BMJ Open

Developing a multivariable prediction model for functional outcome after reperfusion therapy for acute ischaemic stroke: study protocol for the Targeting Optimal Thrombolysis Outcomes (TOTO) multicentre cohort study

Elizabeth Holliday ,[1,2] Thomas Lillicrap,[1,2] Timothy Kleinig,[3,4] Philip M C Choi,[5,6] Jane Maguire ,[7] Andrew Bivard,[8] Lisa F Lincz ,[9,10] Monica Anne Hamilton-Bruce ,[11,12] Sushma R Rao ,[4,13] Marten F Snel ,[4,13] Paul J Trim,[4,13] Longting Lin,[1] Mark W Parsons,[8,14] Bradford B Worrall,[15,16] Simon Koblar ,[11,12] John Attia ,[1,17] Chris Levi[18,19]

To cite: Holliday E, Lillicrap T, Kleinig T, *et al*. Developing a multivariable prediction model for functional outcome after reperfusion therapy for acute ischaemic stroke: study protocol for the Targeting Optimal Thrombolysis Outcomes (TOTO) multicentre cohort study. *BMJ Open* 2020;**10**:e038180. doi:10.1136/bmjopen-2020-038180

► Prepublication history for this paper is available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2020-038180).

Received 01 March 2020
Revised 10 March 2020
Accepted 11 March 2020

Check for updates

© Author(s) (or their employer(s)) 2020. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

For numbered affiliations see end of article.

Correspondence to
Professor Elizabeth Holliday; liz.holliday@newcastle.edu.au

ABSTRACT

Introduction Intravenous thrombolysis (IVT) with recombinant tissue plasminogen activator (rt-PA) is the only approved pharmacological reperfusion therapy for acute ischaemic stroke. Despite population benefit, IVT is not equally effective in all patients, nor is it without significant risk. Uncertain treatment outcome prediction complicates patient treatment selection. This study will develop and validate predictive algorithms for IVT response, using clinical, radiological and blood-based biomarker measures. A secondary objective is to develop predictive algorithms for endovascular thrombectomy (EVT), which has been proven as an effective reperfusion therapy since study inception.

Methods and analysis The Targeting Optimal Thrombolysis Outcomes Study is a multicenter prospective cohort study of ischaemic stroke patients treated at participating Australian Stroke Centres with IVT and/or EVT. Patients undergo neuroimaging using multimodal CT or MRI at baseline with repeat neuroimaging 24 hours post-treatment. Baseline and follow-up blood samples are provided for research use. The primary outcome is good functional outcome at 90 days poststroke, defined as a modified Rankin Scale (mRS) Score of 0–2. Secondary outcomes are reperfusion, recanalisation, infarct core growth, change in stroke severity, poor functional outcome, excellent functional outcome and ordinal mRS at 90 days. Primary predictive models will be developed and validated in patients treated only with rt-PA. Models will be built using regression methods and include clinical variables, radiological measures from multimodal neuroimaging and blood-based biomarkers measured by mass spectrometry. Predictive accuracy will be quantified using c-statistics and R^2. In secondary analyses, models will be developed in patients treated using EVT, with or without prior IVT, reflecting practice changes since original study design.

Ethics and dissemination Patients, or relatives when patients could not consent, provide written informed consent to participate. This study received approval from the Hunter New England Local Health District Human Research Ethics Committee (reference 14/10/15/4.02). Findings will be disseminated via peer-reviewed publications and conference presentations.

Strengths and limitations of this study

- A strength of this study is prospective patient recruitment by multiple Australian Stroke Centres.
- Predictive models will incorporate prognostic measures captured using advanced neuroimaging, which are increasingly used in reperfusion treatment decisions.
- The analysis of blood samples using mass spectrometry has the potential to identify novel biomarkers of intravenous thrombolysis (IVT) and endovascular thrombectomy (EVT) response.
- Statistical analyses are prespecified and the planned sample size provides adequate power to predict outcomes with good precision.
- Because separate predictive models will be developed according to reperfusion therapy type (IVT, EVT or IVT+EVT), individual predictive models may not generalise to patients treated receiving alternate reperfusion therapies.

INTRODUCTION

The emergence of reperfusion therapy in acute ischaemic stroke with the collection of large and well-characterised patient data sets and the concurrent application of advanced brain imaging and 'omics' technologies provide an opportunity to develop a precision medicine approach to acute stroke care. Currently, when

BMJ Open: first published as 10.1136/bmjopen-2020-038180 on 6 April 2020. Downloaded from http://bmjopen.bmj.com/ on June 20, 2021 by guest. Protected by copyright.

facing difficult 'benefit vs risk' decisions, clinicians extrapolate summary estimates of treatment effectiveness or risk derived from clinical trial populations to the individual patient, who may or may not share trial patient characteristics. The greater understanding of pathophysiology and endophenotypes afforded by advanced brain imaging, along with measurement of previously unrecognised factors influencing treatment responsiveness and risk of harm, provide the opportunity to move towards a new paradigm of precision stroke medicine.

Intravenous thrombolysis (IVT) with recombinant tissue plasminogen activator (rt-PA) within a therapeutic window of 4.5 hours (or up to 9 hours guided by multimodal imaging) is the only approved pharmacological reperfusion therapy for acute ischaemic stroke. Treatment of ischaemic stroke patients with rt-PA promotes clot lysis by accelerating the catalytic conversion of plasminogen to plasmin, a protease that dissolves fibrin. In randomised trials and observational studies, patients receiving timely rt-PA treatment demonstrate higher recanalisation rates and more favourable clinical outcomes than patients not receiving reperfusion therapy.[1–4] Despite the proven effectiveness of endovascular thrombectomy (EVT) for ischaemic strokes caused by proximal large artery occlusion in the anterior circulation, rt-PA therapy remains first-line treatment for patients with and without a proximal anterior occlusion, or who present to a hospital where thrombectomy is not available, even if the patient is later transferred to a comprehensive stroke centre (CSC) for EVT ('drip and ship').

While the population benefit of rt-PA therapy is clear, thrombolysis is not equally effective in all ischaemic stroke patients, nor is it without significant risk. Among eligible patients treated within 4.5 hours of symptom onset, only about 35%–50% are alive and independent at 3 months poststroke,[2–8] while 2%–10% (depending on definition) develop symptomatic intracranial haemorrhage (sICH), increasing the risk of early death.[3 8] The treatment response for a given patient cannot be accurately predicted before treatment initiation, making patient selection challenging. Although some predictive factors have been identified, their prognostic value differs between patients, due to the presence of various individual and interacting factors. For example, treatment benefit is known to be time dependent, with shorter onset to treatment (OTT) times associated with higher rates of favourable outcome and reduced risk of sICH.[9–11] However, the absolute benefit of reduced OTT time varies between patients, due to differences in collateral circulation and salvageable brain volume.[12] Thus, IVT therapy in delayed time windows can benefit some patients with larger volumes of mismatch between ischaemic penumbra and core, but has minimal benefit in patients with lower volumes of salvageable brain or large established infarcts.[4 6 13]

High individual variation in rt-PA response has motivated the development of scoring algorithms to predict individual patients' thrombolysis response using baseline characteristics. For a given patient, such scores aim to predict whether potential benefit of treatment outweighs the risk, thus informing treatment decisions. Various prediction models have been proposed,[14–24] although none are routinely used in clinical care. Previously models were developed during the period 2006–2013 and included simple prognostic factors measured during routine clinical/laboratory examinations—for example, age, blood glucose, stroke severity, OTT time and blood pressure—and/or basic neuroimaging, such as early infarct or dense artery signs visualised using non-contrast CT. However, recent randomised trials[6 25–28] demonstrate the central role of advanced neuroimaging modalities such as CT perfusion (CTP), CT angiography (CTA), diffusion-weighted MRI and MRI-fluid-attenuated inversion recovery in guiding ischaemic stroke patient selection for acute stroke therapy. In broader healthcare, there is also emerging interest in precision medicine for tailoring treatment decisions for individual patients using their genetic and/or molecular profiles.

The Targeting Optimal Thrombolysis Outcomes Study was designed to develop clinical decision rules for predicting patient outcomes following rt-PA therapy, using a combination of clinical, basic radiological, advanced radiological and blood-based biomarker measures. Decision rules will be developed in a prospective cohort of Australian ischaemic stroke patients treated with rt-PA. A unique component of the study is large-scale screening for blood-based protein biomarkers for rt-PA response using mass spectrometry. This study was funded by the Australian National Health and Medical Research Council (NHMRC: APP1085550) and is part of the portfolio of studies being conducted by the International Stroke Genetics Consortium (https://strokegenetics.org/) assessing acute endophenotypes and predictors of acute ischaemic stroke outcome.

Study objectives

Primary objective

The primary objective of this study is to develop and internally and externally validate prediction models for treatment outcomes following rt-PA therapy in patients with acute ischaemic stroke, using clinical, radiological and blood-based biomarker variables.

Secondary objective

A secondary objective is to identify individual blood-based protein biomarkers associated with rt-PA treatment response, as potential candidates for diagnostic tests or adjunct therapies. An additional secondary objective is to identify clinical, radiological and biomarker predictors of treatment outcome in ischaemic stroke patients undergoing EVT, with or without prior rt-PA therapy.

METHODS AND ANALYSIS

The design, conduct and reporting of this study will adhere to the Strengthening the Reporting of Observational Studies in Epidemiology[29] and Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis[30] checklists.

BMJ Open: first published as 10.1136/bmjopen-2020-038180 on 6 April 2020. Downloaded from http://bmjopen.bmj.com/ on June 20, 2021 by guest. Protected by copyright.

Participants

Eligible patients will be ischaemic stroke patients aged ≥18 years with clinicoradiological evidence of acute brain ischemia fulfilling standard eligibility criteria for IVT and/or EVT, who present to a participating Australian CSC or Primary Stroke Centre (PSC) from January 2015 to December 2020. The cohort will include eligible patients who have multimodal imaging using either CT or MRI at the baseline evaluation (ie, including angiography and perfusion imaging), with no evidence of intracranial haemorrhage, stroke mimic or other non-stroke pathology, and who are subsequently treated with reperfusion therapies. The principal study objective is to develop prediction models in patients treated with rt-PA. However, since study inception, EVT has been proven as an effective first-line or adjunct therapy for patients with proximal large artery occlusions presenting at, or transferred to, an EVT-capable stroke centre. Eligibility criteria were thus updated to include patients treated with EVT, to facilitate additional, exploratory assessment of prognostic factors for EVT response. Patients with an absolute contraindication for reperfusion therapy, or for whom baseline or follow-up advanced CT and/or MRI is not performed, will be excluded from the study.

Patients will be asked to provide written informed consent for their clinical, imaging and blood sample data to be used for research. Consent will be requested for the use of clinical data, imaging data and blood samples collected at baseline (pretreatment) and 24–36 hours after hospital presentation. Consent will also be sought to store retrieved clots in a subset of patients undergoing EVT. To prevent treatment delay, consent will be sought after reperfusion therapy is administered. All patients will be provided standard acute stroke unit care postreperfusion therapy delivery.

Data collection procedures for predictor variables

Prediction models will include clinical, imaging and biomarker variables as described below. Candidate clinical and imaging predictors have been identified using previously published studies assessing prognostic factors for patient outcome following reperfusion therapy for acute ischaemic stroke, with an emphasis on factors identified by multiple independent studies and/or reported in systematic reviews. Candidate biomarker variables will be identified via mass spectrometry experiments designed to identify proteins differing significantly between groups of patients with low and high values of ischaemic core volume growth from baseline to 24–36 hours follow-up in the current study.

Clinical assessments

Standard clinical assessments will be performed at baseline, at the time of any neurological deterioration and 24–36 hours poststroke. Measured clinical variables will be recognised clinical predictors of stroke outcome including prestroke disability (measured using the modified Rankin Scale (mRS)[31]), age, sex, stroke severity (measured using the National Institutes of Health Stroke Scale (NIHSS) Score), blood glucose, blood pressure, concomitant antithrombotic therapy, OTT time, hospital length of stay, EQ5D5 (5-level EuroQol five dimensions questionnaire), PROMs (patient reported outcome measures) and 90-day mRS. Vascular risk factors and comorbidities such as atrial fibrillation will also be measured. Neurological deterioration will be quantified using the NIHSS. Treatment-related variables will include—as appropriate—IVT therapy type, IVT dose, EVT device type and number of passes during EVT.

Stroke mechanism will be ascertained using both the TOAST (Trial of Org 10172 in Acute Stroke Treatment) criteria[32] and the causative classification system.[33 34] Clinical data will be entered in a web-based data repository for online entry of all clinical variables required to comprehensively phenotype reperfusion therapy-treated patients.

Radiological assessments

All patients will undergo, as a minimum, baseline non-contrast CT, CTP and CTA or multimodal MRI (diffusion, T2, T1, perfusion and MR angiography), as well as postreperfusion therapy MRI or CT. The latter is to be performed 18–36 hours after reperfusion therapy, but accepting occasional logistic limitations, scans up to 5 days post-therapy will be included if earlier imaging is not possible. Patients undergoing EVT will also have a preprocedure and postprocedure angiogram recorded. This combination of imaging is routinely performed during patient work-up for reperfusion therapy in CSCs and PSCs in Australia.

We will assess the standard radiological measures of hyperdense cerebral artery sign, clot length and early infarct signs using the ASPECTS (Alberta stroke program early CT score) scoring system,[35] as well as more advanced pretreatment imaging parameters using automated CTP analysis (MISTAR, Apollo Medical Imaging, Melbourne, Australia), including volumes of ischaemic core, penumbra and severe hypoperfusion, and measures of collateral blood flow. CT occlusion will be assessed at baseline and on follow-up imaging using the thrombolysis in cerebral infarction (TICI) occlusion grading system.[36]

Images will be achieved digitally and uploaded into the secure online International Stroke Perfusion Imaging Registry (INSPIRE), which has been approved by local ethics committees in accordance with the Australian NHMRC guidelines. Images will undergo central ‘core laboratory’ analysis and adjudication of any non-automated measures will be reviewed by an expert neuroradiological panel of three members who will independently classify and characterise vessel occlusion and any degree of haemorrhagic transformation. Disagreement will be resolved by consensus.

BMJ Open: first published as 10.1136/bmjopen-2020-038180 on 6 April 2020. Downloaded from http://bmjopen.bmj.com/ on June 20, 2021 by guest. Protected by copyright.

Blood sample collection and analysis

Blood sample collection

All patients will undergo blood collection for baseline and follow-up (24–36 hours) assessment of circulating biomarkers. Blood samples will be collected in sodium citrate tubes and serum-separator tubes then centrifuged at room temperature to produce platelet free plasma and serum, which will be aliquoted and frozen at −80°C within 2.5 hours of initial sample collection.

Blood sample preparation

A single tube containing the patient's plasma sample will be thawed on ice and used for mass spectrometry analysis. Subsequently, 10 µL of the sample will be incubated with six volumes of ice-cold acetone overnight at −20°C. The tubes will be centrifuged at 1400×g for 10 min at 4°C the following day to precipitate the proteins. The supernatant will be discarded, and the protein pellet air-dried on ice to remove any residual acetone. The protein pellet will be dissolved in 100 µL of 0.1 M triethylammonium bicarbonate buffer (Sigma Aldrich, Australia). The proteins will be incubated with 0.05% sodium dodecyl sulphate and 0.005 M tris(2-carboxyethyl) phosphine hydrochloride solution (Sigma Aldrich, Australia) at 85°C (5 min) and 60°C (30 min) for sequential denaturation and reduction. The cysteine blocking agent, methyl methanethiosulfonate (Sigma Aldrich, Australia) will be added to a final concentration of 20 mM. Proteolytic digestion will be carried out with trypsin (Promega, Australia) for 16 hours at 37°C (1:50 trypsin:protein by weight). The lipids present in the tryptic peptides will be precipitated with 1% formic acid (Sigma Aldrich, Australia), followed by centrifugation at 1500×g for 5 min. The supernatant will be collected, dried under nitrogen gas and dissolved in 0.1% trifluoroacetic acid (Sigma Aldrich, Australia). The samples will then be de-salted using POROS R3 resin (Thermo Scientific). The resulting peptides will be dried, resuspended in 10 µL of 0.1% formic acid and analysed by mass spectrometry.

Liquid chromatography–mass spectrometry (LC–MS) and data acquisition

The peptide samples will be separated by nano-liquid chromatography (nLC) using the NanoElute nLC system (Bruker Daltonics, Germany) coupled online to the timsTOF Pro mass spectrometer (Bruker Daltonics, Germany). A 1–2 µL aliquot of each sample will be loaded onto a 75 µm internal diameter capillary column. Columns are prepared in-house using Reprosil-pur 120 C18-AQ material (1.9 µm beads, Dr Maisch, Germany). The peptides will be separated using Buffer A (0.1% formic acid) and Buffer B (90% acetonitrile and 0.1% formic acid) at a flow rate of 400 nL/min using a 60 min gradient. Three technical replicates of each sample will be run.

LC–MS data processing

The raw data files from each sample generated on the timsTOF Pro mass spectrometer will be processed using the software packages PEAKS X (Bioinformatics Solutions) and MaxQuant (Max Planck Institute of Biochemistry, Germany). The data will be searched against the UniProt reference proteome for Homo sapiens with the following parameters: Variable modifications—deamidation (N/Q), oxidation (M); fixed modification—methylthio (C); enzyme—trypsin; missed cleavages—2; search tolerance—25 ppm; MS/MS tolerance—0.5 Da. Only proteins with a false discovery rate of ≤1% will be reported.

LC–MS data analysis

The proteinGroups.txt output file from MaxQuant will be used for further analysis. Multivariate statistical analysis will be performed using the software packages Perseus (Max Planck Institute of Biochemistry, Germany) and MetaboAnalyst (Xia Lab at McGill University). The proteins and clusters that differ significantly between patient outcome groups will be identified and analysed. The output files from PEAKS X will be analysed for relative protein quantitative differences between patient groups.

Outcome variables

Primary outcome

The primary outcome is good functional outcome at 90 days poststroke, defined as a score of 0–2 on the mRS.[31] Scores of 0–2 define patients with either no symptoms (mRS=0), some symptoms but no significant disability (mRS=1) or slight disability but able to manage their own affairs without assistance (mRS=2). The complementary outcome group will include patients who have either moderate disability (mRS=3), moderately severe disability (mRS=4), severe disability (mRS=5) or who were dead (mRS=6) 90 days poststroke.

Secondary outcomes

Excellent functional outcome will be defined as a score of 0–1 on the mRS. Poor functional outcome, an indicator of treatment futility, will be defined as a score of 4–6 on the mRS. Recanalisation at 24 hours after stroke onset will be classified using the TICI grading system,[36 37] based on angiographic appearances of the treated occluded vessel and vessel branches. The TICI classification consists of five grades (TICI 0, 1, 2a, 2b or 3). Successful recanalisation will be defined as TICI grades 2b–3 (either complete filling of vascular territory although at a slower rate than normal or complete perfusion) and compared with TICI grades 0–2a (no perfusion, minimal perfusion or partial perfusion with less than two-thirds of the vascular territory visualised). Successful reperfusion will be measured as an 80% or greater decrease in the perfusion lesion volume from baseline to 24 hours on perfusion imaging (CT or MRI).[38]

Infarct core growth will be defined as the change in ischaemic core volume from baseline to follow-up (24–36 hours poststroke) imaging, measured using CTP or MRI. Change in NIHSS (ΔNIHSS) will be defined as the difference in NIHSS from baseline to follow-up (24 hours) assessment. In addition to the dichotomous classifications of good and poor functional outcome, the

BMJ Open: first published as 10.1136/bmjopen-2020-038180 on 6 April 2020. Downloaded from http://bmjopen.bmj.com/ on June 20, 2021 by guest. Protected by copyright.

90-day mRS will also be treated as an ordinal variable for shift analysis of functional outcome.[39]

Sample size

Predictive models will be built using multivariable logistic regression, with predictive performance assessed using the area under the receiver-operating characteristic curve (AUC), also known as the c-statistic. To ensure clinical utility, we estimated the sample size necessary to provide 80% power to detect an AUC of 0.9 that is significantly different from a null value of 0.8, for the primary outcome (90-day mRS 0–2), in the sample of patients treated with rt-PA.

Previous randomised controlled trials of rt-PA in various populations have reported response rates of 35%–53.3% for the proportion of patients achieving a good functional outcome, defined either as 90-day mRS 0–1 or 0–2.[2 4–7] A systematic review including 12 rt-PA trials reported 1611/3483 (46.3%) patients treated within 6 hour of onset achieved mRS 0–2 at final follow-up (primarily reported at 90 days).[3] Assuming a response rate of 46% for the primary outcome in our sample, a sample of 136 patients will provide 80% power to detect an AUC of 0.9 at significance 0.05.[40] A sample of 172 patients will provide 90% power assuming the same parameter values. The target size of the entire sample is 400, of which approximately 50% are anticipated to have been treated with rt-PA only. The target sample will thus provide adequate power for predictive model development for the primary outcome and population of interest.

Statistical analysis methods

To address the key objectives of the study, predictive models will be developed for primary and secondary outcomes in patients treated with rt-PA only. As exploratory analyses, predictive models will subsequently be developed for the same outcomes in patient subgroups receiving: (1) any intravenous thrombolytic (rt-PA or TNK (Tenecteplase)); (2) EVT alone or (3) IVT followed by EVT. Results from exploratory analyses will be reported separately. Data management and statistical analyses will be performed using SAS v9.4 software and Stata v15 software.

Model derivation and validation

Due to the modest sample size, a variable selection and shrinkage approach will be used to select predictor variables for inclusion in multivariable models. This helps to develop predictive models that are parsimonious while retaining the most important prognostic variables.[41 42] Models will be simplified using the stepdown procedure described by Ambler *et al*.[41] When some predictors are only weakly associated with the outcome, this method typically produces simplified models with similar or better prognostic performance than the full model, while retaining good operating characteristics. Model selection will be performed using Akaike's information criterion and by assessing model discrimination and calibration. Continuous predictors will be retained in continuous form to maximise power,[43] with fractional polynomials used to model non-linear relationships.[44] Clinically relevant interactions (eg, involving OTT time and neuroimaging variables) will also be tested for inclusion.

The primary estimation model will be a generalised linear model with a binomial response distribution and logit link (logistic regression), with favourable clinical outcome (90-day mRS 0–2) as the response variable. Model calibration will be assessed by comparing observed and expected events by deciles of predicted probability.[45] Test error will be estimated using 10-fold cross validation. For secondary outcomes, models will be estimated using generalised linear models with a response distribution and link function as appropriate for the specified binary, continuous and ordered categorical responses. For all outcomes, model assumptions will be checked using standard diagnostic measures and plots, including plots of residuals and fitted values. The impact of influential observations or collinearity on parameter estimates will be assessed, to increase external validity of reported models.

External validation of clinical and radiological predictors will be performed using the INSPIRE.[46] External validation of identified blood biomarkers will be undertaken in collaboration with colleagues from the International Stroke Genetics Consortium (https://strokegenetics.org/).

Missing data

Complete case analyses will be performed under a missing completely at random assumption. To supplement complete case analyses, missing data will be multiply imputed under a missing at random assumption to assess robustness of results to different presumed missing data mechanisms. Imputation models will include variables included in predictive models and auxiliary variables associated either with observed values of outcome and predictor variables, or the missing data mechanism. Parameter estimates obtained using multiple imputed data will be combined using Rubin's rules.[47]

Model performance

For the primary estimation model, the key measure of predictive performance will be the c-statistic. For a logistic regression model, the c-statistic (equivalent to the area under the receiver operator characteristic curve) is the probability that a randomly selected patient who experienced the event of interest had a higher predicted probability than a randomly selected patient who did not experience the event. Bootstrap resampling will be used to estimate the c-statistic and its 95% CI, to adjust for optimism and internally validate the model. Additional measures of predictive performance to be reported as appropriate for primary and secondary outcomes will include R^2, pseudo R^2[48] and Brier Score.[49]

BMJ Open: first published as 10.1136/bmjopen-2020-038180 on 6 April 2020. Downloaded from http://bmjopen.bmj.com/ on June 20, 2021 by guest. Protected by copyright.

Patient and public involvement

Patients and the public will not be involved in this research, since there were no time or funds allocated for such involvement.

Ethics and dissemination

Patients, or their relatives when patients could not consent, provided written informed consent to participate in the study. This study received approval from the Hunter New England Local Health District Human Research Ethics Committee (reference 14/10/15/4.02). The INSPIRE was approved by the Hunter New England Local Health District Human Research Ethics Committee. Data management will be performed by TL and EH. All acquired data will be de-identified, stored electronically and password protected. Data quality checks will be performed by TL, AB and EH. The final dataset will be curated by EH and access will be at the discretion of study investigators.

Author affiliations
[1]School of Medicine and Public Health, The University of Newcastle, Callaghan, New South Wales, Australia
[2]Hunter Medical Research Institute, The University of Newcastle, New Lambton, New South Wales, Australia
[3]Department of Neurology, Royal Adelaide Hospital, Adelaide, South Australia, Australia
[4]The University of Adelaide, Adelaide, South Australia, Australia
[5]Department of Neurosciences, Eastern Health, Melbourne, Victoria, Australia
[6]Eastern Health Clinical School, Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne, Victoria, Australia
[7]University of Technology Sydney, Sydney, New South Wales, Australia
[8]Melbourne Brain Centre, Parkville, Victoria, Australia
[9]School of Biomedical Sciences and Pharmacy, The University of Newcastle, Callaghan, New South Wales, Australia
[10]Haematology Department, Calvary Mater Newcastle, Waratah, New South Wales, Australia
[11]Adelaide Medical School, The University of Adelaide, Adelaide, South Australia, Australia
[12]Central Adelaide Local Health Network, Adelaide, South Australia, Australia
[13]South Australian Health and Medical Research Institute, Adelaide, South Australia, Australia
[14]Department of Neurology, Royal Melbourne Hospital, Parkville, Victoria, Australia
[15]Department of Neurology, University of Virginia, Charlottesville, Virginia, USA
[16]Department of Public Health Sciences, University of Virginia, Charlottesville, Virginia, USA
[17]John Hunter Hospital, New Lambton Heights, New South Wales, Australia
[18]The Sydney Partnership for Health, Education, Research & Enterprise (SPHERE), Sydney, New South Wales, Australia
[19]School of Medicine and Public Health (SMPH), University of Newcastle (UoN), Callaghan, New South Wales, Australia

Contributors EH, CL, JA, JM, SK, LL and TK secured funding for the study. All authors contributed intellectually to the study design and research methodology. TK, PMCC, MWP, SK and CL provided clinical leadership and supervision of patient recruitment. JM, TL, LL and AB designed and contributed to the administration of clinical assessments. AB, MWP and CL designed the International Stroke Perfusion Imaging Registry and contributed to the administration of imaging data assessment. MFS, PJT and SRR designed the proteomics assessments. EH conducted the power calculation, guided the statistical analysis plan and drafted the manuscript. All authors critically reviewed the manuscript.

Funding This study was supported the Australian National Health and Medical Research Council (NHMRC) grant number APP1085550.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; peer reviewed for ethical and funding approval prior to submission.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/.

ORCID iDs
Elizabeth Holliday http://orcid.org/0000-0002-4066-6224
Jane Maguire http://orcid.org/0000-0001-5722-8311
Lisa F Lincz http://orcid.org/0000-0002-1612-2382
Monica Anne Hamilton-Bruce http://orcid.org/0000-0002-5222-620X
Sushma R Rao http://orcid.org/0000-0002-1773-9747
Marten F Snel http://orcid.org/0000-0002-8502-7274
Simon Koblar http://orcid.org/0000-0002-8667-203X
John Attia http://orcid.org/0000-0001-9800-1308

REFERENCES

1 National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med* 1995;333:1581–8.
2 Hacke W, Kaste M, Bluhmki E, *et al*. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med* 2008;359:1317–29.
3 Wardlaw JM, Murray V, Berge E, *et al*. Recombinant tissue plasminogen activator for acute ischaemic stroke: an updated systematic review and meta-analysis. *Lancet* 2012;379:2364–72.
4 Thomalla G, Simonsen CZ, Boutitie F, *et al*. MRI-guided thrombolysis for stroke with unknown time of onset. *N Engl J Med* 2018;379:611–22.
5 Davis SM, Donnan GA, Parsons MW, *et al*. Effects of alteplase beyond 3 h after stroke in the Echoplanar imaging thrombolytic evaluation trial (EPITHET): a placebo-controlled randomised trial. *Lancet Neurol* 2008;7:299–309.
6 Ma H, Campbell BCV, Parsons MW, *et al*. Thrombolysis guided by perfusion imaging up to 9 hours after onset of stroke. *N Engl J Med* 2019;380:1795–803.
7 Ringleb P, Bendszus M, Bluhmki E, *et al*. Extending the time window for intravenous thrombolysis in acute ischemic stroke using magnetic resonance imaging-based patient selection. *Int J Stroke* 2019;14:483–90.
8 Emberson J, Lees KR, Lyden P, *et al*. Effect of treatment delay, age, and stroke severity on the effects of intravenous thrombolysis with alteplase for acute ischaemic stroke: a meta-analysis of individual patient data from randomised trials. *Lancet* 2014;384:1929–35.
9 Fonarow GC, Zhao X, Smith EE, *et al*. Door-to-needle times for tissue plasminogen activator administration and clinical outcomes in acute ischemic stroke before and after a quality improvement initiative. *JAMA* 2014;311:1632–40.
10 Saver JL, Fonarow GC, Smith EE, *et al*. Time to treatment with intravenous tissue plasminogen activator and outcome from acute ischemic stroke. *JAMA* 2013;309:2480–8.
11 Jahan R, Saver JL, Schwamm LH, *et al*. Association between time to treatment with endovascular reperfusion therapy and outcomes in patients with acute ischemic stroke treated in clinical practice. *JAMA* 2019;322:252–63.
12 Leng X, Lan L, Liu L, *et al*. Good collateral circulation predicts favorable outcomes in intravenous thrombolysis: a systematic review and meta-analysis. *Eur J Neurol* 2016;23:1738–49.
13 Campbell BCV, Ma H, Ringleb PA, *et al*. Extending thrombolysis to 4·5-9 h and wake-up stroke using perfusion imaging: a systematic review and meta-analysis of individual patient data. *Lancet* 2019;394:139–47.
14 Charidimou A, Turc G, Oppenheim C, *et al*. Microbleeds, cerebral hemorrhage, and functional outcome after stroke thrombolysis. *Stroke* 2017;48:2084–90.
15 Cucchiara B, Tanne D, Levine SR, *et al*. A risk score to predict intracranial hemorrhage after recombinant tissue plasminogen activator for acute ischemic stroke. *J Stroke Cerebrovasc Dis* 2008;17:331–3.

BMJ Open: first published as 10.1136/bmjopen-2020-038180 on 6 April 2020. Downloaded from http://bmjopen.bmj.com/ on June 20, 2021 by guest. Protected by copyright.

16 Kamel H, Patel N, Rao VA, *et al*. The totaled health risks in vascular events (THRIVE) score predicts ischemic stroke outcomes independent of thrombolytic therapy in the NINDS tPA trial. *J Stroke Cerebrovasc Dis* 2013;22:1111–6.
17 Kent DM, Ruthazer R, Decker C, *et al*. Development and validation of a simplified Stroke-Thrombolytic predictive instrument. *Neurology* 2015;85:942–9.
18 Kent DM, Selker HP, Ruthazer R, *et al*. The stroke-thrombolytic predictive instrument: a predictive instrument for intravenous thrombolysis in acute ischemic stroke. *Stroke* 2006;37:2957–62.
19 Lou M, Safdar A, Mehdiratta M, *et al*. The HAT score: a simple grading scale for predicting hemorrhage after thrombolysis. *Neurology* 2008;71:1417–23.
20 Mazya M, Egido JA, Ford GA, *et al*. Predicting the risk of symptomatic intracerebral hemorrhage in ischemic stroke treated with intravenous alteplase: safe implementation of treatments in stroke (SITS) symptomatic intracerebral hemorrhage risk score. *Stroke* 2012;43:1524–31.
21 Menon BK, Saver JL, Prabhakaran S, *et al*. Risk score for intracranial hemorrhage in patients with acute ischemic stroke treated with intravenous tissue-type plasminogen activator. *Stroke* 2012;43:2293–9.
22 Saposnik G, Guzik AK, Reeves M, *et al*. Stroke prognostication using age and NIH stroke scale: SPAN-100. *Neurology* 2013;80:21–8.
23 Strbian D, Engelter S, Michel P, *et al*. Symptomatic intracranial hemorrhage after stroke thrombolysis: the SEDAN score. *Ann Neurol* 2012;71:634–41.
24 Strbian D, Meretoja A, Ahlhelm FJ, *et al*. Predicting outcome of IV thrombolysis-treated ischemic stroke patients: the dragon score. *Neurology* 2012;78:427–32.
25 Bracard S, Ducrocq X, Mas JL, *et al*. Mechanical thrombectomy after intravenous alteplase versus alteplase alone after stroke (THRACE): a randomised controlled trial. *Lancet Neurol* 2016;15:1138–47.
26 Campbell BCV, Mitchell PJ, Kleinig TJ, *et al*. Endovascular therapy for ischemic stroke with perfusion-imaging selection. *N Engl J Med* 2015;372:1009–18.
27 Goyal M, Demchuk AM, Menon BK, *et al*. Randomized assessment of rapid endovascular treatment of ischemic stroke. *N Engl J Med* 2015;372:1019–30.
28 Saver JL, Goyal M, Bonafe A, *et al*. Stent-retriever thrombectomy after intravenous t-PA vs. t-PA alone in stroke. *N Engl J Med* 2015;372:2285–95.
29 von Elm E, Altman DG, Egger M, *et al*. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet* 2007;370:1453–7.
30 Collins GS, Reitsma JB, Altman DG, *et al*. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): the TRIPOD statement. *BMJ* 2015;350:g7594.
31 Bonita R, Beaglehole R. Recovery of motor function after stroke. *Stroke* 1988;19:1497–500.
32 Adams HP, Bendixen BH, Kappelle LJ, *et al*. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in acute stroke treatment. *Stroke* 1993;24:35–41.
33 Ay H, Furie KL, Singhal A, *et al*. An evidence-based causative classification system for acute ischemic stroke. *Ann Neurol* 2005;58:688–97.
34 Ay H, Benner T, Arsava EM, *et al*. A computerized algorithm for etiologic classification of ischemic stroke: the causative classification of stroke system. *Stroke* 2007;38:2979–84.
35 Pexman JH, Barber PA, Hill MD, *et al*. Use of the Alberta stroke program early CT score (ASPECTS) for assessing CT scans in patients with acute stroke. *AJNR Am J Neuroradiol* 2001;22:1534–42.
36 Higashida RT, Furlan AJ, Roberts H, *et al*. Trial design and reporting standards for intra-arterial cerebral thrombolysis for acute ischemic stroke. *Stroke* 2003;34:e109–37.
37 Zaidat OO, Yoo AJ, Khatri P, *et al*. Recommendations on angiographic revascularization grading standards for acute ischemic stroke: a consensus statement. *Stroke* 2013;44:2650–63.
38 Lin L, Chen C, Tian H, *et al*. Perfusion computed tomography accurately quantifies collateral flow after acute ischemic stroke. *Stroke* 2020;51:1006–9.
39 Savitz SI, Lew R, Bluhmki E, *et al*. Shift analysis versus dichotomization of the modified Rankin scale outcome scores in the NINDS and ECASS-II trials. *Stroke* 2007;38:3205–12.
40 Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology* 1982;143:29–36.
41 Ambler G, Brady AR, Royston P. Simplifying a prognostic model: a simulation study based on clinical data. *Stat Med* 2002;21:3803–22.
42 Hastie T, Tibshirani R, Friedman J. *The elements of statistical learning. Springer series in statistics*. New York: Springer Science, Business Media, 2009.
43 Harrell FE. *Regression modeling strategies*. 2nd edn. Switzerland: Springer International Publishing, 2015.
44 Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. *Int J Epidemiol* 1999;28:964–74.
45 Hosmer DW, Lemesbow S. Goodness of fit tests for the multiple logistic regression model. *Commun Stat Theory Methods* 1980;9:1043–69.
46 Bivard A, Levi C, Lin L, *et al*. Validating a predictive model of acute advanced imaging biomarkers in ischemic stroke. *Stroke* 2017;48:645–50.
47 Rubin DB. *Multiple imputation for non-response in surveys*. New York: John Wiley, 1987.
48 Mittlböck M, Schemper M. Explained variation for logistic regression. *Stat Med* 1996;15:1987–97.
49 Brier GW. Verification of forecasts expressed in terms of probability. *Mon Weather Rev* 1950;78:1–3.

BMJ Open: first published as 10.1136/bmjopen-2020-038180 on 6 April 2020. Downloaded from http://bmjopen.bmj.com/ on June 20, 2021 by guest. Protected by copyright.