haematoma expansion,[2-4] although there is currently wide variation in the use of these techniques in routine clinical practice.

Haematoma expansion is related to both haemostatic factors and blood pressure; furthermore, haematoma volume can be reduced surgically - all these approaches are potential targets for treatment in intracerebral haemorrhage.

Intensive blood pressure treatment

Lowering blood pressure in patients with intracerebral haemorrhage is a potential therapeutic option to limit haematoma expansion and improve outcome. INTERACT, the largest completed trial to date, assessed intensive blood pressure treatment in 404 intracerebral haemorrhage patients. The study showed that aggressively lowering blood pressure appears safe and may limit haematoma expansion, but did not change outcome.[5] A smaller trial using a different regime again showed a trend to reduction of haematoma expansion in patients randomised to aggressive blood pressure lowering strategies.[6] Some safety concerns remain regarding aggressively lowering blood pressure in acute stroke, and a larger on-going study INTERACT-2 is assessing this area further.[7] Other ongoing trials include ATACH-2 and ENOS (SICH subset).

Surgery for intracerebral haemorrhage

Surgical treatment for intracerebral haemorrhage has proved disappointing, the largest trial STICH [8] failing to show benefit of surgery over conservative treatment. However, patients

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were only recruited when the surgeon was in equipoise and time to surgery was delayed (mean time 30 hours) and thus surgery was unlikely to be able to limit haematoma expansion. The on going STICH-2 [9] study is assessing the efficacy of surgery in patients with lobar haematomas.

Haemostatic therapy

Haemostatic drug therapies have been tested in spontaneous intracerebral haemorrhage, with recombinant factor VIIa being the most widely studied. In a phase IIb trial with 399 patients, rFVIIa restricted haematoma expansion, improved functional outcome and reduced mortality despite a significant increase in arterial thromboembolic events.[10] However in a larger phase III study in 816 patients, rFVIIa had no effect on functional outcome or mortality despite restricting haematoma expansion.[10] Meta-analysis of these and other haemostatic therapies for acute PICH, and containing data from 6 trials and 1398 patients, found no significant benefit on mortality, or death or dependency. There was a trend to improved outcome, but also a trend to increase in thromboembolic events.[11] In a small case series, platelet infusion therapy for patients with intracerebral haemorrhage whilst on anti-platelet therapy did not prevent death or improve outcome.[12]

Tranexamic acid

Tranexamic acid is a licensed anti-fibrinolytic drug that can be administered intravenously or orally and is used in a number of bleeding conditions to reduce bleeding.[13, 14] In a recent mega-trial (CRASH-2) in 20,000 patients with major bleeding following trauma, tranexamic acid significantly reduced mortality, OR 0.91 (0.85-0.97), with no increase in vascular occlusive events.[15] Treatment was most effective when given rapidly; delayed administration was associated with lack of efficacy and potential harm.[16] In a subgroup analysis of patients with traumatic ICH, tranexamic acid showed a non-significant trend to reduced mortality, OR 0.47 (0.21-1.04), and death of dependency, OR 0.66 (0.32-1.36).[17] However, patients in CRASH-2 were younger and had less co-morbidities than those with SICH. In another randomised controlled trial in traumatic intracerebral haemorrhage, tranexamic acid reduced death, OR 0.69 (0.35 -1.39), and death or dependency, 0.76 (0.46 – 1.27), without increased thromboembolic events.[18]

Tranexamic acid has been tested in aneurismal subarachnoid haemorrhage, where it reduced the risk of re-bleeding at the expense of increased risk of cerebral ischaemia.[19]

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However administration was for a week, conferring prolonged exposure to risk of ischaemic events.

Additionally, tranexamic acid has been found to restrict haematoma expansion in acute SICH in a small non randomised study, although this did not report on safety.[20] In another small study (n=156), rapid administration of a bolus of tranexamic acid within 24 hours of stroke was observed to reduce haematoma expansion (17.5% vs. 4.3%).[21] In this study, tranexamic acid was given in combination with intensive blood pressure control, suggesting that it may be possible to combine haemostatic and haemodynamic approaches.
There have been recent calls in the literature for large clinical trials to examine the use of tranexamic acid in SICH.[22]

DETAILS OF INVESTIGATIONAL MEDICINAL PRODUCT(S)

Description and Manufacture

Intravenous tranexamic acid (Cyklokapron, 100mg/ml 5ml ampoules, Pfizer Manufacturing Authorisation: PL 00057/0952) or matched placebo of intravenous Sodium Chloride 0.9% 5ml ampoules (Hameln, Manufacturing Authorisation: 1502 / 0006R).

Tranexamic acid 100mg/ml 5ml ampoules are a licensed product and the summary of the product characteristics is available for investigator.

Packaging and labelling

Sharp Clinical Services Ltd (previously known as Bilcare GCS (Europe) Ltd) (MIA(IMP): 10284) will prepare blinded individual treatment packs containing four 5ml glass ampoules of tranexamic acid 500mg or sodium chloride 0.9% which will be identical in appearance by the addition of a heat shrink sleeving. Ampoules and the secondary carton will be labelled in accordance with Annex 13 of Volume 4 of The Rules Governing Medicinal Products in the EU: Good Manufacturing Practices, assuming that the primary and secondary packaging remain together throughout the trial. To facilitate identification the carton and the ampoules contained within it will be labelled with the same unique pack number.

Detailed prescribing and administration instructions will be provided in the treatment pack.

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The final product will be QP released by the designated person at Sharp Clinical Services to provide blinded trial treatment packs for this trial.

Storage, dispensing and return

Sharp Clinical Services Ltd (previously known as Bilcare GCS (Europe) Ltd) (MIA(IMP): 10284) will store the treatment packs and distribute to pharmacies within trial sites using a web-based system control. Pharmacy at each participating site will take receipt of numbered supplies from Sharp Clinical Services.

The web-based system operates as follows. Participating centres will be allocated a batch of trial treatment. The container numbers for these batches are tracked by the web-based system to the participating site and once receipt has been confirmed they are released for use in the trial. When the supplies at the participating centre reach a pre-determined level then a re-order is triggered and a further supply of trial treatment is sent to the corresponding participating site.

The packs will be stored at room temperature and protected from excessive heat and freezing in a restricted access area. Stability data exists which demonstrates that Tranexamic Acid is stable at temperatures between -20°C and 50°C. [23] Temperature monitoring will not be required. The IMP will be clearly labelled for clinical trial use only. Each pack will be a numbered box containing either tranexamic acid or placebo according to a computer-defined sequence.

The local site investigator is responsible for ensuring trial treatment accountability, including reconciliation of trial treatment and maintenance of trial treatment records, throughout the course of the study in accordance with UK regulatory requirements. Responsibility can be delegated to the site pharmacy clinical trials staff.

Following randomisation the participant will be allocated a treatment pack number. Specifically authorised personnel will retrieve the appropriate pack number and complete the participant name, date of randomisation and participant number. Pack number allocation will be checked and countersigned by the research staff and the nursing staff administering the treatment. The treatment will be prescribed on the participant's treatment chart as trial medication.

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Dispensing will be recorded on the appropriate trial specific accountability forms. Trial treatment must not be used for any other purpose than the present study. Returned trial treatment that has been dispensed to a participant must not be re-dispensed to a different participant. Any unused drug will be returned to pharmacy.

Placebo

The placebo will be supplied, packaged, labelled, OP released and distributed as for the active IMP.

Known Side Effects

Gastrointestinal disorders (nausea, vomiting, diarrhoea) may occur but disappear when the dosage is reduced. Hypotension has occasionally been reported after rapid intravenous infusion. Rare instances of colour vision disturbances have been reported following long-term use. Rare cases of thromboembolic events have been reported. Rare cases of allergic skin reactions have also been reported (see Appendix A).

Tranexamic acid will counteract the thrombolytic effect of fibrinolytic preparations but these would be contra-indicated in patients with haemorrhagic stroke.

TRIAL / STUDY OBJECTIVES AND PURPOSE

PURPOSE

To assess in a pragmatic phase III prospective double blind randomised placebo-controlled trial whether tranexamic acid is safe and reduces death or dependency after spontaneous intracerebral haemorrhage (SICH). The results will determine whether tranexamic acid should be used to treat SICH, which currently has no proven therapy.
There will also be a sub-study using MRI scans (referred to hereafter as the TICH-2 MRI sub-study) that will determine the effects of tranexamic acid on the perihaematoma oedema, the presence of remote diffusion weighted imaging hyperintense lesions (DWIHL), and extent of end-stage tissue injury surrounding the haematoma cavity.

PRIMARY OBJECTIVE

To assess whether tranexamic acid is safe and reduces death or dependency after spontaneous intracerebral haemorrhage (SICH).

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SECONDARY OBJECTIVES

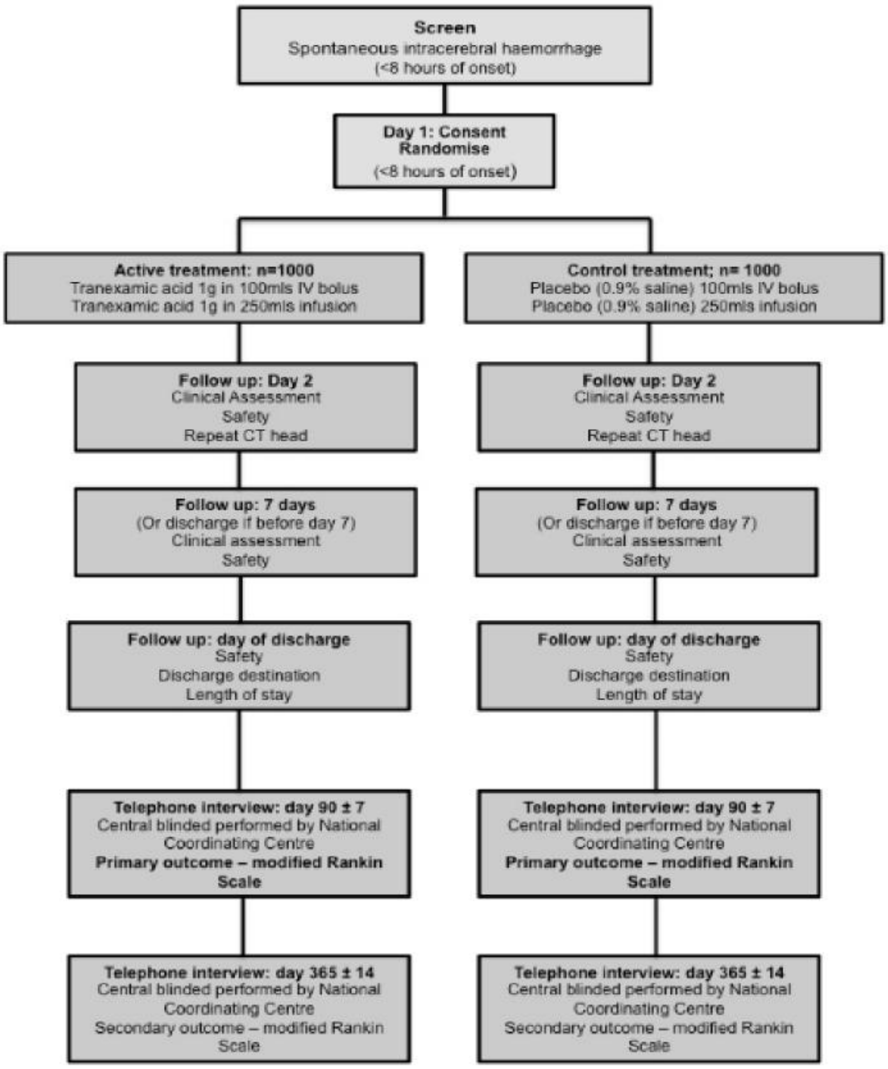
To assess the effect of tranexamic acid on secondary outcomes: clinical outcomes, safety outcomes, costs, radiological efficacy and plasma biomarkers.

TRIAL / STUDY DESIGN

TRIAL / STUDY CONFIGURATION

Pragmatic, phase III, prospective double blind randomised placebo-controlled trial performed in two phases: 18 month start up phase (activate 30 centres, recruit a minimum of 300 participants) then main phase (120 centres recruit to a total of 2,000 participants). There will be no break in recruitment as the trial proceeds from the start-up phase to the main phase provided the stopping criteria are not met.

See trial flow chart



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The trial will consist of a UK base with UK participating sites and an international element involving a number of international sites. Separate local approvals will be sought for the international sites, all applicable local regulations will be adhered to and a contract will be in place between the University of Nottingham and those sites apportioning liabilities and responsibilities for the conduct of the study.

Assessments:

Clinical assessment; Baseline (Day 1), end of treatment (day 2) and day 7 (or earlier if discharged). Day 90 – telephone interview. Day 365 – telephone interview.
Radiological – Day 2 CT brain scan to assess for haematoma expansion. MRI scan (optional, in centres participating in the TICH-2 MRI sub-study) at Days 5 (+/- 2 days) and 90 (+/- 5 days).

Plasma biomarkers (optional, Nottingham University Hospital only) – baseline (before randomised treatment is given), day 2 and day 7 (or discharge if sooner than 7 days).

Primary endpoint

Death or dependency (modified Rankin Scale, mRS) at day 90.

Secondary endpoint

1. Neurological impairment (NIHSS) at day 7 or discharge if sooner.
2. modified Rankin Scale (mRS) at day 365
3. Disability (Barthel index) at day 90 and day 365
4. Quality of Life (EuroQol) at day 90 and day 365
5. Cognition and mood at day 90 and day 365 (TICS and ZDS).
6. Costs: length of stay in hospital, re-admission, institutionalisation.
7. Radiological efficacy/safety (CT scan): change in haematoma volume from baseline to day 2, haematoma location and new infarction.

Safety endpoints

Recorded until end of follow-up (Day 90):

1. Death (cause);
2. VTE; ischaemic events (stroke/TIA/MI/PAD);
3. Seizures;
4. Serious adverse events in first 7 days

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Tertiary end points (TICH-2 MRI sub-study)

1. Prevalence of remote DWIHL on Day 5 MRI scan
2. Perihaematoma oedema volume and perihaematoma diffusion restriction on Day 5 MRI scan
3. Combined volume of the residual haematoma cavity and surrounding gliosis on the Day 90 MRI scan.

Tertiary end points (TICH-2 Plasma biomarker sub-study)

1. Plasma biomarkers levels including haemostatic and inflammatory markers

Stopping rules and discontinuation

Participants may withdraw consent at any time. Study medication maybe stopped at any time by the investigator or treating physician if deemed advisable.

RANDOMIZATION AND BLINDING

All participants eligible for inclusion will be randomised centrally using a secure internet site in real-time. Randomisation involves minimisation on key prognostic factors: age; sex; time since onset; systolic blood pressure; stroke severity (NIHSS); presence of intraventricular haemorrhage; known history antiplatelet treatment used immediately prior to stroke onset. This approach ensures concealment of allocation, minimises differences in key baseline prognostic variables, and slightly improves statistical power.[24]
Randomisation will allocate a number corresponding to a treatment pack and the participant will receive treatment from the allocated numbered pack.
In the event of computer failure (for example: server failure), investigators will follow the working practice document for computer system disaster recovery, which will allow the participant to be randomised following standardised operating procedure.

Maintenance of randomisation codes and procedures for breaking code

Clinicians, patients and outcome assessors (research nurse and radiologist) will be blinded to treatment allocation.

In general there should be no need to unblind the allocated treatment. If some contra-indication to antifibrinolytic therapy develops after randomisation (e.g. clinical evidence of thrombosis), the trial treatment should simply be stopped. Unblinding should be done only in those rare cases when the doctor believes that clinical management depends importantly upon knowledge of whether the patient received antifibrinolytic or placebo. In those few

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cases when urgent unblinding is considered necessary, the emergency telephone number should be telephoned, giving the name of the doctor authorising unblinding and the treatment pack number. The caller will then be told whether the patient received antifibrinolytic or placebo The rate of unblinding will be monitored and audited.

In the event of breaking the treatment code this will normally be recorded as part of managing a SAE (see below for more details) and such actions will be reported in a timely manner. The Chief Investigator (delegated the sponsor's responsibilities) shall be informed immediately (within 24 hours) of any serious adverse events and shall determine seriousness and causality in conjunction with any treating medical practitioners

TRIAL MANAGEMENT

Day-to-day management of the trial will be the responsibility of the Trial Management Group. The Trial Management Group will report to the independent Trial Steering Committee. An independent Data Safety Monitoring Committee will monitor safety of participants, and will report to the Trial Steering Committee. Trial co-ordination will be through the Stroke Trials Unit, in conjunction with the Nottingham Clinical Trials Unit (NCTU).

The Chief Investigator is the data custodian and has overall responsibility for the study and shall oversee all study management.

Trial Management Group

The Trial Management Group (TMG) will include the Chief Investigator, Study Trial Manager, Trial Statistician, and other project staff. This group, based at the Stroke Trials Unit, will meet regularly, at least every four weeks.

Trial Steering Committee

The independent Trial Steering Committee (TSC) will provide oversight of the trial. It will meet (in person or by telephone conference) prior to commencement of the trial, and then at regular intervals until completion (at least annually). Specific tasks of the TSC are: · to approve the trial protocol

· to approve necessary changes to the protocol based on considerations of feasibility and practicability

· to receive reports from the Data Monitoring Committee

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· to resolve problems brought to it by the co-ordinating centre and TMG ·
to ensure publication of the trial results

· to advise on whether the main phase of the trial is feasible

Data Safety Monitoring Committee

An independent Data Monitoring Committee (DSMC) will be established. The DSMC will receive safety reports every six months, or more frequently if requested and perform unblinded reviews of efficacy and safety data. The DSMC will perform a formal interim analyses after 800 participants have been recruited (comprising both trial phases) and followed-up at 90 days.
A DSMC Charter will be prepared containing details of membership, terms and conditions and full details of stopping guidelines. The DSMC will report their assessment to the independent chair of the TSC who will report to the HTA.

Collaborators, and all others associated with the trial, may write through the trial office to the DSMC, to draw attention to any concern they may have about the trial interventions, or any other relevant issues.

DURATION OF THE TRIAL / STUDY AND PARTICIPANT INVOLVEMENT

The study will be run in two phases: an 18 month start-up phase (activate a minimum of 30 centres, recruit a minimum of 300 participants) then main phase (25 months, 120 centres. recruit to a total of 2,000 participants). There will be no break in recruitment as the trial proceeds from the start-up phase to the main phase unless the TSC and funder consider the trial no longer feasible or stopping criteria are met.

The participant's involvement in the trial will last for one year, from randomisation (day 1) until final follow up at 365 days. Treatment period will be for 1 day (approximately 8 hours). Enrolment will begin when the study has obtained full regulatory approval and cease when the final participant has been recruited.

End of the Trial

The trial will end when the final participant has completed the treatment period and follow up (Day 365).

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SELECTION AND WITHDRAWAL OF PARTICIPANTS

Recruitment

The trial setting is in secondary care, in acute stroke services across the UK and worldwide; 30 in the start up phase, 120 in the main phase. Estimated 80 UK sites, 40 non-UK sites. UK participants will be recruited from NIHR Stroke Research Network sites (adoption will be sought from the NIHR Stroke Research Network). These sites have dedicated Stroke Research Network Local Research Network nurses to facilitate recruitment and follow-up.

Participants will be recruited from the acute stroke unit or emergency admissions department. The initial approach will be from a member of the patient’s usual care team (which may include investigators).

The investigator or their nominee will inform the participant or their legal representative, of all aspects pertaining to participation in the study.

If needed, the usual hospital interpreter and translator services will be available to assist with discussion of the trial, the participant information sheets, and consent forms, but the consent forms and information sheets will not be available in other languages.

It will be explained to the potential participant or their legal representative that entry into the trial is entirely voluntary and that their treatment and care will not be affected by their decision. It will also be explained that they can withdraw at any time but attempts will be made to avoid this occurrence. In the event of their withdrawal it will be explained that their data collected so far cannot be erased and we will seek consent to use the data in the final analyses where appropriate.

Some centres will participate in a sub-study, the TICH-2 MRI sub-study, where participants will have 2 MRI scans – one at Day 5 (+/- 2 days) and one at Day 90 (+/- 5 days). Only patients who are likely to be able to tolerate the MRI scan will be approached to participate in this sub-study. Participation is optional and participants may be approached from randomisation up to 7 days (i.e. Day 5 +2 days) from their stroke. Written informed consent from the participant or a personal legal representative will be obtained.

Nottingham University Hospital will be the only participating centre for the plasma biomarkers sub-study. Investigators may approach participants at the time of randomisation to participate. In addition, investigators from this site will also approach relative or friend of

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participating patients to recruit them as healthy volunteers. Participation of patients and healthy volunteers in this sub-study is optional and voluntary. Written informed consent from the participant or a personal legal representative will be obtained for patients' participation and from healthy volunteers themselves for healthy volunteers' participation.

Inclusion criteria

Adult (≥18 years) patients with acute SICH within 8 hours of stroke onset. (Where stroke onset time is unknown, the time of when last known well will be used.)

Exclusion criteria

1) Patients with intracerebral haemorrhage secondary to anticoagulation, thrombolysis or known underlying structural abnormality such as arterial venous malformation, aneurysm, tumour, venous thrombosis as cause for the intracerebral haemorrhage. Note it is not necessary for investigators to exclude underlying structural abnormality prior to enrolment, but where an underlying structural abnormality is already known, these patients should not be recruited.
2) Patients for whom tranexamic acid is thought to be contraindicated.
3) Patients with pre-morbid dependency (mRS>4).
4) Pre-stroke life expectancy <3 months (eg. advanced metastatic cancer).
5) Coma – Glasgow coma scale <5
6) Randomising event was secondary to trauma
7) Female patient of childbearing potential, pregnant or breastfeeding at randomisation
8) Geographical or other factors that prohibit follow up at 90 days e.g. no fixed address or telephone contact number, or overseas visitor.
9) Participation in another drug or devices trial concurrently, with the exception of the secondary prevention trial, RESTART. Participants enrolled in TICH-2 may be enrolled into the RESTART trial after 21 days.

Additional eligibility criteria for participation in the TICH-2 MRI sub-study

Inclusion criteria

1. Written informed consent from the participant or a personal legal representative to participate in the MRI sub-substudy
2. Participant is able to safely undergo MRI scanning (according to the clinical assessment and MRI safety procedures in place at the recruiting centre)

Exclusion criteria

1. Contraindication to MRI scan (eg, non-MRI compatible implant, intra-ocular / intracranial metallic device or fragment, claustrophobia, etc, as defined by the MRI safety procedures in place at the recruiting centre)

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2. Clinical instability (for example cardiorespiratory or neurological instability) such that MRI scan would introduce additional clinical risk.

Additional eligibility criteria for participation in the TICH-2 plasma biomarkers sub-study

Inclusion criteria:

1. Patient or legal representative consent to plasma biomarkers sub-study

Exclusion criteria

1. Evidence of coagulopathy
2. Evidence of systemic hypotension at the time of recruitment
3. Admission to ICU at the time of recruitment
4. Mechanical ventilation at the time of recruitment

Additional eligibility criteria for controls:

Inclusion criteria:

1) Age > 18 years
2) Volunteers consent to plasma biomarkers study
3) Agree for data to be taken

Exclusion criteria:

1) Intracerebral haemorrhage or ischaemic stroke in the previous 3 months
2) Venous thromboembolism in the previous 3 months
3) Any illness that warrants admission to hospital in the past 3 months.
4) Taking tranexamic acid whether per oral or parenteral in the past 7 days.
5) Taking anticoagulants in the past 7 days.

Expected duration of participant participation

Study participants will be participating in the study for one year.

Removal of participants from therapy or assessments

Participation in the trial is voluntary and patients are free to withdraw from the trial at any stage without giving a reason. Study medication may be stopped at any time by the investigators or any treating clinician if deemed in the patient's best interest. Treatment (with tranexamic acid/placebo) will be given on top of 'best medical care'.

Participants may be withdrawn from the trial either at their own request or at the discretion of the Investigator. The participants will be made aware that this will not affect their future care. Participants will be made aware (via the information sheet and consent form) that should they withdraw the data collected to date cannot be erased and may still be used in the final analysis.

Enrolled participants who withdraw before randomisation can be replaced (though keeping their trial ID), but participants who withdraw after randomisation will not be replaced.

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Informed consent

Intracerebral haemorrhage is a medical emergency, for which there is no proven effective treatment and rapid deterioration can occur early, leading to brain damage, long term disability or death. Intracerebral haemorrhage can cause significant brain injury and many patients may not be physically or mentally capable of giving informed consent to participate in a clinical trial. There is evidence from trials in traumatic bleeding that 2g of tranexamic acid (the regime being utilised in TICH-2) is safe (with no significant increase in harm in 20,000 patients), and improves survival. In addition, tranexamic acid is more effective when given early[16]. The need for urgent treatment, in an attempt to prevent potentially fatal deterioration, means that it would be inappropriate to delay treatment until fully informed consent can be obtained from an incapacitated patient.

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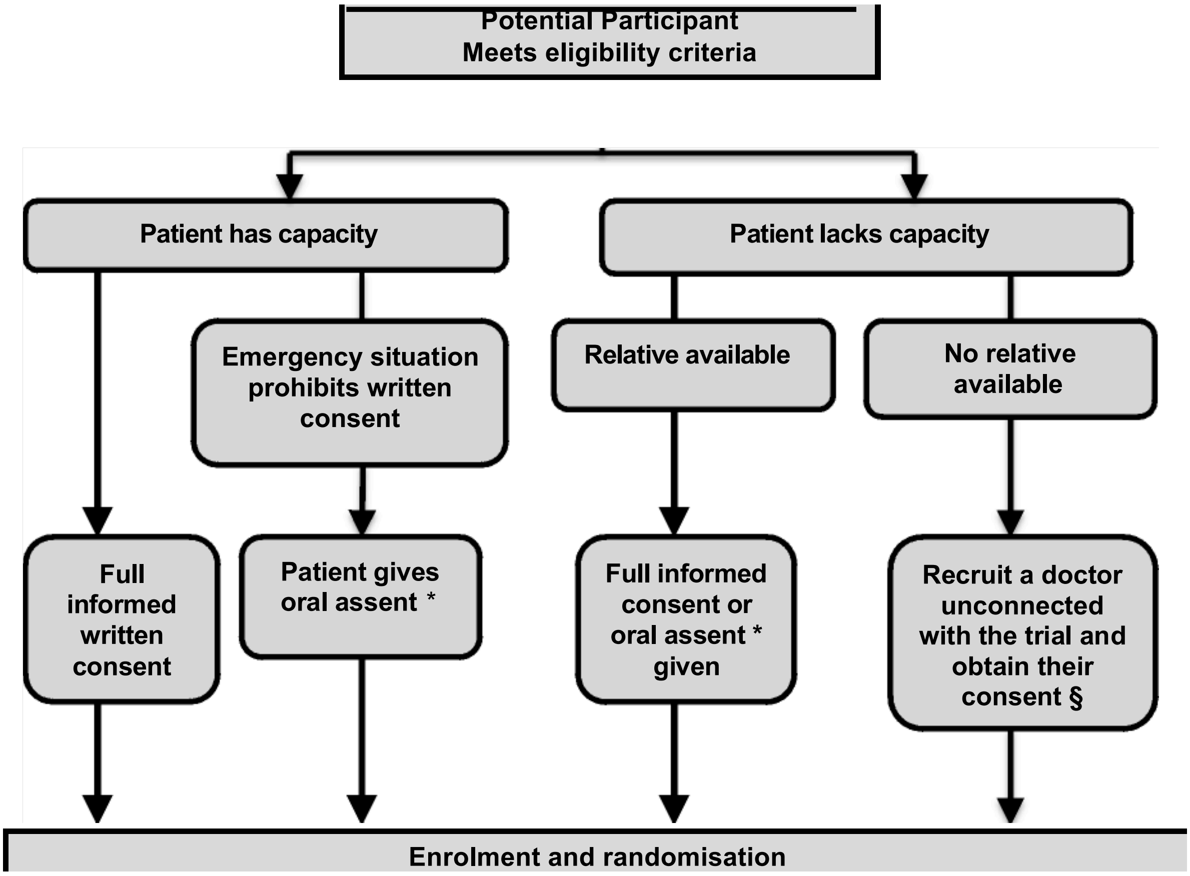
Therefore the following procedure will be used for giving information and obtaining informed consent for the TICH-2 trial:



*** Patients or relatives will be approached to give oral assent in circumstances where the therapeutic time window does not allow investigators to seek full informed written consent, and only if the attending clinicians consider it appropriate. Patients or relatives will not be approached if there is insufficient time to give a brief oral summary of the trial, or they do not speak fluent English and no translator is available. How long will be required for oral assent will depend on factors such as time window for treatment efficacy before brain damage. If the patient or relative does not give oral assent they will not be recruited.**

If oral assent for recruitment is given:

- **Participants (or relatives if the participant lacks capacity) will be approached as soon as possible after recruitment to give written consent to participation in follow up and access to their data**
- **Oral assent will be monitored by Trial Steering Committee**

§ Patients who lack capacity to consent (e.g unconscious) with no relative present will be discussed with a doctor unconnected to the trial. Information relating to the trial is provided verbally and their verbal consent for the patient's inclusion in the trial is obtained. If a doctor unconnected with the trial is not available, patients will not be entered.

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Patient has capacity to provide consent:

All participants who are able to will provide written informed consent. The Informed Consent Form will be signed and dated by the participant before they enter the trial. The Investigator (or nominee) will explain the details of the trial and provide a Participant Information Sheet. The Investigator will answer any questions that the participant has concerning study participation. Potential participants will be given as long as they need to consider whether to consent, however we recommend that a maximum of 15 minutes should be taken obtaining consent. It will be explained to the potential participant that as this is an emergency treatment, with a small therapeutic time window before permanent brain damage occurs, previous work suggests that the earlier the treatment is commenced the greater the potential for benefit.

If the participant is unable to write (e.g. in the presence of dominant hand weakness, ataxia or dyspraxia), witnessed verbal consent may be recorded on the consent form.

Patient has capacity but time prohibits written consent:

If the therapeutic time window before brain damage occurs does not allow investigators to seek informed written consent and the attending clinician considers it appropriate, the potential participant will be asked if they are willing to be recruited.

Specifically, the responsible doctor will explain to the patient that they will receive the usual care for haemorrhagic stroke but that in addition to this, the patient can be enrolled in a research study that aims to improve the treatment of patients with this condition. It will be explained that the study is being done to see whether using a drug called tranexamic acid will help patients with haemorrhagic stroke by reducing the amount of bleeding into the brain therefore preventing further brain damage. If enrolled the patient will be given an infusion into a vein over 8 hours of either the tranexamic acid or a dummy medicine (a salt water liquid which does not contain tranexamic acid). The doctor will explain that tranexamic acid has been shown to improve outcome in patients with other types of severe injury and that whilst we hope that it will also improve recovery after haemorrhagic stroke, at present we cannot be sure about this. Further information will be provided on request. If requested, an information sheet will be provided. If they say yes, the potential participant will be randomised. Written informed consent will be sought for access to medical notes and for participation in the trial follow up, including additional CT scan. The participant information sheet will be provided to the participant at this time if not already provided.

Patient lacks capacity to give consent:

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Lack of capacity will be determined by the participant's attending stroke physician.
If the potential participant lacks capacity to give meaningful consent (e.g. in cases of dysphasia, confusion, or reduced conscious level) the following procedure will be employed.
Relatives available: If relatives (or other legal representative such as partner or close friend, able to represents the patients views and wishes) are present, bearing in mind the clinical situation and their level of distress, they will be provided with brief information about the trial. Specifically, the responsible doctor will explain to the relatives that the patient will receive the usual care for haemorrhagic stroke but that in addition to this, the patient can be enrolled in a research study that aims to improve the treatment of patients with this condition. It will be explained that the study is being done to see whether using a drug called tranexamic acid will help patients with haemorrhagic stroke by reducing the amount of bleeding into the brain therefore preventing further brain damage. The relative will be informed that the patient will be given an infusion into a vein over 8 hours of either the tranexamic acid or a dummy medicine (a salt water liquid which does not contain tranexamic acid). The doctor will explain that tranexamic acid has been shown to improve outcome in patients with other types of severe injury and that whilst we hope that it will also improve recovery after haemorrhagic stroke, at present we cannot be sure about this. Further information will only be provided on request. If requested, a brief information sheet will be provided. If relatives object to the inclusion of the patient in the trial, their views will be respected and the patient will not be enrolled. Full informed written consent will be obtained from the patient or legal representative afterwards as soon as practicable.
If relatives are not physically present but are available and happy to speak on the telephone, the same procedure will be followed. If the relative is unhappy to speak on the telephone or unable to decide, the patient will not be enrolled.
Relatives not available: If no relatives are available, we intend to recruit a doctor, wherever possible unconnected with the trial, provide them with verbal information relating to the trial and obtain their verbal consent for the patient's inclusion in the trial. If a doctor unconnected with the trial is not available patients will not be entered into the trial.
If enrolled, full informed written consent will be obtained from the patient or their legal representative afterwards as soon as practicable.

Participants who originally lacked capacity (and were entered into the study following agreement from a legal representative) but then regain capacity will need to give informed written consent to continue in the study. The participants' decision to withdraw would overrule the decision of the legal representative.

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Participants may discontinue treatment either at their own request or if it is felt in their best interest by the attending physician. Participants who discontinue treatment (for whatever reason) will not be withdrawn from the trial unless the participant specifically withdraws consent for further follow-up. Participants maybe withdrawn from the trial either at their own request (if they regain capacity) or at the request of the legal representative. The participants and the legal representative will be made aware that this will not affect the participant's future care. Participants who withdraw from the trial will be informed that data already collected prior to withdrawal cannot be deleted.

The requirements of the relevant ethics committee will be adhered to at all times.
Should there be any subsequent amendment to the final protocol, which might affect a participant's participation in the trial, continuing consent will be obtained using an amended consent form which will be signed by the participant.

Where the patient is being assessed and treated via telemedicine (as is often standard care in many stroke services out of hours) by a member of the medical team who is appropriately trained and listed on the delegation log, the process is as above, with the exception that the paper consent form will be countersigned by a witness, and signed by the investigator upon their return to the hospital site. If the patient does not wish to decide via telemedicine they will not be enrolled.

TICH-2 MRI Sub-study

Participants who are eligible and either have the capacity to consent or where a personal legal representative is present will be approached by a member of the research team during the period between randomisation and Day 7 to explain the MRI sub-study to them. They will be provided with an information sheet and if they agree to participate, will be required to provide written informed consent. Participants and (where appropriate) their personal legal representative, will be made aware that participation is optional and they can remain part of the main TICH-2 trial without taking part in the sub-study.

TICH-2 Plasma Biomarkers Sub-study

All patients will be required to sign and date a separate consent form than that is used in the main TICH-2 study before entering the sub-study. Investigators will explain about the sub-study, provide patient information sheet and answer any questions the patient or legal

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representative may have. Patients' relatives or friends will be asked to participate in sub-study as control volunteers. Participation in the sub-study is voluntary and patients or volunteers can withdraw from sub-study at any point.

TRIAL / STUDY TREATMENT AND REGIMEN

Trial treatment is administered as tranexamic acid 1g (10ml in 100ml sodium chloride 0.9% infusion bag) through a venous cannula with a loading dose infusion over 10 minutes followed by 1g infusion (10ml in 250ml sodium chloride 0.9% infusion bag) over 8 hours. Placebo treatment replaces tranexamic acid 100mg/ml with sodium chloride 0.9%

Participants will be assessed before treatment (day 1), after treatment (day 2), after 7 days, 90 days, and 365 days. See flow chart for more details.

Patients will be assessed clinically at baseline, in line with local clinical practice pre-inclusion (day 1), end of treatment (day 2) and day 7. Follow up at day 90 and day 365 will be via a telephone interview as is standard practice in clinical stroke trials.

Brain imaging (CT head scan) will be performed as part of routine clinical management prior to enrolment (all acute stroke patients have a CT head scan performed on admission to hospital). For the purpose of the study the CT head scan will be repeated after treatment, day 2, (tranexamic acid/placebo) to assess haematoma expansion.

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Participant measures

Assessments	Screening	Day 1	Day 2	Days 2-7	Day 5 (+/-2 days)	Day 7 (or discharge if sooner)	Day 90 (+/- 5 days)	Day 90 (telephone interview)	Day 365 (telephone interview)
CT Scan	X*		X^						
Clinical Assessment	X**		X			X			
Consent	X								
Eligibility screening		X							
Randomisation		X							
Administer Intervention		X							
NIHSS	X*		X			X			
mRS		X						X	X
Barthel Index								X	X
EuroQOL (EQ5D)								X	X
Cognition (TICS)								X	X
Mood (ZDS)								X	X
SAEs		X	X			X		X	
Eligibility and consent (MRI)^^		X	X	X	X				
MRI^^					X		X		
Plasma Biomarkers¶		X	X			X			
Plasma Biomarkers¶ (volunteers)		X							

mRS modified rankin scale; NIHSS National institute of Health Stroke Score; SAEs: serious adverse events; TICS Telephone Interview Cognitive Status; ZDS Zung Depression Score

* Routine clinical scan for index stroke

** Routine clinical assessment

^ Additional scan to assess for hematoma expansion

^^ Optional and only available in centres participating in the TICH-2 MRI sub-study. . Consent for the MRI sub-study may be obtained from randomisation into the main TICH-2 study to Day 7.

¶ Optional and only available in centres participating in TICH-2 plasma biomarkers sub-study. Assessment to be performed after full consent obtained.

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Follow Up

Face to face assessment at the end of treatment (day 2) and day 7 with telephone follow up at day 90 and day 365.

Researchers will not contact the participant or their family directly at Day 90 or Day 365, they will first contact the participants general practitioner (GP) or obtain information through the Medical Research Information Service, at the NHS Information Centre to check their health status. Permission to contact the GP at day 90 and day 365 will be sought at the time of consent.

Participants taking part in the TICH-2 MRI sub-study will have their scans at Days 5 (+/- 2 days) and 90 (+/- 5 days). They will not be contacted for the Day 90 MRI until their status has been ascertained. The MRI scans will be performed according to a standardised MRI protocol. The MRI protocol will comply with the essential imaging sequences specified by the Standards for Reporting vascular Neuroimaging (STRIVE) neuroimaging standards. Clinical reading and reporting of MRI scans will be performed by the clinical radiologists at the recruiting centre, and any clinically relevant findings will be communicated to the clinical team caring for the participant via the locally approved channels for clinical communication of radiological reports. The MRI images will be transferred (in an anonymous format) to the coordinating centre via the internet-based image submission portal for quantitative analysis as outlined below.

Concomitant and Rescue Medications and Treatments

The intervention (tranexamic acid or placebo) will be given in addition to routine care. There are no prohibited concomitant treatments.

Compliance

Compliance will be assessed by examining the participant's drug chart and recording evidence of treatment administration. Compliance will also be assessed by recording, and returns of residual/unused trial medications. Compliance will be recorded on the case report forms at end of treatment (day 2).

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Accountability for drugs & placebos

The pharmacist will maintain records of the dispensing of the drug and the research nurse will record administration of the drug to the patient. Dispensing details will be recorded on each participant's CRF. Unused and partially used supplies will be returned to pharmacy. This will be recorded in the pharmacy study log.

Management of study drug overdose

No specific antidotes are available. The study drug will be administered by slow intravenous injection by qualified nursing staff so the potential for overdose is not anticipated.

Criteria for terminating trial

The trial may be terminated by either the TSC, the sponsor or the funders as a result of a formal or informal interim analysis and based on overwhelming evidence of major safety concerns, new information, or issues with trial conduct (e.g. poor recruitment, loss of resources). Any decision to stop the study prematurely will be based on asymmetric stopping rules.

The trial may be stopped at one centre due to unacceptable performance in recruitment and/or failure to comply with protocol.

RADIATION EXPOSURE

Details of diagnostic or therapeutic ionising radiation

The participant will receive a routine clinical non-contrast single run CT head scan at the time of presentation with stroke and an additional non-contrast single run CT head scan at the end of the treatment. The CT head scan at the time of stroke is part of routine clinical care whether or not the patient goes on to participate in the trial. For patients who are randomised into the trial the results will be used as baseline data.

Trial Procedures

Single run non-contrast CT head scan *1 day post recruitment (day*

2). **Details of radioactive materials and dose**

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From NRPB –W67 ‘Doses from computed tomography (CT) examinations in the UK (2003 review), the doses from CT scans will vary between sites with different models of equipment and between different sizes of patient.

A CT of the brain will give an average of 1.5mSv but this could be up to a maximum of 5mSv. So a typical dose from CT due to research exposures would be 1.5mSv, but could be as high as 5mSv.

A 1.5mSv dose would be roughly equivalent to 8 months of exposure to natural background radiation to a member of the public resident in the UK. A 5mSv dose is roughly equivalent to 21/4 years of background received by a member of the public resident in the UK.

Risk Assessment (induction of fatal cancer)

Based on a risk coefficient for developing fatal radiation induced cancer (all ages) of 5%/Sv (ICRP), this would lead to a risk of radiation exposure incurred as part of the trial. similar to the annual risk of dying from an accident in the home.

This is classed as an intermediate risk and the required benefit should be aimed directly at diagnosis, cure or prevention of disease.

Clinical Assessment

The scan itself takes about half a minute and does not involve any injections. The scan uses x-rays, which in large amounts can be harmful, but for this extra CT head scan the additional risk to the participant from the scan has been judged to be extremely small.

The objective of the exposure is to assess the extent of the bleeding (haematoma) in the brain to see if it has got worse (larger) or better (smaller) following treatment.

An alternative would be MRI brain scan but this takes longer and many patients are unsuitable or unable to tolerate it due to claustrophobia. The procedure for CT and any doses in lay terms are explained in the participant information sheet.

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STATISTICS

Sample size and justification

The null hypothesis (H_0) is that tranexamic acid does not alter death or dependency in participants with acute PICH. The alternative hypothesis (H_A) is that death or dependency differ between those participants randomised to tranexamic acid versus saline. A total sample size of 2,000 (1,000 per group) participants with acute primary intracerebral haemorrhage are required, assuming overall *significance (alpha) = 0.05*; *power (1-beta) = 0.90*; distribution in mRS (mRS 0=4%, 1=17% 2=16% 3=19% 4=24% 5=7% 6(death)=13% based on data from participants with primary intracerebral haemorrhage in the ENOS trial); *ordinal odds ratio of 0.79*; increases due to *losses to follow-up* of 5%; and a reduction of 20% for *baseline covariate adjustment*.[25] In summary, a trial of 2,000 participants (1,700 from main phase and 300 from start-up phase) will have 90% power to detect an ordinal shift of mRS outcome with odds ratio 0.79.

For the TICH-2 MRI sub-study

Primary hypothesis: Based on previous studies finding prevalence of DWIHL of 20% in SICH, sample size calculations were performed to allow a detection of different percentage increases in DWIHL prevalence in the TA group above a 20% baseline, using the sample size formula for prevalence

$= \frac{z_{1-\alpha/2}^{2} \times p(1-p)}{m^2}$ where Z = confidence interval, P = prevalence, and M = margin of error (standard is 5%). Results are shown in the table:

Prevalence	Sample size if confidence level 90%	Sample size if confidence level 95%
Prevalence of 20%	174	246
10% increase (prevalence 22%)	186	264
25% increase (prevalence 25%)	204	290
50% increase (prevalence 30%)	228	324

Assuming a small increase in prevalence of DWIHL by 10% in the TA group, and with 95% confidence levels, a sample size of 264 is required. Allowing 5% imaging data rejection (eg due to excessive patient motion), a sample size of 280 is required. Based on pilot data from the TICH-1 study this, this sample size will also be sufficient to detect a group difference in increase in relative perihaematoma oedema with power of 0.89 and α=0.05 (independent samples t-test, 2-tails).

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For the TICH-2 Plasma Biomarkers sub-study

Based a previous study, where mean D-dimer level for placebo group is 3612.5 ng/ml, tranexamic acid group 1125 ng/ml and pooled standard deviation of 1308.6 ng/ml; and assuming an type 1 error (α) of 0.05 and aiming for a power of 90%(independent samples t-test, 2-tails), the estimated sample size is 8 patients per group. The minimum sample size required is 16 patients.

Assessment of efficacy

Detailed information regarding analyses will be provided in a separate statistical analysis plan to be prepared and finalised prior to database lock.

Primary outcome: Death or dependency (ordinal shift on mRS) at day 90 will be compared between tranexamic acid and saline by intention-to-treat using ordinal logistic regression (OLR), with adjustment for minimisation factors. The assumption of proportional odds will be tested using the likelihood ratio test.

Sub-group analyses: The comparison of tranexamic acid and saline on the primary outcome will be performed in pre-specified subgroups, including the minimisation criteria, and: start of treatment (≤3, >3 hours), spot positive (yes,no) and intraventricular haemorrhage (yes, no). Analysis of the primary outcome in these pre-specified sub-groups does not comprise the primary analysis and has not informed the sample size calculation. The interpretation of any subgroup effects will be based on interaction tests (i.e. evidence of differential treatment effects in the different subgroups).

Secondary analyses: Binary logistic regression will be used for binary outcomes, including death, SAEs and thromboembolic events. ANCOVA will be used for continuous measures, including hematoma expansion. Wilcoxon rank sum test will be used for continuous measures which are not normally distributed, including Barthel Index. Regression analyses will be performed with adjustment for minimisation factors. As it is likely that haematoma volumes will be skewed, potential transformations of the data will be explored to permit an analysis based on ANCOVA, so as to exploit the baseline haematoma volumes and maximise statistical power. The impact of tranexamic acid on quality of life will be assessed using the EuroQoL. A full health-economic analysis will only be performed after completion of the main phase of the trial.

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Analyses of MRI data from the TICH-2 MRI sub-study: A risk model (binary logistic regression model) for presence of DWIHL post-SICH will be constructed, based on treatment group, patient factors (age, gender, race, cardiovascular and cerebrovascular risk factors) and imaging markers of small vessel disease (CMB, WMH) and likely diagnostic classification for CAA (based on CMB distribution and presence of superficial siderosis), allowing stratification of risk of developing ischaemia in patients presenting with ICH. To look at the effects of treatment group on the perihaematoma tissue changes, a group comparison test of perihaematoma volume, diffusion metrics and day 90 haematoma cavity / gliosis volume between participants in the TA and standard treatment groups will be performed treating initial haematoma volume as a covariate of no interest.

Analyses of data from the TICH-2 Plasma Biomarkers sub-study: We will compare plasma biomarkers levels between the two groups using multiple linear regression. The model will be tested for goodness of fit. We will perform analysis on relation of plasma biomarkers on primary outcome (modified Rankin Scale) using ordinal logistic regression. We will test correlation between plasma biomarkers and haematoma volume using Pearson product-moment correlation if the samples are normally distributed and Spearman rank-order correlation if not normally distributed.

DSMC Analyses: The DSMC will perform a formal interim analyses after 800 participants have been recruited (comprising both trial phases) and followed-up at 90 days. Safety analyses will be performed 6 monthly.

Decision to proceed to main phase – stopping criteria: The decision to recommend proceeding from start-up to main phase will be made by the Trial Steering Committee at 17 months on the basis of information on feasibility (at least 27 active sites and 270 recruited participants, i.e. at least 90% of target of both measures) and safety (as assessed by the independent Data safety Monitoring Committee). The recommendation will be given to HTA for ratification.

The DSMC will recommend to the TSC that the trial be stopped if there is 'proof beyond reasonable doubt' of overall benefit with the active treatment or if there is a lower level of evidence suggesting overall hazard. Evidence of hazard will include effect on the primary outcome (shift analysis of mRS) and analysis of safety endpoints, as specified in the protocol (death, VTE, ischaemic events, seizures, serious adverse events).

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To guide the DSMC in their deliberations the following guidelines are included in the protocol to illustrate the corresponding levels of evidence:

- Shift analysis of mRS favours the active (benefit) with P<0.001 (2-sided). The significance level of P<0.001 amounts to 'proof beyond reasonable doubt'.
- Shift analysis of mRS favours the control (hazard) with P<0.02 (2-sided)
- Analysis of death favours the control (hazard) with P<0.02 (2-sided)

Procedures for missing, unused and spurious data

Missing data will be reported, rules/methods for handling missing data will be detailed in the statistical analysis plan.

Definition of populations analysed

Safety population: All randomised participants.
Intention-to-treat population: All randomised participants, who receive any study medication. The intention-to-treat population will be defined in a blinded review prior to database lock.

Per protocol population: All participants in the intention-to-treat population who are deemed to have no major protocol violations that could interfere with the objectives of the study. The per-protocol population will be defined in a blinded review prior to database lock.

Analyses
All efficacy analyses will be performed on the intention-to-treat population; the robustness of the primary and key secondary analyses will be assessed in the per-protocol population. Safety analyses will be performed on the safety population.

Protocol Violations and Deviations

The study should be conducted in accordance with the approved protocol and changes to the protocol will only be made to protect the safety, rights, or welfare of the subject.

Protocol Violation

A protocol violation is a major deviation from the trial protocol where a participant is enrolled in spite of not fulfilling all the inclusion and exclusion criteria, or where deviations from the protocol could affect the trial delivery or interpretation significantly.

The following baseline characteristics constitute a protocol violation:

1. Randomisation > 8 hours from onset of symptoms

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2. Participant less than 18 years of age
3. Failure to obtain appropriate consent
4. Pre-morbid dependency (mRS) >4
5. Baseline cranial imaging shows underlying structural abnormality such as tumour or arterial venous malformation
6. On anticoagulation
7. Randomising event was secondary to trauma
8. Glasgow Coma Score < 5
9. Known probable life expectancy of less than 3 months
10. Female patient of childbearing potential, pregnant or breastfeeding at randomisation
11. Existing contra-indication to tranexamic acid known at the time of randomisation
12. Existing participation in another drug or devices trial, with the exception of the secondary prevention trial, RESTART. Participants enrolled in TICH-2 may be enrolled in RESTART after 21 days.

The following practice during the trial constitutes a protocol violation:

1. Subsequent randomisation into another drug or devices trial with the exception of the secondary prevention trial, RESTART. Participants enrolled in TICH-2 may be enrolled in RESTART after 21 days.
2. Patient does not receive randomised treatment
3. Failure to complete SAEs where appropriate
4. Failure to complete outcomes where appropriate
5. Follow-up assessments are performed (as opposed to submitted) outside the specified time as shown below:

a. 2-day follow-up: >2 days past the due date
b. 7-day follow-up: >7 days past the due date
c. Discharge and Death form: >30 days past the due date
d. 90-day follow up: >30 days past the due date
e. 365 day follow up: > 30 days past the due date

Protocol Deviation

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. Examples of Deviations are given below but this is not exhaustive.

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Follow-up assessments are performed (as opposed to submitted) outside the specified time as shown below:

a. 2-day follow-up: >1day past the due date
b. 7-day follow-up: >3days past the due date
c. Discharge and Death form: >7days past the due date
d. 90-day follow-up: >7 days past the due date
e. 365 day follow-up: > 14 days past the due date

Review of Protocol Violations and Deviations

Protocol Violations will be reviewed annually by both the Data Safety Monitoring Committee (using unblinded data) and the Trial Steering Committee (with blinding to treatment assignment). The list of protocol violations and deviations will be updated, as necessary, in a working practice document which will be uploaded and available on the trial website.

ADVERSE EVENTS

Definitions

An adverse event is any unfavourable and unintended sign, symptom, syndrome or illness that develops or worsens during the period of observation in the study.

An AE does include a / an:

1. Exacerbation of a pre-existing illness.
2. Increase in frequency or intensity of a pre-existing episodic event or condition.
3. Condition detected or diagnosed after medicinal product administration even though it may have been present prior to the start of the study.
4. Continuous persistent disease or symptoms present at baseline that worsen following the start of the study. An AE does not include a / an:

1. Medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction, transfusion); but the condition that lead to the procedure is an AE.
2. Pre-existing disease or conditions present or detected at the start of the study that did not worsen.
3. Situations where an untoward medical occurrence has not occurred (e.g., hospitalisations for cosmetic elective surgery, social and / or convenience admissions).
4. Disease or disorder being studied or sign or symptom associated with the disease or disorder unless more severe than expected for the participant's condition.
5. Overdose of concurrent medication without any signs or symptoms.

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A Serious Adverse Event (SAE) is any adverse event occurring following study mandated procedures, having received the IMP or placebo that results in any of the following outcomes:

1. Death
2. A life-threatening adverse event
3. Inpatient hospitalisation or prolongation of existing hospitalisation
4. A disability / incapacity
5. A congenital anomaly in the offspring of a participant

Important medical events that may not result in death, be life-threatening, or require hospitalisation may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

A distinction is drawn between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined using the criteria above. Hence, a severe AE need not necessarily be serious.

Serious adverse events are common in haemorrhagic stroke, for a full list of expected SAE that are not subject to expedited reporting, investigators should refer to Appendix A. As the IMP is administered once and has a short half life, serious adverse events occurring within the first 7 days will be assessed for seriousness, expectedness and causality. In addition fatal SAEs and safety outcome events (VTE, recurrent stroke, TIA, MI, PAD and seizures) will be reported until day 90.

Causality

Not related 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 “related” for notification purposes.

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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.

An AE whose causal relationship to the study IMP is assessed by the Chief Investigator as “possible”, “probable”, or “definite” is an Adverse Drug Reaction.

With regard to the criteria above, medical and scientific judgment shall be used in deciding whether prompt reporting is appropriate in that situation.

Reporting of adverse events

Participants will be asked to contact the study site immediately in the event of any serious adverse event. All adverse events will be recorded and closely monitored until resolution, stabilisation, or until it has been shown that the study medication or treatment is not the cause. The Chief Investigator (delegated the sponsor’s responsibilities) shall be informed immediately (within 24 hours) of any serious adverse events, occurring within the first 7 days and shall determine seriousness and causality in conjunction with any treating medical practitioners.

In the event of a pregnancy occurring in a trial participant or the partner of a trial participant monitoring shall occur during the pregnancy and after delivery to ascertain any trial related adverse events in the mother or the offspring. Where it is the partner of trial participant consent will be obtained for this observation from both the partner and her medical practitioner.

All serious adverse events occurring within the first 7 days will be recorded and reported to the MHRA and REC as part of the annual reports. SUSARs will be reported within the statutory timeframes to the MHRA and REC as stated below. The Chief Investigator shall be responsible for all adverse event reporting.

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SUSARs

A serious adverse event that is either sudden in its onset, unexpected in its severity and seriousness or not a known side effect of the IMP *and* related or suspected to be related to the IMP is classed as Suspected Unexpected Serious Adverse Reaction and requires expedited reporting as per the clinical trials regulations.

All serious adverse events that fall or are suspected to fall within these criteria shall be treated as a SUSAR until deemed otherwise.

The event shall be reported immediately (within 24 hours) of knowledge of its occurrence to the Chief Investigator.

The Chief Investigator will:

- Assess the event for seriousness, expectedness and relatedness to the study IMP
- Take appropriate medical action, which may include halting the trial and inform the Sponsor of such action
- If the event is deemed a SUSAR, shall, within seven days, enter the required data on the MHRA's eSUSAR web site.
- Shall inform the REC using the reporting form found on the NRES web page within 7 days of knowledge of the event
- Shall, within a further eight days send any follow-up information and reports to the MHRA and REC.
- Make any amendments as required to the study protocol and inform the ethics and regulatory authorities as required

Trial Treatment Related SAEs

A serious adverse event that is unexpected in its severity and seriousness *and* deemed directly related to or suspected to be related to the trial treatment but not the IMP shall be reported to the ethics committee that gave a favourable opinion as stated below.

The event shall be reported immediately of knowledge of its occurrence to the Chief Investigator.

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The Chief Investigator will:

- Assess the event for seriousness, expectedness and relatedness to the trial treatment.
- Take appropriate medical action, which may include halting the trial and inform the Sponsor of such action.
- If the event is deemed related to the trial treatment shall inform the REC using the reporting form found on the NRES web page within 7 days of knowledge of the event.
- Shall, within a further eight days send any follow-up information and reports to the REC.
- Make any amendments as required to the study protocol and inform the REC as required

Participant removal from the study due to adverse events

Any participant who experiences an adverse event may be withdrawn from the study at the discretion of the Investigator. Should the participant not receive the complete intervention due to, for example, an adverse event, they will remain in the study until the end of the trial at day 90 (±7), as completeness of follow-up is essential. However, should they wish to do so, any participant is free to withdraw from the trial at any time and without giving reason.

ETHICAL AND REGULATORY ASPECTS

ETHICS COMMITTEE AND REGULATORY APPROVALS

The trial will not be initiated before the protocol, informed consent forms and participant and GP information sheets have received approval / favourable opinion from the Medicines and Healthcare products Regulatory Agency (MHRA), Research Ethics Committee (REC), and the respective National Health Service (NHS) Research & Development (R&D) department. Should a protocol amendment be made that requires REC approval, the changes in the protocol will not be instituted until the amendment and revised informed consent forms and participant and GP information sheets (if appropriate) have been reviewed and received approval / favourable opinion from the REC and R&D departments. A protocol amendment intended to eliminate an apparent immediate hazard to participants may be implemented immediately providing that the MHRA, R&D and REC are notified as soon as possible and an approval is requested. Minor protocol amendments only for logistical or administrative changes may be implemented immediately; and the REC will be informed.

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The trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, 1996; the principles of Good Clinical Practice, in accordance with the Medicines for Human Use Regulations, Statutory Instrument 2004, 1031 and its subsequent amendments and the Department of Health Research Governance Framework for Health and Social care, 2005.

INFORMED CONSENT AND PARTICIPANT INFORMATION

The process for obtaining participant informed consent will be in accordance with the REC guidance, and Good Clinical Practice (GCP) and any other regulatory requirements that might be introduced. The investigator or their nominee and the participant or other legal representative shall both sign and date the Consent Form, if possible this will be before enrolment into the study. If this is not possible, where permission for enrolment is given verbally or by a relative or legal representative, written consent will be obtained as soon as practicable.

The participant will receive a copy of the signed and dated forms and the original will be retained in the Trial Master File. A second copy will be filed in the participant's medical notes and a signed and dated note made in the notes that informed consent was obtained for the trial.

The decision regarding participation in the study is entirely voluntary. The investigator or their nominee shall emphasize to them that consent regarding study participation may be withdrawn at any time without penalty or affecting the quality or quantity of their future medical care, or loss of benefits to which the participant is otherwise entitled. No trial-specific interventions will be done before informed consent has been obtained.

The investigator will inform the participant of any relevant information that becomes available during the course of the study, and will discuss with them, whether they wish to continue with the study. If applicable they will be asked to sign revised consent forms.

If the Informed Consent Form is amended during the study, the investigator shall follow all applicable regulatory requirements pertaining to approval of the amended Informed Consent Form by the REC and use of the amended form (including for ongoing participants).

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RECORDS

Drug accountability

Drug supplies will be kept in a secure, limited access storage area under the storage conditions specified by Pharmacy.

The investigator and the local site pharmacist shall maintain records of the study drug's delivery to the pharmacy, an inventory at the site, the distribution to each participant, and the return to the pharmacy or alternative disposition of unused study drugs. These records will include dates, quantities received, batch / serial numbers, expiration dates, and the unique code numbers (patient trial number) assigned to the trial participant. Investigators and /or the local site pharmacists will maintain records that document adequately that the participants were provided with the correct study medication. These records will be part of each patient's Case Report Form (CRF). All study medication packs and bottles received by the pharmacy shall be accounted for.

Case Report Forms

Each participant will be assigned a trial identity code number (country number, centre number and randomisation number), allocated at randomisation, for use on CRFs other trial documents and the electronic database. The documents and database will also use their initials (of first and last names separated by a hyphen or a middle name initial when available). The date of birth (dd/mm/yy) is entered into the database once for the use of data verification and is not visible when entering study data.

CRFs will be treated as confidential documents and held securely in accordance with regulations. The investigator will make a separate confidential record of the participant's name, date of birth, local hospital number or NHS number, and Participant Trial Number (the Trial Recruitment Log), to permit identification of all participants enrolled in the trial, in case additional follow-up is required.

CRFs shall be restricted to those personnel approved by the Chief or local Principal Investigator and recorded on the 'Trial Delegation Log.'

All paper forms shall be filled in using black ballpoint pen. Errors shall be lined out but not obliterated by using correction fluid and the correction inserted, initialled and dated.

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The Chief or local Principal Investigator shall sign a declaration ensuring accuracy of data recorded in the CRF.

Source documents

Source documents shall be filed at the investigator's site and may include but are not limited to, consent forms, current medical records, laboratory results and pharmacy records. A CRF may also completely serve as its own source data. Only trial staff as listed on the Delegation Log shall have access to trial documentation other than the regulatory requirements listed below.

Direct access to source data / documents

The CRF and all source documents, including progress notes and copies of laboratory and medical test results shall made be available at all times for review by the Chief Investigator, Sponsor's designee and inspection by relevant regulatory authorities (MHRA).

DATA PROTECTION

All trial staff and investigators will endeavour to protect the rights of the trial's participants to privacy and informed consent, and will adhere to the Data Protection Act, 1998. The CRF will only collect the minimum required information for the purposes of the trial. 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 (see above). Computer held data including the trial database will be held securely and password protected. All data will be stored on a secure dedicated web server. Access will be restricted by user identifiers and passwords (encrypted using a one way encryption method).
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.

Electronic data will be backed up every 24 hours to both local and remote media in encrypted format.

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QUALITY ASSURANCE & AUDIT

INSURANCE AND INDEMNITY

Insurance and indemnity for trial participants and trial staff is covered within the NHS Indemnity Arrangements for clinical negligence claims in the NHS, issued under cover of HSG (96)48. There are no special compensation arrangements, but trial participants may have recourse through the NHS complaints procedures.

The University of Nottingham as research Sponsor indemnifies its staff, research participants and research protocols with both public liability insurance and clinical trials insurance. These policies include provision for indemnity in the event of a successful litigious claim for proven non-negligent harm.

TRIAL CONDUCT

Trial conduct will be subject to systems audit of the Trial Master File for inclusion of essential documents; permissions to conduct the trial; Trial Delegation Log; CVs of trial staff and training received; local document control procedures; consent procedures and recruitment logs; adherence to procedures defined in the protocol (e.g. inclusion / exclusion criteria, correct randomisation, timeliness of visits); adverse event recording and reporting; drug accountability, pharmacy records and equipment calibration logs.
The Trial Coordinator, or where required, a nominated designee of the Sponsor, shall carry out a site systems audit at least yearly and an audit report shall be made to the Trial Steering Committee.

TRIAL DATA

Monitoring of trial data shall include confirmation of informed consent; source data verification; data storage and data transfer procedures; local quality control checks and procedures, back-up and disaster recovery of any local databases and validation of data manipulation. The Trial Coordinator, or where required, a nominated designee of the Sponsor, shall carry out monitoring of trial data as an ongoing activity.

Entries on CRFs will be verified by inspection against the source data. A sample of CRFs (10% or as per the trial risk assessment) will be checked on a regular basis for verification of all entries made. In addition the subsequent capture of the data on the trial database will be checked. Where corrections are required these will carry a full audit trail and justification.

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Trial data and evidence of monitoring and systems audits will be made available for inspection by the regulatory authority as required.

RECORD RETENTION AND ARCHIVING

In compliance with the ICH/GCP guidelines, regulations and in accordance with the University of Nottingham Code of Research Conduct and Research Ethics, the Chief or local Principal Investigator will maintain all records and documents regarding the conduct of the study. These will be retained for at least 7 years or for longer if required. If the responsible investigator is no longer able to maintain the study records, a second person will be nominated to take over this responsibility.

The Trial Master File and trial documents held by the Chief Investigator on behalf of the Sponsor shall be finally archived at secure archive facilities at the University of Nottingham. This archive shall include all trial databases and associated meta-data encryption codes.

DISCONTINUATION OF THE TRIAL BY THE SPONSOR

The Sponsor reserves the right to discontinue this trial at any time for failure to meet expected enrolment goals, for safety or any other administrative reasons. The Sponsor shall take advice from the Trial Steering Committee and Data Monitoring Committee as appropriate in making this decision.

STATEMENT OF CONFIDENTIALITY

Individual participant medical information obtained as a result of this study are considered confidential and disclosure to third parties is prohibited with the exceptions noted above. Participant confidentiality will be further ensured by utilising identification code numbers to correspond to treatment data in the computer files.

Such medical information may be given to the participant's medical team and all appropriate medical personnel responsible for the participant's welfare.

Data generated as a result of this trial will be available for inspection on request by the participating physicians, the University of Nottingham representatives, the REC, local R&D Departments and the regulatory authorities.

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PUBLICATION AND DISSEMINATION POLICY

Reporting, dissemination and notification of the results

Trial results will be published in a peer reviewed academic journal. Reporting will be in compliance with CONSORT [26, 27] recommendations. The focus of that article will be to discuss the effectiveness and safety of tranexamic acid in haemorrhagic stroke. When the study is complete summary findings will post on the support group website. Findings will also be presented at conferences such as UK Stroke Forum, European Stroke Conference and World Stroke Congress.

Policy for publication and authorship

The trial results will be published by named members of the trial team, on behalf of the TICH 2 Trial Collaborative Group. Members of the collaborative group will be listed in the publication, based on contributorship. Any secondary publication may be published by named individuals, but with appropriate acknowledgement of the collaborative group.

USER AND PUBLIC INVOLVEMENT

The project and protocol was discussed at the Nottingham Stroke Survivor Consumer Group meeting on January 30th 2012. The group reviewed the trial design and were highly supportive of the project. A member of the Group (Malcolm Jarvis) was involved in the subsequent designing of the definitive study and is a lay member of the Trial Steering Committee. The Stroke Consumer Group will also help with dissemination of the results via the user group website. The consent proceedure was reviewed and disucssed with the group on July 30th 2012, the group feel strongly that potential participants should not be denied access to the study because they lack capacity, have no relative present or an independent doctor cannot be accessed. The group, all of whom are stroke survivors, would like the treating stroke physician to be able enroll patients if the stroke physician feels it is in the patients best interest.

STUDY FINANCES

Funding source

This study is funded by NIHR HTA project code 11_129_109.

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Participant stipends and payments

Participants will not be paid to participate in the trial. Travel expenses will be offered for any hospital visits in excess of usual care.

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SIGNATURE PAGES

Signatories to Protocol:

Chief Investigator: (name) NIKOLA SPRIGG.

Signature: Nikola Sprigg

Date: 18/07/16

Trial Statistician: (name) Katie Robson

Signature: KRobson

Date: 12/7/16

Trial Pharmacist: (name) PAULA BLANCE

Signature: [signature]

Date: 01AUG2016

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Appendix A: Expected events not subject to expedited reporting

After tranexamic acid the following events are expected and therefore not subject to expedited reporting:

== Gastro-intestinal ==
Abdominal pain
Diarrhoea
Gastrointestinal disturbance
Nausea
Vomiting
== Cardiovascular ==
Arterial thrombosis any site
Deep vein thrombosis (DVT)
Collapse
Hypotension
Ischaemic stroke
Peripheral artery embolism
Pulmonary embolism (PE)
Tachycardia
Venous thrombosis any site

== Central nervous system ==
Convulsions
Disturbance in colour vision
Dizziness
Headache
Seizure

== General ==
Anaphylaxis
Fatigue
Flushing
Hypersensitivity including oropharangeal swelling, urticaria, angiodema
Musculoskeletal pains
Rash

After haemorrhagic stroke the following events are expected and therefore not subject to expedited reporting:

== Cardiovascular ==
Acute coronary syndrome (ACS)
Atrial fibrillation (AF)
Bradycardia
Cardiac arrest
Cardiac failure
Cardiac dysrhythmia
Carotid endarterectomy
Chest pain
Collapse
Deep vein thrombosis (DVT)
Hypertension

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Hypotension
Myocardial infarction (MI)
Pulmonary embolism (PE)
Sudden cardiac death
Systemic embolism
Tachycardia
Unstable angina

== Central nervous system ==
Agitation
Anxiety
Cerebral oedema
Complication of initial stroke
Dementia
Depression
Dysphagia
Extension of initial haemorrhagic stroke – haematoma expansion
Extension of initial ischaemic stroke –infarct expansion
Haemorrhagic transformation of infarct (HTI)
Headache
Intracerebral bleed
Recurrent stroke - ischamaemic
Sedation
Seizure
Sensory loss
Transient ischaemic attack (TIA)
Vertigo
Visual loss
Weakness

== Gastro-intestinal ==
Abdominal pain
Cholecystitis
Constipation
Diarrhoea
Dysphagia
Feeding tube insertion and/or complication
Gall Stones
Gastrointestinal bleed
Gastrointestinal disturbance
Incontinence, faecal
Heartburn
Hepatitis
Maelena
Nausea
Oral ulceration
Pancreatitis
Vomiting
Weight loss

== Genito-urinary ==
Sexual dysfunction

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Incontinence, urinary

Renal impairment
Urinary retention
Urinary tract infection (UTI)

== Haematological ==
Anaemia
Leukopenia
Thrombocytopenia

== Miscellaneous ==
Acid base disturbance
Bacteraemia
Diaphoresis
Electrolyte disturbance
Fall
Fatigue
Hyperglycaemia
Hyperuricaemia
Hypoglycaemia
Lymphadenopathy
Malignancy/Cancer –new diagnosis
Muscle twitching
Osteoarthritis

== Respiratory ==
Asthma
Bronchitis
Bronchospasm
Chest infection
Chronic obstructive pulmonary disease (COPD)
Emphysema
Exacerbation of COPD
Hypoxia
Pleural effusions
Pneumonia
Pulmonary embolism (PE)
Shortness of breath

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Appendix B: TICH-2 MRI sub-study

MRI Evaluation of effects of Tranexamic Acid in spontaneous intracerebral haemorrhage: The TICH-2 MRI study.

Summary

Spontaneous intracerebral haemorrhage (SICH) is common and has high associated morbidity and mortality. Tranexamic acid (TA), an antifibrinolytic drug proven to be safe and effective for acute traumatic haemorrhage, has potential to reduce haematoma expansion, a determinant of outcome in SICH. The TICH-2 trial is a NIHR-funded randomised controlled trial (n=2000) assessing therapeutic benefit of TA on outcome in SICH. However, TA has potential to moderate brain injury in other ways through interaction with the plasminogen activator / plasmin cascade, which has putative roles in modulating neuroinflammation, extracellular matrix stabilisation and apoptosis. TICH-2 provides a unique opportunity to use MRI scanning to study effects of TA on the brain in patients with an acute cerebrovascular injury in a blinded randomised trial, and to relate MRI-detected treatment effects to the detailed TICH-2 outcome measures. Results may be generalisable to other types of brain injury where TA has a potential therapeutic role.

Background to the project and pilot data

Tranexamic acid (TA) is an anti-fibrinolytic drug that reduces bleeding in surgical patients and reduces the risk of death in patients with traumatic bleeding [1, 2]. The therapeutic potential of TA to restrict haematoma expansion and improve outcome in patients with spontaneous intracerebral haemorrhage (SICH), a common condition associated with 1-year survival rates of 46% [3] and high rates of disability in survivors [4], is being evaluated in a NIHR-HTA funded multicentre randomised placebo-controlled trial (the TICH-2 study).

TA binds reversibly to the lysine binding site of plasminogen / plasmin [5], leading to potent inhibition of the interaction of plasmin with fibrin that results in reduced fibrinolysis. In the context of SICH it has been postulated that this will result in more rapid cessation of bleeding at the point of vessel rupture and hence limit haematoma expansion (a factor shown to be associated with both mortality and disability [6]). However, there are alternative mechanisms by which TA could alter the pathophysiology of the evolving brain injury that accompanies SICH via its interaction with the plasminogen activation axis.

Firstly, there is potential for TA to precipitate ischaemic events. Diffusion weighted imaging (DWI) hyperintense lesions (DWIHL), which are indicative of acute or subacute ischaemic lesions, are known to co-occur with SICH with a prevalence (8-35%) [7-10] and are associated with a higher risk of dependence or death at 3 months (OR 4.8; 95% CI 1.7–13.9; *P*=0.004) [11]. The mechanisms underlying the high prevalence of DWIHL in SICH are not known. Endothelial failure is now thought to be a key event in the mechanism of small vessel disease and lacunar infarction [12]. A sequential process of endothelial failure, non-occlusive wall associated microthrombosis, vessel rupture and microbleed, and finally reactive occlusive thrombosis leading to infarction has been proposed based on animal models [13]. The inhibition of fibrin degradation by TA could potentiate this process by exacerbation of both non-occlusive and occlusive microvascular thrombosis. Cerebral microbleeds (CMB) are known to have an association with silent infarction, particularly when associated with associated with cerebral amyloid angiopathy (CAA) [9, 14], and therefore SICH patients with CMB and / or underlying CAA may be at increased risk of DWIHL following TA administration. Recent findings in CAA patients show that asymptomatic DWIHL

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lead to chronic local microstructural injury which may cumulatively contribute to vascular cognitive impairment [15].

There are no completed large RCTs of TA for SICH, or trials of TA in for other clinical indications that include brain MRI as an outcome measure. The outcome measures for TICH-2, which is the largest on-going RCT of TA for SICH, will allow documentation of cerebral ischaemic events that present clinically or that are large enough and sufficiently mature to be visible on the day 2 CT scan; however, without MRI there is potential for ischaemic lesions that are not clearly visible on the CT scan to be missed. Similarly, the published protocol for the STOP-AUST study [16], which will also evaluate the use of TA in improving outcome in spontaneous ICH will collect clinical and CT outcome data, and hence may also miss ischaemic lesions not seen on CT. There do not appear to be any ongoing RCTs of TA includes brain MRI.

Support for a possible potentiating effect of TA on cerebral ischaemia comes from a meta-analysis of TA administration in spontaneous subarachnoid haemorrhage (SAH) which found pooled relative risk for reported cerebral ischaemia of 1.41 (95% CI 1.04-1.91) [17]. However, it should be noted that all but one study of TA included in the meta-analysis used prolonged (>10 days) administration of TA, and that SAH has a known association with cerebral infarction due to vasospasm, which is unlikely to be the dominant mechanism driving DWIHL in SICH. Against a possible potentiating effect of TA on cerebral ischaemia are multiple studies of TA in a variety of disease settings, including traumatic and spontaneous bleeding (non-SAH) showing no increase in cerebral ischaemia with TA use [1, 2, 18, 19]. The TICH-1 study [20], designed as a feasibility study for the administration of TA in the setting of SICH, found no cases of symptomatic ischaemic stroke and no infarction on computed tomography (CT) scan performed 48 hours after TA administration.

Secondly, there are potential beneficial effects of TA in reducing neurotoxicity and neuroinflammation. Plasmin is an important protease involved in remodelling of the extracellular matrix [21]. Direct effects of plasmin demonstrated in animal models include degradation of neuronal cell adhesion molecule and extracellular laminin (an event that precedes neuronal death) [22, 23]. Indirectly the plasminogen activation axis may contribute to neurotoxicity by plasmin-mediated activation of the matrix metalloproteinase (MMP) pathway of extracellular proteolysis [21]. Levels of circulating MMP-3 are independently associated with perihaematoma oedema (PHE) volume and clinical outcome at 90 days [24], and clinical and biologic markers of inflammatory response at presentation are predictors of early neurological deterioration in SICH [25]. Furthermore, modulation of brain inflammation by Fingolimod attenuates neurological dysfunction and promotes recovery [26]. Notably a study in surgical patients has shown that TA attenuates the inflammatory response [27], thought to be mediated by inhibition of the plasminogen activation cascade.

Modulation of the plasminogen activation cascade by TA provides alternative mechanisms for the potential therapeutic effects of TA in the TICH-2 trial. Measurement of the PHE volume and diffusion characteristics provides a potential tool to evaluate the inflammatory response around SICH [26]. PHE increases rapidly during the first 48 hours and peaks towards the end of the second week following SICH [28]. Studies have shown that the PHE is predominantly not ischaemia-driven, in that DWI most commonly shows elevated diffusion [29, 30]; the implication is that PHE results from mechanisms such as increased permeability of the neurovascular unit or alterations in the extravascular ultrastructural environment resulting in increased tissue water content and mobility. Conflicting results have been found between PHE volume and functional outcome [31, 32], but the trial of Fingolimod therapy in ICH found that reduced PHE in fingolimod-treated patients was associated with reduced neurologic deficits and improved recovery [26]. In cases where restricted diffusion (indicating

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cytotoxic oedema) is detected in PHE, an association with poor clinical outcome has been observed [33]. Through its potential beneficial effects in reducing neurotoxicity and neuroinflammation, TA may be expected to affect volume and diffusion properties of PHE, and the consequent end-stage damage in brain surrounding the haematoma

The TICH-2 study provides a unique opportunity to address the knowledge gap of how TA modifies the evolution of parenchymal brain injury (beyond the potential limitation of haematoma expansion). The CT scanning performed in the main TICH-2 trial is designed to detect group difference in haematoma expansion; the proposed TICH-2 MRI study will use MRI performed in a sub-group of the TICH-2 population to test a primary hypothesis regarding treatment-related differences in DWIHL prevalence, and will allow exploratory analyses of treatment effects on PHE volume and diffusion properties, and end-stage SICH-related tissue damage at day 90

Pilot data: Analysis of TICH-1 CT data showed that for every 1ml ICH volume, the mean relative PHE volume increased between the Day 0 and Day 2 CT scan by 0.29ml (s.d. 0.34) in TA-treated patients (n=16) and by 0.41ml (s.d. 0.27ml) in the placebo group (n=7). This difference in rPHE expansion was not significant in the pilot data, but has been used to support a power calculation below. Figure 1 is a composite image showing ROI analysis of PHE DWI properties using data from one of the potential recruiting centres to illustrate the proposed analysis method.

Primary hypothesis
Prevalence of remote DWIHL on the day 5 MRI scan will be greater in the TA group compared to controls

Exploratory hypothesis
1) Perihaematoma oedema volume and perihaematoma diffusion restriction on the day 5 MRI scan will be reduced in the TA group compared to controls
2) Combined volume of the residual haematoma cavity and surrounding gliosis on the day 90 MRI will be reduced in the TA group compared to controls, controlling for initial haematoma volume

In addition to testing these hypotheses, differences in these MRI measures will be related to outcome at 90 days adjusting for haematoma expansion. Investigations will be performed to determine whether imaging markers of co-existing small vessel disease (CMB and WMH), and imaging markers of CAA (CMB distribution, superficial siderosis) are associated with the presence of DWIHL in TA treated patients.

Experimental details and design of proposed investigation

Patient selection and recruitment:
Recruitment will be restricted to those centres participating in the TICH-2 MRI sub-study. Patients being recruited to the TICH-2 trial will be invited to participate in the TICH-2 MRI sub-study up to 7 days after randomisation into the main study, provided they meet the additional following inclusion / exclusion criteria:

· Additional TICH-2 MRI study inclusion criteria:
  1. participant freely gives informed consent to participate in the TICH-2 MRI study
  2. Able to safely undergo MRI scanning

· Additional TICH-2 MRI study exclusion criteria:
  1. Contraindication to MRI scan (eg, non-MRI compatible implant, intra-ocular / intracranial metallic device or fragment, claustrophobia, etc)

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2. Clinical instability (for example cardiorespiratory or neurological instability) such that MRI scan would introduce additional clinical risk

Participation in the MRI sub-study will be optional and participants may continue in the main TICH-2 trial without participation in the MRI sub-study. Only participants who have capacity to provide consent will be invited to participate in the sub-study.

Image acquisition: MRI scanning performed on day 5 (+/- 2) and day 90 (+/-5) will comply with the essential imaging sequences specified by the Standards for Reporting vascular Neuroimaging (STRIVE) neuroimaging standards [12], including:

- DWI: axial single-shot spin-echo EPI, with standardised multi-b-value acquisition optimised to minimise distortion (parallel imaging factor =2 and partial Fourier EPI based scan)
- T2*-weighted axial gradient echo
- 2D axial T1-weighted, T2-weighted and T2-weighted FLAIR acquisitions
- If available, manufacturer optimised SWI

All axial acquisitions to have 32-42 slices to cover the brain, with slice thickness 4mm or less, slice gap 0.4mm or less, typical FOV 230mm, typical matrix 180x256 or higher (except the DWI which will have matrix in the range 112-128). Where practical, MRI will be performed at 1.5T to minimise spatial distortion in DWI data, but a pragmatic approach will be taken based on local scanner availability. The study MRI physicist will standardise imaging protocols across sites, within the limitations of scanner make / model. Imaging data quality checks and testing of transfer of data using dummy data will be carried prior to sending patient data.

Image analysis: Manual or semi-automated delineation of DWIHL, CMB, PHE, WMH and day 90 lesion volume will be performed using standard off-line image processing tools. DWI scans will be analysed for presence, number and distribution of DWIHL and for quantifying the diffusion properties of PHE (fig 1). Axial T2-weighted images will be used to quantify PHE. SWI / T2* will be analysed for number and distribution of CMB and presence of superficial siderosis, and used for classification of ICH patients into probable CAA and non-CAA groups [34]. FLAIR will be images to quantify gliosis and final haematoma cavity on the day 90 scan, as well as for scoring of WMH using an established 4 point scale [35] on the day 5 scan. Training will use existing imaging datasets, and a high intra and interobserver reliability must be demonstrated prior to commencing analysis of the trial dataset.

Protection against bias: In addition to recruitment prior to treatment randomisation, multiple measures will be taken to minimise bias: recruitment according to pre-defined inclusion/exclusion criteria; central data registration with real time validation and concealment of allocation; blinded adjudication of MRI scans; research staff trained in protocol and processes; analysis by intention to treat; analyses adjusted for baseline prognostic variables.

Data archiving: individual participant data (trial database, anonymised neuroimages) will be stored for sharing with data repositories, e.g. Cochrane, VISTA, NeuroGRID as explained in the patient information sheet.

Statistical analysis: To analyse the primary hypothesis, a risk model (binary logistic regression model, in SAS using the GENMOD procedure) for presence of DWIHL post-SICH will be constructed, based on treatment group, patient factors (age, gender, race, cardiovascular and cerebrovascular risk factors) and imaging markers of small vessel disease (CMB, WMH) and likely diagnostic classification for CAA (based on CMB distribution and presence of superficial siderosis), allowing stratification of risk of developing ischaemia in patients presenting with ICH. As the sample size is 280, it would be unwise to add too many (unnecessary) covariates into the model. To determine the best set of covariates for

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the model a stepwise selection will be used in the model fitting process. The significance that each variable needs to have on the outcome can be altered in the stepwise selection to ensure that only the most relevant ones are chosen.

To look at the exploratory hypotheses, a group comparison test of PHE volume, PHE diffusion metrics and day 90 haematoma cavity / gliosis volume between participants in the TA and standard treatment groups will be performed treating initial haematoma volume as a covariate of no interest. Patient factors, as above, may also be added into the model and again a stepwise selection process will be used. Other proposed analysis will include using a univariate analysis of association between the MRI measures and 90 day functional outcome: dependency (modified Rankin scale), disability (Barthel Index), quality of life (EQ-5D), cognition (TICS-M) and mood (Zung Depression Scale), controlled for haematoma expansion. We will also explore whether treatment-driven differences in number and distribution of imaging-identified lesions (SICH, DWIHL, CMB) relates to seizure activity.

Power calculations
Primary hypothesis: Based on previous studies finding prevalence of DWIHL of 20% in SICH, we performed calculations for sample size to allow us to detect different percentage increases in DWIHL prevalence in the TA group above a 20% baseline, using the sample size formula for prevalence:

$= \frac{z_{1-\alpha/2}^{2} \times p(1-p)}{m^{2}}$ where Z = confidence interval, P = prevalence, and M = margin of error (standard is 5%). Results are shown in the table:

Prevalence	Sample size if confidence level 90%	Sample size if confidence level 95%
Prevalence of 20%	174	246
10% increase (prevalence 22%)	186	264
25% increase (prevalence 25%)	204	290
50% increase (prevalence 30%)	228	324

Assuming a small increase in prevalence of DWIHL by 10% in the TA group, and with 95% confidence levels, a sample size of 264 is required. Allowing 5% imaging data rejection (eg due to excessive patient motion), a sample size of 280 is required.

Exploratory hypotheses: Pilot data from the TICH-1 study showed that for every 1ml of SICH volume, the mean relative PHE volume increased between the Day 0 and Day 2 CT scan by 0.29ml (s.d. 0.34) in TA-treated patients and by 0.41ml (s.d. 0.27ml) in the placebo group. Assuming a similar effect size is present on the day 5 MRI scan using the sample size proposed for the main hypothesis (n=280) we will be able to detect a group difference in increase in relative PHE with power of 0.89 and $\alpha=0.05$ (independent samples t-test, 2-tails).

Expected value of results
TA may have biological effects in the brain tissue that could alter the outcome in SICH patients independent to its effects on haematoma expansion. Understanding these effects is vital, particularly if the results of TICH-2 and STOP-AUST trials show an effect of TA on outcome that is not (or only partially) explained by an effect of TA on haematoma expansion. This study will allow quantification of treatment related brain changes (particularly prevalence of ischaemic lesions, PHE volume and diffusion characteristics, and late post-SICH tissue damage) in SICH patients in the context of a multicentre RCT. If the results of the TICH-2

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and SPOT-AUST trials support the use of TA, then the opportunity to study the potential therapeutic effects of TA in acute cerebrovascular brain injury in a controlled trial is unlikely to occur again. By including imaging markers of small vessel disease and CAA in the analysis of DWIHL burden, investigations can take place to determine whether there is a subgroup of patients at risk of DWIHL following TA treatment for SICH, which will inform future studies of TA in SICH and could ultimately influence clinical practice. If the study shows a beneficial effect of TA on PHE, then this study may trigger studies of TA in other acute brain pathologies associated with oedema and inflammation.

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Appendix C TICH-2 Plasma Biomarkers sub-study

Background & Literature review

Intracerebral haemorrhage (ICH) is a common medical emergency affecting approximately 5 million people per year worldwide[1]. It associated with high mortality and morbidity. The 30-days mortality rate for ICH is about 40% [1, 2] and approximately 60% of the patients remained dependent after ICH[2]. To date, it remains a largely untreatable disease. Exploration of mechanisms of brain injury after ICH is crucial for targeted therapy.

Mechanisms of brain injury

Various pathophysiological mechanisms are involved in brain injury after ICH. At the time of ictus, the rupture of an abnormal intracranial vessel resulted in extravasation of blood into the extravascular spaces and thus forming a haematoma. The direct physical disruption and mass effect of haematoma cause death of adjacent neurons[3]. Large haematoma causes midline shift and fatal herniation syndromes[4]. The haematoma may also cause an increased intracranial pressure leading to a reduced cerebral perfusion pressure[5]. The volume of haematoma is an independent factor of poor prognosis[6]. Haematoma expansion occurs in 38% of ICH patients within 24 hours leading to early neurological deterioration and poor outcome[7]. Subsequently, brain injury may occur due to various secondary mechanisms.

A cascade of inflammatory response is initiated at the onset of ICH and this leads to a breach in blood-brain barrier and worsening oedema[8, 9]. Within an hour of insult, microglials are activated followed neutrophils infiltration[10]. Various cytokines are produced including tumour necrosis factor-α (TNFα), Interleukin-1β (IL1β), Interleukin-6 (IL-6), chemokines, adhesive molecules and matrix metalloproteinases [10, 11]. The complement system plays additional role as chemotactic agent and also forming membrane attack complexes leading to cell lysis of erythrocytes, neurons and glials[12].

Plasmin-mediated pathways are activated following an ICH. Plasminogen is converted to plasmin by urokinase-type plasminogen activator and tissue-type plasminogen activator. Plasmin is a fibrinolytic agent that binds to fibrin, breaking it down to fibrin degradation products such as D-dimer[13]. Apart from acting on the coagulation cascade, plasmin can act via protease-activated receptor 1, 2, 3 and 4 (PAR-1, 2, 3, 4) to mediate inflammation and healing[13]. Plasmin stimulates neutrophils and macrophage infiltration as well as the production of IL-1 and tissue growth factor β (TGF-β), fibroblast growth factor-2 (FGF-2), and hepatocyte growth factor (HGF)[14]. Plasmin enhances the production of growth factor via proteolysis of extracellular matrix. Plasmin also mediates the production of MMP and modifies ECM, both important elements of inflammatory process. In addition, plasmin promotes angiogenesis and platelet aggregation. In summary, plasmin-mediated pathways lead to neuroinflammation and neurotoxicity [15].

Thrombin is formed rapidly following ICH to enable stable thrombus formation. Thrombin acts on fibrinogen, converting it to fibrin, an essential component of a thrombus. While it is important to prevent further bleeding in ICH, it can lead to further brain injury by stimulating inflammatory infiltrates, inducing apoptosis, disrupting the blood-brain barrier and causing early peri-haematomal oedema [16-18]. Thrombin also causes neuronal injury via extracellular signal-regulated kinase pathways[19]. Thrombin induces activation of complement, TNFα and matrix metalloproteinases [20, 21]. Animal studies have shown that while a high concentration of thrombin is harmful, a low concentration may actually have neuroprotective effects[22].

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Delayed neuronal injury occurs as erythrocyte breakdown into haemoglobin and its byproducts including haem, carbon monoxide, biliverdin and iron [23]. Haemoglobin and its degradation products are cytotoxic to neurons and glials. Studies targeting the therapeutic effects of reducing haem and iron load by using haem oxygenase inhibitor, haptoglobulin and iron chelator deferoxamine demonstrated possible beneficial effects[24-26].

Additional effects of tranexamic acid

Tranexamic acid is an antifibrinolytic agent that acts on plasminogen and preventing lysis of thrombin by plasminogen [27]. It is postulated that tranexamic acid reduces haematoma expansion as stopping continuing bleed or prevent rebleeding. However, by acting on plasminogen, tranexamic acid may also affect plasmin-mediated pathways including neuroinflammation. Animal studies showed that tranexamic acid inhibits plasminogen and thrombin-induced neuronal injury[28]. Tranexamic acid may also reduce matrix metalloproteinase-9 (MMP-9) levels thus conferring neuroprotection [29]. However, tranexamic acid may also potentiate the effect of thrombin, which results in cerebral ischaemia as we see in the subarachnoid haemorrhage trials[30].

Tranexamic acid has been shown to have an anti-inflammatory effect based on clinical studies. Jimmenez et al conducted a randomised trial recruiting patients undergoing extracorporeal circulation in cardiac surgery to receive tranexamic acid versus placebo. The trial reported significant reduction in inflammatory response in tranexamic acid group as well as a reduction in inflammatory markers including IL-6 and soluble TNF-α receptor antigen[31]. In a second randomised controlled trial, Jimmenez et al reported reduced incidence of inflammatory response in patients given higher dose of tranexamic acid (40mg/kg given twice) than those given a lower dose (40mg/kg given once)[32].

In conclusion, tranexamic acid may have anti-inflammatory and haemostatic effects in patients with spontaneous ICH. This hypothesis needs to be examined by a study of plasma biomarkers.

Research Objectives

The primary objective of the sub-study is:
1) To assess whether tranexamic acid reduces the levels of plasmin and inflammatory markers including IL-1a, IL-1b, IL2, IL4, IL6, IL8, IL10, IL12, IL17A, IFNγ, TNFα, and GM-CSF

The secondary objectives are:
1) To assess there is any association between plasma biomarkers and clinical outcome as assessed by modified Rankin Scale at 90 days
2) To assess there is any association between plasma biomarkers and haematoma size
3) To assess there is any association between plasma biomarkers and peri-haematomal oedema

Research hypothesis:

1) Tranexamic acid reduces plasma D-dimer levels significantly compared to patients not receiving tranexamic acid
2) Tranexamic acid reduces plasma plasmin levels significantly compared to patients not receiving tranexamic acid
3) Tranexamic acid reduces plasma inflammatory markers (IL-1a, IL-1b, IL2, IL4, IL6, IL8, IL10, IL12, IL17A, IFNγ, TNFα, and GM-CSF) significantly compared to patients not receiving tranexamic acid
4) Lower plasma plasmin and inflammatory markers levels (IL-1a, IL-1b, IL2, IL4, IL6, IL8, IL10, IL12, IL17A, IFNγ, TNFα, and GM-CSF) are associated with better clinical outcome

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5) Lower plasma plasmin and inflammatory markers levels (IL-1a, IL-1b, IL2, IL4, IL6, IL8, IL10, IL12, IL17A, IFNγ, TNFα, and GM-CSF) are associated with smaller haematoma size
6) Lower plasma plasmin and inflammatory markers levels (IL-1a, IL-1b, IL2, IL4, IL6, IL8, IL10, IL12, IL17A, IFNγ, TNFα, and GM-CSF) are associated with lesser degree of peri-haematomal oedema

Sample size

Based a previous study, where mean D-dimer level for placebo group is 3612.5 ng/ml, tranexamic acid group 1125 ng/ml and pooled standard deviation of 1308.6 ng/ml [33, 34]; and assuming an type 1 error (α) of 0.05 and aiming for a power of 90%, the estimated sample size is 8 patients per group. The minimum sample size required is 16 patients.

Assessment of Plasma biomarkers

Approximately 10mls of venous blood will be drawn from consenting patients at baseline (before randomised treatment is given), day 2 and day 7 (or discharge if sooner than 7 days). The blood samples filled in EDTA tubes will then be centrifuged and the supernatant stored at a temperature of -20 degree Celsius. Samples will be stored in linked anonymised format at the University of Nottingham and labeled according to patients' trial identity numbers and initials to allow accurate linkage to clinical data and consent form in accordance with the Human Tissue Authority Code of Practice. All laboratory analyses will be performed at the Clinical Sciences Building, City Hospital Campus, the University of Nottingham. Once analysed, all blood samples will be disposed of in accordance with the Human Tissue Authority Codes of Practice. However, if the participants consent, blood samples may be kept for future researches.

The following plasma parameters will be assayed:
1) Plasma Plasminogen levels using Plasminogen (PLG) Human SimpleStep ELISA® Kit
2) Plasma Plasmin levels using Plasmin Activity Assay Kit (Fluorometric)
3) Plasma D-dimer levels using D-Dimer Human SimpleStep ELISA® Kit
4) Plasma urokinase-type plasminogen activator (uPA), and tissue-type plasminogen activator (tPA) levels
5) Plasma pro-inflammatory cytokine and chemokines levels(IL-1a, IL-1b, IL2, IL4, IL6, IL8, IL10, IL12, IL17A, IFNγ, TNFα, and GM-CSF can be studied using a multi-analyte ELISArray kit)
6) Plasma thrombin levels using Thrombin (Factor II) Human ELISA Kit
7) Plasma MMP-2 & MMP-9 levels (MMP-2/MMP-9 Inhibitor III – Calbiochem)
8) Coagulation profile (prothrombin time/partial thromboplastin time/international normalised ratio) and fibrinogen levels (on day 1 and 2 only, where coagulation profile would be done as part of routine medical care on day 1)

The plasma biomarkers will also involve healthy volunteers as controls. For healthy controls, information collected includes age, gender, medical condition(s) and medication history. Healthy volunteers shall have the above blood tests only once on the same day as day 1 for patients.

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