INDIVIDUAL PATIENT PROFILING USING CLINICAL AND NEURORADIOLOGICAL BIOMARKERS IN ACUTE ISCHEMIC STROKE: APPLICATION OF ADVANCED MULTIMODAL NEUROIMAGING

SONU BHASKAR
Mismatch showing salvageable tissue (penumbra) and dead tissue (core) in an acute ischemic stroke patient.
INDIVIDUAL PATIENT PROFILING USING CLINICAL AND NEURORADIOLOGICAL BIOMARKERS IN ACUTE ISCHEMIC STROKE: APPLICATION OF ADVANCED MULTIMODAL NEUROIMAGING

SONU BHASKAR

Department of Neurology, John Hunter Hospital
Priority Research Centre for Translational Neuroscience and Mental Health
Hunter Medical Research Institute (HMRI), Hunter Stroke Services
School of Medicine & Public Health, and School of Health Sciences
Faculty of Health & Medicine
University of Newcastle

A Thesis by Publication Submitted for the Degree of

Doctor of Philosophy (Medicine)

School of Medicine & Public Health
School of Health Sciences
Faculty of Health and Medicine
University of Newcastle, Australia
SUPERVISORS

A/PROF PETER STANWELL, PHD
Associate Professor
School of Health Sciences
Faculty of Health and Medicine
Convenor Imaging Section, Priority Research Centre for Translational Neuroscience & Mental Health, Hunter Medical Research Institute (HMRI), University of Newcastle, Callaghan, NSW, Australia

PROF CHRISTOPHER LEVI, MD
Senior Staff Neurologist, Comprehensive Stroke Centre, Department of Neurology, John Hunter Hospital
Director of Clinical Research and Translation, Hunter New England Local Health District, New South Wales (NSW)
Conjoint Professor of Medicine (Neurology), School of Public Health & Medicine, University of Newcastle
Practitioner Fellow, National Health & Medical Research Council (NHMRC), Australia

PROF MICHAEL NILSSON, MD, PHD
Director, Hunter Medical Research Institute (HMRI)
Professor, School of Medicine & Public Health, Faculty of Health and Medicine, University of Newcastle, Australia
Burges Professor of Medical Science, Department of Clinical Neuroscience, Institute of Neuroscience and Physiology, Sahlgrenska University Hospital, University of Gothenburg, Sweden

PROF JOHN ATTIA, MD, PHD, FRCPC, FRACP
Professor of Medicine and Clinical Epidemiology, University of Newcastle
Director, Clinical Research Design, IT, and Statistical Support Unit, Hunter Medical Research Institute
Academic Director, Department of General Medicine, John Hunter Hospital

DR ANDREW BIVARD, PHD
Hunter Medical Research Institute (HMRI), University of Newcastle
Kookaburra Circuit, New Lambton Heights, Australia
STROKE – NO POSTCODE UNTouched IN AUSTRALIA*

*Based on the 2014 report of Deloitte Access Economics, commissioned by National Stroke Foundation, to estimate the impact of stroke across Australia.
DECLARATIONS

Statement of Originality
I, Sonu Bhaskar, hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying subject to the provisions of the Copyright Act 1968.

Statement on Thesis by Publication
I, Sonu Bhaskar, hereby certify that this thesis is submitted in the form of a series of published papers of which I am a joint author. I have included as part of the thesis a written statement from each co-author and endorsed by the Faculty Assistance Dean (Research Training), attesting to my contribution to the joint publications.

Statement of Collaboration
I, Sonu Bhaskar, hereby certify that the work, embodied in this thesis, have been done in collaboration with other researchers. I have included as part of the thesis a statement clearly outlining the extent of collaboration, with whom and under what auspices.

Signed

Date
ACKNOWLEDGEMENTS

“Attention is the rarest and purest form of generosity.”
- Simone Weil

First, and foremost, I would like to start by expressing my gratitude towards my clinical and academic supervisors A/Professor Peter Stanwell, Professor Christopher (Chris) Levi, Professor Michael Nilsson, and Dr Andrew Bivard. I am also grateful to Professor John Attia for his generous guidance and feedback on research methodology and statistical analyses. Thank you, John, for your advice and support. I would like to acknowledge Professor Mark Parsons for his overall guidance gratefully. Special thanks to A/Professor Neil Spratt for his critical feedback, valuable edits to my “confirmation thesis”. I could not have asked for better scientific mentors at this early stage of my career.

Chris, I have been incredibly lucky to have a supervisor like you, and I sincerely want to express my utmost gratitude for everything you’ve done for me – for giving me the opportunity to work with you, for believing in me, and for making me realise my potential. Your breadth and depth of clinical knowledge about stroke and neurology, in general, have been inspirational and invaluable. Thank you for being a great mentor, guide and a teacher; and for inspiring me to pursue this doctoral project (at a time when going was tough!). The best part of Chris has been his attention to details, inexhaustible patience, and generosity. Every time I have had an opportunity to meet him, I was assured that he is there to look after me. Thank you, Chris, for all that you have done. I am not sure this section would do justice to the enormous gratitude I owe to you for your continued support. I will always remember our chats regarding new ideas and coming up with so many that could change the world but quickly having to get back on track and focus on the achievable goals for the short-term. I hope I can come up with as many excellent ideas throughout my career. You have been a role model to me - one who I look up to with reverence, and shall always, for your humility, kindness, and awesomeness in every way. I hope, in you, I have a mentor and friend for life. Gracias!

Peter, thank you for being an excellent mentor. Your passion for radiology/neuroimaging is infectious. I could not have succeeded without your care and patience in guiding me through the radiological analyses. I have many fond memories of the catch-ups over coffee at cafeterias located at the Bar on the Hill, and the Hunter Building. Thank you for agreeing to meet me at short notices, for your critical feedback, and discussions on the manuscripts under revision. Your humility, forthrightness and grace have been truly inspirational that I would like to imbibe within me for the rest of my life. Thank you for just being there for me and helping me take my mind off research when I needed to. Beyond research, you have also been a great friend who taught me how to strike a much-needed work and family-life balance.
I owe special thanks to Professor Robert (Bob) Callister, the Deputy Head of the Faculty of Health & Medicine and Assistant Dean Research Training, for his support during the initial stages of my study, and to Shirley Savy, Alli Johns, Kristy Brooks, and Annalese S. Johnson for their continued administrative support and encouragement. Thank you to the Hunter Stroke team at the Lodge, and at the Department of Neurology, John Hunter Hospital. Clinical research is a collaborative endeavour, and I have been fortunate enough to collaborate with all of you on a daily basis. In particular, I would like to thank Louise-Anne Jordan, Malcolm Evans, Christine Selmes, Erin Kerr, Kristy Morris, Nicole Starkie, Rhonda Walker, Heidi Janssen, Shelagh Garfield, and Gemma Kitsos who I have had the privilege of working closely with. Louise has been supportive, both as a colleague and as a friend. She has been a kind mentor, and I would like to express my gratitude for her cooperation and enterprising spirit during my involvement in the clinical trials. Also, thanks to staff members of Clinical Governance Unit who were extraordinarily welcoming while I had my office at the Lodge. Thank you, Louise & Allan Evans, for being a great support all this while, for those delicious cupcakes, and for your generosity in introducing me to some of the finest restaurants, and tourist spots in Newcastle. You both are undoubtedly great ambassadors of what Newcastle stands for.

Big thanks go to my family, parents in particular, without whom I will not be here where I am placed today. Mum and Dad, I am forever indebted for your selfless love, tenacity, unflinching support, and faith in me. You both have been a pivotal anchor to my life. I would also like to thank my other-half for her continued love, motivation, and patience. My five-year-old daughter has been a blessing all this while. I love you so much, my angel, and I promise to spend more time with you in future. I could not have embarked on an endeavour like this without their support. They have kept me going despite the odds of being far away from them. At my heart, they are with me always. Last, but not the least, I would like to remember my late grandfather who used to enthuse me to pursue medicine and delve into clinical research. He continues to be my greatest inspiration. On the closing note, thank you G-d for your blessings to me, and my family; for the strength, you bestow every passing day and for all the beautiful people and surprises that you bring our way! Baruch Hashem.

Thank you!

Sonu Bhaskar
25/01/2017
Newcastle, Australia
“Every man, if he so desires, becomes the sculptor of his own brain.”

- Santiago Ramón y Cajal, Noble Prize in Medicine, 1906
The photo of the sculpture, located in front of the Hunter Building at the University of Newcastle (Callaghan), depicts time-compass with an engraved adage “use time to make better world” (top), and an equation of time (bottom).

Dated 8th of August 2016.
PEER REVIEWED PUBLICATIONS INCLUDED IN THIS THESIS

PUBLICATION I:


PUBLICATION II:


PUBLICATION III:


PUBLICATION IV:

CONFERENCE PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS THESIS


VI. **Bhaskar S**. Frontiers in Stroke Imaging: Applications to Diagnostics, Thrombolysis and Treatment. *Department of Radiology, Kaunas University Hospital, Kaunas (Lithuania)*; January 15, 2015; (Oral).

VII. **Bhaskar S**. Neuroimaging in Stroke: Clinical and Neuroradiological Perspectives. *Clinical Grand Rounds, Manning Base Hospital, Taree (Australia)*; October 16th, 2014; (Oral).
AWARDS, SCHOLARSHIPS AND GRANTS (2013-16)

I. Grant in Aids (4000 AUD) award (2016-17), Faculty of Health & Medicine, University of Newcastle.

II. Nominated for “Young Investigator Awards oral paper category” at Brain Ischemia Conference, Rome, Italy (2014).

III. Hunter Medical Research Institute (HMRI) & Centre for Translational Neuroscience & Mental Health (CTNMH) Top-Up Scholarship (15000 AUD).

IV. Doctoral Scholarship, University of Newcastle (2013-2016).

V. Travel Grant (1200 AUD), Hunter New England (HNE) Health, Newcastle.

VI. HMRI small project infrastructure grant (5000 AUD).

EXTRA-CURRICULAR

1. Clinical research associate for TnT and T3 stroke clinical trials (conjointly with Australian Catholic University, John Hunter Hospital, HMRI, and University of Newcastle).

2. Conjoint Lecturer, University of Newcastle.

3. Leadership role as iLEAD Executive for the Faculty of Health & Medicine, University of Newcastle. Organised seminars and talks at the faculty including one guest lecture by the Sydney nodal officer of Médecins Sans Frontières (Doctors without Borders) to encourage medical volunteering initiatives among young doctors.

4. Certificate course in ‘Concept Development Workshop for Trials and Translational Research Studies’ held on 31st October 2014 at the Clinical Trials Centre, the University of Sydney.


“I slept and dreamt that life was joy. I awoke and saw that life was service. I acted and behold, service was joy.”

- Rabindranath Tagore, Noble Prize in Literature, 1913
PREFACE

The author, Dr Sonu Bhaskar, was working as a doctoral researcher/clinical research fellow jointly at Department of Neurology, John Hunter Hospital, and Schools of Public Health & Medicine, and School of Health Sciences, at the Faculty of Health & Medicine, the University of Newcastle during doctoral research. The topic of the doctoral research was “Individual patient profiling using clinical and neuroradiological biomarkers in acute ischemic stroke: application of advanced multimodal neuroimaging”. The scientific research on this thesis was undertaken at the Department of Neurology and Hunter Medical Research Institute (HMRI), John Hunter Hospital from 2013 to 2016 during the author’s engagement as a clinical research associate/fellow at the Department of Neurology, John Hunter Hospital.

A/Prof Peter Stanwell, Prof Christopher Levi, Prof Michael Nilsson, and Dr Andrew Bivard at the Department of Neurology (John Hunter Hospital), Stroke Research Team (HMRI), School of Public Health & Medicine, and School of Health Sciences (University of Newcastle) supervised the project. This project has received funding from the University of Newcastle, HMRI and Hunter New England (HNE) Health.

A/Prof Peter Stanwell
Signed
17.02.2017
Date

Prof Christopher Levi
Signed
13.02.2017
Date
“God is the light shining in the midst of darkness, not to deny that there is darkness in the world but to reassure us that we do not have to be afraid of the darkness because darkness will always yield to light. As theologian David Griffin puts in, God is all-powerful, His power enables people to deal with events beyond their control, and He gives us the strength to do those things because He is with us.”

Harold S. Kushner
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<td>ADC</td>
<td>Apparent diffusion coefficient</td>
</tr>
<tr>
<td>AIS</td>
<td>Acute Ischemic Stroke</td>
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<tr>
<td>ASL</td>
<td>Arterial spin labelling</td>
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<tr>
<td>ASITN/SIR</td>
<td>American Society of Interventional and Therapeutic Neuroradiology/Society of Interventional Radiology</td>
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<td>ASPECTS</td>
<td>Alberta Stroke Program Early Computed Tomography Score</td>
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<td>ATLANTIS</td>
<td>The Alteplase ThromboLysis for Acute Noninterventional Therapy in Ischemic Stroke</td>
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<td>BAO</td>
<td>Basilar artery occlusion</td>
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<td>BASICS</td>
<td>Basilar Artery International Cooperation Study</td>
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<td>BASIS</td>
<td>Boston Acute Stroke Imaging Scale</td>
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<tr>
<td>BI</td>
<td>Barthel Index</td>
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<td>BOLD</td>
<td>Blood-oxygen-level dependent</td>
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<td>CASES</td>
<td>Canadian Alteplase for Stroke Effectiveness Study</td>
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<tr>
<td>CBF</td>
<td>Cerebral blood flow</td>
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<td>CBV</td>
<td>Cerebral blood volume</td>
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<tr>
<td>CC</td>
<td>Correlation coefficient</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<tr>
<td>CTA</td>
<td>Computed tomography angiography</td>
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<td>CTA-SI</td>
<td>Computed tomography angiographic source image</td>
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<td>CTP</td>
<td>Computer tomography perfusion</td>
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<td>CCT</td>
<td>Cranial computed tomography</td>
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<td>DSC–PWI</td>
<td>Dynamic susceptibility contrast-enhanced perfusion-weighted imaging</td>
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<td>DWI</td>
<td>Diffusion-weighted imaging</td>
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<td>ECASS</td>
<td>European Cooperative Acute Stroke Study</td>
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<td>ECG</td>
<td>Electrocardiogram</td>
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<td>EPI</td>
<td>Echo planar imaging</td>
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<td>FA</td>
<td>Flip angle</td>
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<td>FLAIR</td>
<td>Fluid-attenuated inversion recovery</td>
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<td>FWHM</td>
<td>Full width at half maximal</td>
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<tr>
<td>GOLIATH</td>
<td>General or Local Anaesthesia in Intra-arterial Therapy</td>
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<tr>
<td>IPC</td>
<td>Inferior parietal cortex</td>
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<td>ICA</td>
<td>Internal carotid artery</td>
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ICH  Intracerebral haemorrhage
IST-3  Third international stroke trial
IQR  Interquartile range
IV/IA  Intravenous/Intra-arterial
LFF  Low-frequency fluctuations
LVO  Large vessel occlusion
M1/M2  Proximal portion/insular portion of the MCA
MCA  Middle cerebral artery
MCI  Mild cognitive impairment
MNI  Montreal Neurological Institute
MOST  Multi-Arm Optimisation of Stroke Thrombolysis
MPC  Maximal peak concentration
MPFC  Medial prefrontal cortex
MRA  Magnetic resonance angiography
MRA-SI  Magnetic resonance angiography-source imaging
MR CLEAN  Multicenter Randomised Clinical Trial of Endovascular Treatment for Acute Ischemic Stroke in the Netherlands
MR  Recanalization of Stroke Clots Using Embolectomy
RESCUE  Magnetic resonance imaging
mRS  Modified Rankin score
MT  Mechanical thrombectomy
mTICI  Modified Thrombolysis in Cerebral Infarction
MTT  Mean transit time
NCCT  Non-contrast computed tomography
NIHSS  National Institutes of Health Stroke Scale
NINDS  National Institute of Neurological Disorders and Stroke
NNT  Number needed to treat
PCA  Posterior cerebral artery
PCC  Posterior cingulate cortex
PET  Positron emission tomography
PROACT  Prolyse in Acute Cerebral Thromboembolism
PWI  Perfusion-weighted imaging
RACE  Rapid Arterial occlusion Evaluation
REVASCAT  Randomised Trial of Revascularization with Solitaire FR Device Versus Best Medical Therapy in the Treatment of Acute Stroke Due to Anterior Circulation LVO Presenting within Eight Hours of Symptom Onset
ROC  Receiver-operating characteristics
ROI  Region-of-interest
ROSIE  ReoPro Retavase Reperfusion of Stroke Safety Study
rtPA  Recombinant Tissue Plasminogen Activator
SAH  Subarachnoid Haemorrhage
SI  Source imaging
sICH  Symptomatic intracerebral haemorrhage
SIESTA  Sedation vs. Intubation for Endovascular Stroke Treatment
SITS  Safe Implementation of Thrombolysis in Stroke
SK  Streptokinase
SNR  Signal-to-noise ratio
SOFIA  Soft Torqueable Catheter Optimized For Intracranial Access
<table>
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>SPECT</td>
<td>Single-photon emission computed tomography</td>
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<tr>
<td>SPM</td>
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<td>THRACE</td>
<td>Mechanical thrombectomy after intravenous alteplase versus alteplase alone after stroke</td>
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<td>TIA</td>
<td>Transient ischemic attack</td>
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<td>TICA</td>
<td>Terminal internal carotid artery</td>
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<tr>
<td>TIMI</td>
<td>Thrombolysis in Myocardial Infarction</td>
</tr>
<tr>
<td>TR</td>
<td>Repetition time</td>
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<tr>
<td>TSA</td>
<td>Time-shift analysis</td>
</tr>
<tr>
<td>TTP</td>
<td>Time to peak</td>
</tr>
<tr>
<td>UK</td>
<td>Urokinase</td>
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SYNOPSIS

THESIS TITLE: INDIVIDUAL PATIENT PROFILING USING CLINICAL AND NEURORADIOLOGICAL BIOMARKERS IN ACUTE ISCHEMIC STROKE: APPLICATION OF ADVANCED MULTIMODAL NEUROIMAGING

ABSTRACT

The aim of the thesis is to study clinical and neuroradiological biomarkers towards individual patient profiling in acute ischaemic stroke (AIS). There is existing evidence that individual patient profiling using a combination of initial stroke severity, infarct topography and surrogate neuroimaging markers provide clinicians with prognostically relevant information that can guide the selection of patients for acute interventions and/or planning and conduct of rehabilitation. Perilesional brain perfusion neuroimaging and cortical venous drain angiography may be additional measures of prognostic relevance. Combinations of these prognostic variables could potentially allow the development of more sophisticated approaches to selection of post-stroke patients for recovery assistance interventions and AIS patients for thrombolytic and/or endovascular treatments. The overall goal of the thesis is to examine specific subgroups of AIS patients and assess functional outcome, where subgroups are defined by, (a) clinically in terms of stroke severity profile (measured using National Institute of Health Stroke Severity (NIHSS) scale), and (b) neuroradiologically depending upon: lesion topography, presence or absence of peri-lesional hyperperfusion, and presence or absence of delayed late venous phase cortical vein filling (delayed-LCVF). Advanced neuroimaging such as arterial spin labelling (ASL) magnetic resonance imaging (MRI) and dynamic computed tomography angiography (dCTA) can be effectively used to discern surrogate imaging biomarkers, investigate underlying post-ischemic pathophysiological mechanisms, identify stroke subtype, and predict the clinical course of AIS patients receiving reperfusion therapy.
“Gam zu l'tova. This too is for the good.”

- Nachum Ish Gamzu, Talmud, Taanit, 21a
OVERVIEW OF THE THESIS

Intravenous thrombolysis, in conjunction with the endovascular intra-arterial thrombolysis, is the only approved treatment for acute ischemic stroke (AIS). The advent of thrombolysis has revolutionised how a subgroup of stroke patients is managed in clinical settings and are evaluated using neuroimaging techniques; so that the effective treatment can be tailored to achieve maximum clinical benefit in the interest of the patients. The identification of precise stroke-subtype using advanced multimodal neuroimaging can guide clinicians to choose and prescribe optimal therapy to the stroke patients. The patient selection or individual profiling for thrombolytic trials can be improved by applying a combined approach where both clinical and imaging metrics could be factored in the prognostic models. Furthermore, the PhD thesis will also enhance our knowledge on the use of clinical (baseline stroke severity), and advanced neuroimaging (arterial spin labelling (ASL)-magnetic resonance imaging (MRI) and computed tomographic angiography (CTA) in particular) prognostic markers in selecting patients who will benefit from the thrombolytic therapy even beyond the therapeutic time-window. We believe that the findings of our study may be incorporated in future thrombolytic trials so that efficient selection of a group of patients will provide synergistic treatment benefits to those who will receive the therapy and those who are currently disqualified due to the time-window restrictions.

Outline of the PhD thesis

The goal of the thesis is to pursue an individual patient profiling based approach towards diagnosis, treatment and recovery of stroke patients. The underlying mechanism of recovery following stroke remains intriguing and poses a challenge for clinicians interested in vascular neurology. Clinically, depending on the location/site of lesion, stroke comes as an interesting and challenging construct owing to its specific pathogenesis, distinct neuropsychological features and reasonable prognosis. To study the underlying mechanisms of stroke recovery, selection of patients based on aetiology, imaging parameters is essential for the efficient and comprehensive design of the clinical trials to assess the efficacy of drugs/intervention in stroke. In the first part of the thesis, we will give an overview of stroke epidemiology, pathophysiology and role of neuroimaging in diagnosis, patient selection for thrombolytic trials, and
follow-up treatment (Chapter 1), followed by the review on thrombolysis and patient profiling based approach in AIS (Chapter 2). Subsequently, in the following chapter (Chapter 3) [1], we investigated putative (and degree of) impact of stroke severity in predicting the overall functional outcome, in-hospital placement and death at 90 days, in comparison with age, admission to the stroke unit and thrombolytic treatment. Prediction models for short-term and long-term mortality are important in determining a prognostic prescription for the patients.

In chapter 4, the recovery profiles of ischemic stroke patients with middle cerebral artery occlusion using the topographical characterization of ischemic lesions and neuroradiological study of peri-lesional hyperperfusion on ASL-MRI were investigated [2]. The analysis included a review of CT (non-contrast CT (NCCT)/ perfusion computed tomography (CTP)/CTA) and MRI (diffusion-weighted imaging (DWI)/perfusion weighted imaging (PWI)/ASL) imaging datasets. The classification of patients based on the topography of the lesion; and studying its impact on the recovery profile of the patients may give us insights into the haemodynamic basis. Also, the role of patency of collateral status was also investigated to study its causative association with peri-lesional hyperperfusion. In Chapter 5, we report the incidence of delayed-cortical vein filling in late venous phase in AIS patients as a potential biomarker [3]. We also investigated its association with collateral status. Subsequently, in Chapter 6, follow-up studies were conducted to determine the association of delayed-cortical vein filling with clinical outcomes in AIS patients [4]. Furthermore, the prognostic link of delayed cortical vein filling with early angiographic reperfusion, 90 days functional outcome, and site of occlusion were also studied.

The thesis is based on four original research papers [1-4]. These studies were carried out on a cohort of AIS patients admitted to a comprehensive stroke centre, Department of Neurology, John Hunter Hospital.

**Paper I: Stroke Severity profiling study [1]:** In Chapter 3 [1], we explore the prognostic role of initial stroke severity in AIS. Epidemiological studies on the extent of the interaction and/or influence of stroke severity on clinical outcomes are necessary. Initial severity of stroke and age are both recognised to be important influences on the likelihood of death following AIS - however, the extent of the interaction is uncertain. AIS patients treated with IV-rtPA have only a 4–16% chance
of good outcome at one year with baseline stroke severity of NIHSS > 20, assessed using National Institute of Health Stroke Scale (NIHSS), and for patients with NIHSS<10 the chance increases to 60–70% [5, 6]. The findings were recently reconfirmed [7]. These results suggest that NIHSS is a significant covariate in the selection of patients for an appropriate thrombolytic therapy, or for the assessment of stroke outcome. Individual profiling based on pre-defined NIHSS thresholds will potentially achieve the desired outcome for the patient with improved treatment efficacy. However, these findings need further validation. The aim of the present study was to investigate the putative (and degree of) impact of initial stroke severity in predicting the overall functional outcome, in-hospital placement and mortality in AIS, in comparison with age, admission to the stroke unit and thrombolytic treatment. We looked at the independent effects of the baseline stroke severity measured using the NIHSS scores as compared with age and other covariates. By strength of significance, we meant to quantify the independent effect of stroke severity in predicting mortality using receiver-operating characteristics (ROC) area and odds ratio (OR). We hypothesised that NIHSS would be the dominant clinical determinant of stroke prognosis.

**Paper 2: Peri-lesional hyperperfusion in acute ischemic stroke: An arterial spin labelling (ASL) study [2]:** In Chapter 4, we used ASL in the pathophysiological investigation of perilesional hyperperfusion (PLH). Focal hyperperfusion after AIS could be of prognostic value depending upon its spatial localisation and temporal dynamics. A recent study using ASL MRI has shown that a select group of patients with ischemic stroke show focal regions of PLH on ASL at 24 hours and that these patients have better clinical recovery from their initial stroke [8]. Factors associated with late-stage (12-24 hours) PLH, identified using ASL, are poorly defined. Leptomeningeal collaterals are relatively dense in and around the cortical superior, middle cerebral vascular territory. Therefore, arterial collaterals supplying the peri-lesional areas around the infarct topographies involving superior cortical MCA, and lenticulostriate (feeding the striatocapsular region) arterial territories may have a role in focal PLH patterns observed on ASL. To this end, a study of the association of baseline collateral status and the topography of infarcts with late-stage (12-24 hours) PLH may be useful in understanding the underlying pathophysiological mechanism. In this prospective study, we sought to examine clinical and neuroradiological correlates
of late-stage (12-24 hours) post-ischaemic focal PLH in a group of AIS patients using ASL mapping of brain perfusion. The specific aims of the study were:

(1) To investigate the association of PLH at 24 hours with baseline collateral status.
(2) To identify infarct topographies that associate with PLH at 24 hours (identified using MRI ASL blood flow measurement).
(3) To investigate the factors associated with early PLH in these infarct topographies.

Our underlying hypotheses were: (a) that better collateral flow grades will be associated with PLH, (b) that there will be variation in the degree of PLH seen depending on the topography of the ischaemic lesion, and (c) that the infarction in the superior cortical MCA and deep MCA territories such as in striatocapsular region (as per Marchal et al. [9]) would more commonly show evidence of PLH than other infarct topographies.

**Paper 3: Delay of late venous phase cortical vein filling in acute ischemic stroke patients: a novel neuroradiological biomarker [3].** In Chapter 5[3], we reported a novel phenomenon, delayed late venous phase cortical vein filling (LCVF), observed on four-dimensional (4D) dynamic computed tomography angiography (dCTA) obtained using 320-detector row 640-slice multidetector CT (MDCT) scanner, in AIS patients. Delayed late venous phase cortical vein filling (delayed-LCVF) is characterised by late venous phase opacification of cortical veins despite contrast clearance from contralateral cortical veins on dCTA [3]. The aim of this study was to examine the frequency of delayed-LCVF and assess its association to baseline collateral status. Neuroradiological assessment of the venous system may be beneficial in stroke prognostication and patient selection for acute intervention strategies. MDCT scanners allow for whole-brain, sub-second, and volumetric acquisition of four-dimensional (4D) dynamic time-resolved dCTA studies of the brain, in particular, visualisation of changes in perfusion [10, 11]. In addition to obtaining three-dimensional (3D) evaluation of intracranial vasculature, time-resolved dCTA allows visualisation of contrast flow from its arterial to venous phases and has demonstrated superior diagnostic accuracy in comparison to the single-phase conventional CT-angiography [11, 12]. Currently, clinical-neuroradiological assessments using CTA focus primarily on the cerebral arterial circulation. Information
on the haemodynamics and the drainage topography of the cerebral venous circulation may be useful for prognostication in stroke.

**Paper 4: Association of cortical vein filling with clot location and clinical outcomes in acute ischaemic stroke patients [4]**. In Chapter 6 [4], we examined the association of late stage cortical vein drainage in a prospective cohort of AIS patients treated with IVT, with tissue at risk, clot location, and clinical outcome. The background to this work was our previous study (Chapter 5) where we reported delayed-LCVF as a novel dCTA finding in AIS patients [3]. The specific objectives of the study were:

1. To examine the association of delayed-LCVF with ischemic infarct core and tissue at risk.
2. To study the association of delayed-LCVF with clot location.
3. To investigate if delayed-LCVF is associated with clinical outcomes including reperfusion status at 24 hours and 90-day functional outcome.

There are limited studies on the role of cortical veins in stroke pathophysiology, and their associations with thrombus location and clinical outcome [13-18].

Finally, in Chapter 7, we will revisit the overall findings of our studies reported in first six chapters, and provide further insights on the use of advanced neuroimaging in individualised patient profiling for prognostication and to guide acute and long-term intervention strategies in AIS.
“Science does not know its debt to imagination.”

- Ralph Waldo Emerson
CHAPTER 1: BACKGROUND - PATHOPHYSIOLOGY OF STROKE AND NEUROIMAGING APPROACHES TO DIAGNOSTICS AND TREATMENT OF ACUTE ISCHEMIC STROKE
In this chapter, we review the pathophysiology and epidemiology of stroke. Classification, clinical diagnosis, and evaluation of stroke subtypes using neurological assessment and neuroimaging assessment are discussed. The chapter draws its material from two sources [19, 20], along with other references duly cited in the content.

1.1. BACKGROUND: STROKE PATHOPHYSIOLOGY

1.1.1. Introduction to Stroke

Stroke, also called cerebrovascular accident (CVA), is the rapid loss of brain function due to the global or focal disturbance of cerebral function, which occurs either (i) due to the lack of blood flow or due to the sudden blockage of artery supplying blood to the brain (ischemic stroke), or (ii) due to bleeding (haemorrhagic stroke) [21, 22]. Stroke is a medical emergency that may cause permanent neurological damage, complications, and death [23]. A plethora of studies has identified putative and confirmed risk factors, which make stroke a disorder of multifactorial origin [24-29]. Modifiable risk factors for stroke include arterial hypertension, diabetes mellitus, heart disease, previous stroke or transient ischemic attack (TIA), obesity, platelet hyperaggregability, alcoholism, smoking, elevated blood lipid levels, hyperuricemia, and infections [22]. Hypertension is an important modifiable risk factor for stroke. The non-modifiable risk factors for stroke include old age, racial or ethnic factors, low birth weight, and genetic susceptibility (genetic and familial factors). A task force constituted by the World Health Organisation (WHO) advocated that prevention strategies should focus on the modifiable risk factors to prevent the occurrence of stroke [22].

The risk assessment and prevention of stroke rely on risk profiles in a population [30, 31]. Such risk models are used to determine an individual's chances of developing stroke. The Framingham Stroke Profile (FSP) is a gender-specific algorithm that uses a Cox proportional-hazards model, with risk factors as covariates, to estimate the risk of developing a stroke within a specified amount of time, usually over a period of 10 to 30 years [31-33]. The FSP is widely used to predict first stroke events and is especially reliable in populations from United States, Australia, and New Zealand [23, 34-36]. However, it is yet to be validated in many populations where it has been found to overestimate the risk, for instance, in the European, Japanese, Hispanic, and Native American cohorts [36-41]. Nevertheless, FSP, like other risk scores, provides an
indication of the likely benefits of prevention. They act as a useful tool, for both patients and clinicians alike, in helping them make informed decisions on choices of lifestyle modification and preventive medical treatment for the management of specific risk factors for stroke, and gross cardiovascular disease risk [42]. It is recommended for asymptomatic patients aged 20 or above to undergo periodic cardiovascular risk assessment including for risk of stroke. The revised 2008 Framingham cardiovascular risk score uses following risk factors to predict fatal or nonfatal stroke risk: age, gender, diabetes mellitus, systolic blood pressure, use of hypertensive drugs, total and HDL cholesterol, and current smoker. The family history of cardiovascular disease was dropped from the revised model. Other risk scores include Reynolds risk score to predict nonfatal stroke (2007/08), American College of Cardiology/American Heart Association (ACC/AHA) pooled cardiovascular risk calculator to predict fatal and nonfatal stroke (2013), and China-PAR score for predicting fatal or nonfatal stroke (2016).

1.1.2. Prevalence & Burden of Stroke

Stroke is the second leading cause of mortality for people above the age of 60 years [43], fifth leading cause for people in the age group of 15-59 years [43], and third most common cause of long-term disability [44, 45] [46, 47]. On an average, stroke claim lives of six people every 60 seconds worldwide, and for every 60 seconds, 30 incidents of stroke (1 every two seconds) are reported [48-52]. An estimated 6.2 million lives are lost due to stroke every year [53]. The incidence of stroke is decreasing in high-income countries including in the United States [54]. However, the incidence shows an upward trend in low-income countries [47]. Although in past two decades, stroke mortality rates have decreased worldwide, an absolute number of people with stroke, stroke survivors, stroke-related deaths, and global burden of stroke-related disability is high and increasing [47]. Worldwide, approximately 80% of all strokes are due to ischemic stroke making it the most common type of stroke [55]. Some epidemiological studies including the Northern Manhattan Study [56], the Greater Cincinnati/Northern Kentucky Stroke Study [57], and others [58, 59] have reported an increased risk of stroke among Blacks and Hispanics in comparison to non-Hispanic whites in the United States. There is geographical, based on the economy [47, 53], an imbalance in the burden of stroke as 80% of stroke survivors currently live in resource-poor
countries, and the overall incidence rates of stroke in low to middle-income countries were twenty percent more than that of high-income countries [53]. Stroke is the second leading cause of disability in resource-limited, low and mid-income countries [47]. The risk of stroke is also age and gender dependent. The incidence and mortality rates of ischemic stroke are higher in men [60]; however, the differences are not significant for haemorrhagic stroke [60]. Regarding disability index, as measured in disability-adjusted life years (DALYs) and years lived with disability (YLDs), stroke is the second-leading cause of disease burden after dementia in low to mid-income countries [47]. Robust data from some epidemiological studies show that the overall case fatality ranged from 16 to 23 percent within the first month and increased by approximately five percent each year [24, 28, 61].

Across Australia, about 8,300 individuals died due to stroke in 2009, contributing to about 6% of all deaths and 18% of deaths due to cerebrovascular disease (CVD). According to the Australian Institute of Health and Welfare (AIHW), stroke is the second biggest cause of mortality in Australia, after coronary heart disease, and is a leading cause of disability [62]. The World Stroke Organisation predicts that 1 in 6 people will have a stroke in their lifetime [63]. The Deloitte Access Economics (DAE) audit report estimated that stroke, with 51,000 occurrences each year in Australia [64], cost the Australian economy whopping 5 billion dollars [65], including three billion dollars in lost productivity and about one billion dollars in lost wages [62]. The report was commissioned by the National Stroke Foundation (NSF, Australia) to estimate the national economic and social impact of stroke. Over 437,000 people were living with the effects of stroke in 2014 which is projected to increase to 709,000 in 2032 [66]. Stroke also causes a significant social and economic burden given that about 65% of stroke survivors suffer from one or more forms of disability that impede their ability to carry out activities of daily living independently [65, 66].

1.1.3. Stroke Aetiology
Ischemia and haemorrhage are two primary mechanisms that cause brain damage in stroke [67, 68] as shown in Figure 1. Ischemic and haemorrhagic stroke represents about 80% and 17% of all the strokes, respectively. Depending upon the etiological mechanism, ischemic stroke can be divided into four subtypes: (a) thrombotic (20%), (b) embolic (20%), (c) lacunar or small vessel disease (25%), and (d) cryptogenic and
other known cause (30%) [68]. Haemorrhagic stroke can be classified into (a) intracerebral haemorrhage (59%), and (b) subarachnoid haemorrhage (41%) [67, 68]. The primary cause of an ischemic stroke is the temporary or permanent occlusion or stenosis of a feeding artery, extracranially or intracranially, or rarely of a vein or dural sinus. During ischemic stroke, decreased or absent cerebral blood flow deprives neurons of necessary substrates such as glucose, lactate, and oxygen indispensable for the survival of neurons [69-72]. In the absence of oxygen, the brain switches to the glycolytic pathway (anaerobic glycolysis), during which glial cells produce lactate, for most basic energy requirements, before a cascade of events occurs that lead to neuronal cell damage and apoptosis [70, 73-75]. The events following ischemia induced by stroke unfold rapidly and may cause irreversible damage to the brain tissues [70]. On the other hand, the rupture of an artery or arteriole, due to an aneurysm or an arteriovenous malformation, results in a hemorrhagic stroke. The relative occurrence of these two types of stroke varies from country to country [22]. Clinically, it is difficult to distinguish between ischemic and hemorrhagic stroke [44]. Next, we will discuss the two pathological substrates of stroke, namely haemorrhagic and ischemic infarction.

1.1.4. Haemorrhage
The ischemic infarct that appears red due to the varying amounts of blood cells prevalent within the necrotic tissue or bleeding is referred to as hemorrhagic stroke [76-78]. The rupture of the vasculature or an aneurysm secondary to ischemia causes early infarct hematoma between 6 and 18 hours after stroke. On the other hand, haemorrhagic transformation (HT) that develops within 48 hours to two weeks is caused by the leakage from the damaged vessels because of the increased vascular permeability in ischemic tissue [79].

1.1.4.1. Pathophysiology of Intracerebral haemorrhage (ICH)
Intracerebral haemorrhage (ICH) represents around 10–15% of all strokes in Europe, USA, and Australia, while in Asia it accounts for about 20–30%; and is associated with high morbidity and mortality [80, 81]. ICH occurs predominantly in the deep areas of the cerebral hemispheres (typical ICH) and is caused due to the bleeding from an arterial source, mainly from arterioles or small arteries, into the brain parenchyma leading to the formation of a localised hematoma [82]. Depending on the underlying
cause of bleeding, ICH is classified into primary (80 to 85%) and secondary (15 to 20%) causes [83, 84]. Primary ICH is more prevalent and is mainly caused by chronic hypertension (hypertensive vasculopathy), illicit drug use (mostly amphetamines and cocaine), trauma, bleeding diatheses or amyloid angiopathy [85, 86]. ICH induces primary injury due to structural damage and mass effect. More than 50 % of primary ICH typically occur in basal ganglia. On the other hand, secondary ICH is associated with: (i) vascular abnormalities (e.g. arteriovenous malformations, sinus-venous thrombosis and aneurysmal rupture), (ii) HT of ischemic stroke, (iii) coagulopathy (impaired coagulation), (iv) brain tumours, and (v) moyamoya disease [73, 84].

![Figure 1.1](image_url)

Figure 1.1. Two types of stroke, namely (A) Ischemic and (B) Hemorrhagic Stroke. Schematic shows three different mechanisms of ischemic stroke, and two types of haemorrhage stroke.

The secondary injury takes place over an extended period of several days or possibly weeks, in the areas surrounding the hematoma, also known as a peri hemorrhagic region (PR). The devastating clinical outcome of secondary brain injury, including high mortality, following ICH could be associated with inter-related complex mechanisms.
mediated by cytotoxic, excitotoxic, oxidative, and inflammatory effects of intraparenchymal blood caused by the hematoma expansion (HE), local compression, neuronal apoptosis, peri hemorrhagic oedema (PRE) [82, 87-90], thrombin [91], haemoglobin [92], oxidative stress [81], neuroinflammation [93], and peroxidases [91]. An MRI study on 32 hyperacute ICH patients concluded that the hypoperfusion in PR might be caused by the diaschisis (reduced metabolic demand) rather than ischemia [89]. This study debunked the hypothesis of the presence of a PR ischemic penumbra in hyperacute ICH settings [89]. However, by the use of perfusion-weighted magnetic resonance imaging (MRI) to track perfusion changes in PR region over time, Pascual AM et al. provided radiological confirmation in support of the hypothesis based on the presence of hypoperfusion around the hematoma, which disappeared completely after the first week [94, 95]. Haematoma expansion is halted by the haemorrhage decompression when hematoma empties into the ventricular system by itself or by the increase in the surrounding pressure.

The presentation of early symptoms of ICH is based on the location of hematoma [96]. For instance, (i) left limb motor and/or sensory signs of weakness are caused by the hematoma in the region of right putamen, and internal capsule, (ii) difficulty in walking seen in patients with the hematoma in the cerebellar region, and (iii) aphasia is seen in ICH cases with hematoma in the left temporal lobe. The neurologic symptoms associated with ICH increase gradually over minutes or a few hours in contrast to subarachnoid haemorrhage (SAH) or brain embolism. In SAH, clinical presentation is more abrupt. Large hematomas are associated with a rise in intracranial pressure, and shifts in the intracranial axis or features. Common symptoms observed in ICH cases with large hematomas include a headache, vomiting, and fading levels of consciousness. Large hematomas are often associated with severe morbidity and significantly increased the risk of death [97]. Hypertensive putaminal haemorrhage (HPH) is a common form of ICH [97-99]. Fisher classified HPH into four groups: nuclear, paranuclear, paraventricular, and subcortical [99].

The treatment goal in ICH is to limit the bleeding and prevent its recurrence. Recurrence of ICH is common in patients with lobar hematoma associated with cerebral amyloid angiopathy (CAA) [100]. Management of hypertension and bleeding diathesis can reduce the high risk of repeat bleeding following symptomatic ICH [100].
1.1.4.2. Pathophysiology of Subarachnoid Haemorrhage (SAH)

The bleeding into the CSF within the subarachnoid space surrounding the brain is referred to as SAH. The arterial aneurysmal rupture at the base of the brain and bleeding from vascular malformations lying near the pial surface are the two principal causes of SAH. Other factors such as trauma, amyloid angiopathy, bleeding diatheses, and illicit drug use are less common. Symptoms of SAH are more abrupt in contrast to the more gradual onset of ICH. A severe headache at the start is very common followed by vomiting in SAH cases. The pain may radiate down to the neck or even further down to the back of the legs.

A previous study found a sentinel headache, characterised by a sudden and severe manifestation, preceded a major SAH in approximately 30 percent of patients [101]. Therefore, a diagnosis of minor SAH should be considered if a patient makes a complaint of the sudden onset of a severe headache. An onset headache was prevalent in almost all the patients with SAH, and in about 50 percent of those with ICH. Sentinel headache, onset headache, and vomiting were not frequent in IS patients. Another study prospectively done on 148 patients in Netherlands, presenting with a sudden and severe headache, found the prevalence of SAH: in a quarter of the overall population, and in 12 percent of patients in whom a headache was the only symptom [102].

1.1.5. Pathophysiology of Ischemic Stroke

Identification of the mechanism of ischemic stroke is important for many reasons, including (i) grouping of patients into distinct subtypes for the study of different aspects of prognosis, which is crucial for patient management, and (ii) selection of patients for specific therapies or intervention. The pathological substrate of ischemic stroke is infarction of brain tissue [72].

At a gross tissue level - the location, extent and shape of infarcts depends on a number of factors [103, 104], including (a) collateral circulation (e.g. a good collateral circulation is associated with a better outcome) [105-108], (b) size of the occluded vessel [109], (c) duration and rate of onset (e.g., an ischemic event of shorter duration or one with slow start is better tolerated) [110], (d) mechanism of arterial obstruction
[111], (e) temperature (e.g. cerebral ischemic injury is greater at elevated body temperature) [112-114], (f) glucose metabolism (e.g. the size of an infarct is adversely affected in case of hyper/hypoglycaemia) [114-117], (g) state/health of systemic circulation (e.g. systemic hypotension from any reason can result in global cerebral ischemia; constant cerebral perfusion pressure depends on adequate systemic blood pressure) [118, 119], and (h) haematological factors (e.g., amyloid angiopathy or a hypercoagulable state may exacerbate the spread of thrombi) [120, 121]. A detailed stratification of ischemic stroke based on the hemodynamic patterns is shown in Fig 1.2.

![Figure 1.2. Depending on the variable hemodynamic patterns, various types and sizes of infarcts are shown.](image)

(a) Occlusion of large arteries due to atherothrombosis or embolizations, along with inadequate collateral supply leads to territorial infarct. (b) Core infarct, meningeal anastomosis supply peripheral zones. (c) Territorial infarct in the centre of supply area, due to branch occlusion. (d) Borderzone infarction in watershed areas due to stenotic lesions in arteries supplying adjacent areas. They typically develop at the borderzone between vascular territories and are the result of a critically reduced cerebral perfusion pressure (low flow infarctions) (e) Lacunar infarctions are mainly caused due to the small-vessel disease. (Adapted from Zülch K-J, 1985 [122])

The three primary mechanisms causing ischemic strokes are (a) thrombosis, (2) embolism, and (3) systemic hypotension or global ischemia (hypotensive) stroke. Other causes of stroke include (i) blood and coagulation disorders, and (ii) transient ischaemic attack (TIA).
1.1.5.1. Thrombosis
Thrombosis refers to a local in situ partial or complete obstruction of an artery due to a disease of the arterial wall (e.g., arteriosclerosis, fibromuscular dysplasia, or dissection) with or without superimposing thrombosis [123-130]. Thrombotic stroke is caused: (i) by reduced blood flow distal (low flow) to the site of vascular obstruction due to thrombus and/or atherosclerotic lesions, or (ii) by an embolic fragment that travels to a more distant vessel after breaking off from the stenosis or intrinsic lesions on the arterial lumen (artery-to-artery embolism) [129, 130], or (iii) by a combination of mechanisms. The formation of thrombi may be accentuated by the severe stenosis from which it may dissociate and embolize. Furthermore, the wash out of the emboli is compromised due to the reduced blood flow caused by the vascular obstruction. There are two subtypes of thrombotic strokes depending upon the site of the occlusion: (a) large vessel disease, and (b) small vessel disease. Large vessel disease may occur extracranially (common internal carotid artery, internal carotid artery, vertebral artery) and/or intracranially (circle of Willis and proximal branches, middle cerebral and basilar arteries). Several pathological conditions are associated with thromboses, such as: (I) atherosclerosis, (II) hypercoagulability, (III) arteritis (Giant Cell and Takayasu), (IV) fibromuscular dysplasia, and (V) dissection of the vessel wall [131]. Atherosclerosis is the primary cause of thrombosis [132].

1.1.5.2. Embolism
Embolism refers to the debris or fragments originating elsewhere but blocks the arterial supply to a particular brain region [133]. Depending on the etiological source, embolic strokes can be classified into four types: (a) those with known cardiac sources (atrial fibrillation and paroxysmal AF, rheumatic mitral or aortic valve disease, recent myocardial infarction within one month, dilated cardiomyopathy, coronary artery bypass graft (CABG) surgery [134]), (b) those with a possible cardiac or aortic source based upon transesophageal and/or transthoracic echocardiographic findings, (c) those with an arterial source (artery to artery embolism), and (d) those with unknown/undetermined sources (in which embolic sources are negative). Embolic infarcts are formed when a small clot breaks off from a larger thrombus and is carried to other places in the bloodstream, and blocks an artery [103]. Blockage of the artery
causes infarction of distal brain tissue because of the deprivation of essential nutrients and oxygen. An embolus can also cause vasospasm, by irritation in the vascular segment or the whole arterial tree, by lodging itself into that particular arterial segment. Vasospasm causes an adverse impact on the overall neurological functioning of the patient.

1.1.5.3. Systemic Hypoperfusion or Global Ischaemic/Hypotensive stroke

Systemic hypotension or global ischemia is a phenomenon which is characterised by the diminution of cerebral blood flow (CBF) over the entire brain [135]; unlike the focal ischemia in which there is reduction in regional CBF in a specific vascular territory and is usually encountered clinically as an “ischemic stroke” due to thromboembolic or atherothrombotic vaso-occlusive disease [136]. Systemic hypotension or global ischemia occur commonly at the intersection of major cerebral and cerebellar arteries, commonly known as the watershed area or the boundary zone [137-141]. Boundary zone areas are at high risk of developing systemic hypoperfusion. With regards to the prevalence, watershed infarcts amount to approximately 10% of all ischemic strokes [137, 142-144]. Also, 40% of ischemic stroke patients with watershed infarcts happen to have carotid stenosis or occlusion [144]. Clinically speaking, infarction in the borderzone area causes weakness of shoulders and thighs, paralysis and sensory loss predominantly involving the arm; however, the face, feet, and hands are not affected, and speech remain intact (also referred to as “man in a barrel” syndrome), stupor, cortical blindness, or the bilateral visual loss [144]. One of the main reasons behind systemic hypotension is a sudden/abrupt profound reduction in systemic blood pressure.

1.1.6. Focal Ischemia

Clinically, ischemic stroke, due to the thromboembolic or atherothrombotic vaso-occlusive disease, is caused by focal ischemia that entails a reduction in regional CBF in a specific vascular territory [136]. Ischemic depolarization occurs when CBF falls to levels of approximately 18 mL/100 g of brain tissue per minute. Neuronal cell death starts when CBF falls below 10mL/100g/min, causing irreversible neuronal injury. The area in the ischemic vascular bed that experiences a severe diminution of CBF during focal ischemia is called ischemic core, whereas, the ischemic penumbra is a more
distal region including the sectors that are marginally perfused and are irrigated by collateral vascular channels [145-147].

1.1.6.1. Mechanism of Neuronal Injury in Focal Ischemia

Focal ischemia causes a cascade of events that eventually lead to neuronal damage and death (see Figure 1.3) [148-151]. Owing to severe diminution of CBF during ischemia, the production of high-energy phosphates, adenosine triphosphate (ATP), is depleted. The shortage in the energy supply affects the functioning of sodium-potassium (Na+/K+-ATPase) pump. In normal conditions, sodium potassium pump sustains the gradients of potassium and sodium ions across the neuronal membrane. During ischemia, failure of sodium-potassium pump leads to increase in the intracellular concentrations of sodium and calcium ions, and extracellular potassium ions, which result in an imbalance in ion homoeostasis, or loss of neuronal membrane permeability. Moreover, failure of high-energy metabolism also results in an increase in extracellular concentrations of certain neurotransmitters such as aspartate and glutamate [150-153]. Activation of the N-methyl-D-aspartate receptor (NMDAR), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), and metabotropic glutamate receptors (mGluR) by binding to corresponding excitatory neurotransmitters, such as aspartate or glutamate, induces recalcitrant and progressive depolarization of neurons referred to as anoxic depolarization. Furthermore, anoxic depolarization facilitates the influx of calcium ions leading to an increase in the intracellular calcium concentration. The excessive accumulation of glutamate caused by decreased uptake of glutamate by astrocyte glutamate transporters triggers the process called excitotoxicity [154]. An increase in receptor concentration activates downstream pathways of apoptosis and necrosis, causing neuronal injury and death. The concentration of NMDA modulates the activity of nitric oxide synthase (NOS). For instance, neuronal NOS (nNOS) and induced NOS (iNOS) leads to the production of large amounts of free radicals such as nitric oxide (NO). NO also interacts with superoxide, another free radical, to produce peroxynitrite (PON). PON, along with NO, causes intracellular and/or deoxyribonucleic acid (DNA) damage that causes tissue injury and/or cell death.

One of the first cell types to respond to hypoxia is endothelial cells [148, 155-160]. The inflammatory response to tissue injury caused by ischemia starts with the release of
inflammatory mediators such as tumour necrosis factor [161-165]. Vasodilation, vasoconstriction, increased permeability, increased platelets aggregation [160], increased leukocyte adherence to the endothelial wall [157-159], and immunoregulation are some of the consequences of cellular effects of these mediators [156]. Within 30 minutes of ischemia onset, leukocytes are recruited to the ischemic areas [165]. The leukocytes adhere to the endothelial cells and trigger the release of vasoactive factors such as NO, arachidonic acid metabolites, reactive oxygen species, and vasoactive peptides [166, 167].

Figure 1.3. Pathogenesis of neuronal tissue damage in acute ischemic stroke [149]. ATP = adenosine triphosphate, nNOS= neuronal nitric oxide synthase, NO = nitric oxide, NMDAR = N-methyl-D-aspartate receptor, AMPAR = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, mGluR = metabotropic glutamate receptor.

1.1.6.2. Oedema Formation

Cerebral oedema is swelling of the brain tissue due to the accumulation of fluids caused by leaky blood vessels. Cytotoxic (cellular) and vasogenic oedema are the two
principal types of Ischemic brain oedema [168]. Acute hypoxia initially causes cytotoxic oedema, after which within the next hours to days is followed by the development of vasogenic oedema, causing the morphological enhancement of the infarcted tissue [168]. Cytotoxic oedema is characterised by swelling of neurons, astrocytes, and all other cellular elements of the brain that trigger a potentially reversible process evolving over minutes to hours [169]. Vasogenic oedema is caused by the accumulation of water and plasma proteins such as albumin in the interstitial fluid. The vasogenic oedema evolves over a time-span ranging from hours to days. Unlike cytotoxic oedema, it causes irreversible damage [170].

Concerning perfusion, oedema is the result of a critical disturbance in cerebral blood flow (CBF) with CBF falling below ten cm³/100g/min. This results in oxygen depletion, energy failure, terminal depolarization, and ion haemostasis failure. Cytotoxic oedema represents the bulk of final infarct. The ischemic penumbral tissue has CBF between 10-20 cm³/100g/min which is salvageable using appropriate thrombolytic treatment or neuroprotective drugs. Evolution from ischemia to infarction depends on a multitude of factors including hyperglycaemia, and collateral status.
1.2. STROKE DIAGNOSIS

Due to the emergency condition arising because of the stroke event, it is important to make therapeutic decisions as quickly as possible after stroke onset; considering time is a precious asset as minutes can make the difference between benefit and harm to the stroke patient. Immediate stroke diagnosis is crucial after stroke onset and typically achieved by a combination of neurological examination and neuroimaging. Stroke scales and stroke-subtype classification systems are not only useful for assessing the impact of therapeutic interventions in clinical trials; they too are used in the routine clinical settings as aids to improve diagnostic accuracy, determine the appropriateness of specific treatments, monitor change in neurologic deficits, and predict and gauge/measure the clinical outcomes.

1.2.1. Stroke assessment scales

*Stroke Diagnostic Scales:* Due to poor recognition of stroke symptoms, stroke diagnostic scales aid general community and emergency responders, in the identification of individuals with acute stroke, to hasten the transport of stroke patients to nearest medical centre. Some of the commonly used scales are Recognition of Stroke in the Emergency Room (ROSIER), Cincinnati Prehospital Stroke Scale (CPSS), Face Arm Speech Test (FAST), and Los Angeles Prehospital Stroke Screen (LAPSS).

*Stroke Impairment Scales:* The stroke impairment scales used in clinical trials are: National Institutes of Health Stroke Scale (NIHSS) [171], European Stroke Scale (ESS) [172], Canadian Neurologic Scale (CNS) [173, 174], Scandinavian Stroke Scale [175, 176], and Paediatric National Institutes of Health Stroke Scale (pedNIHSS) [177]. NIHSS is the most widely used reliable stroke scale with high interrater reliability. It has become a standard stroke impairment scale for use in both clinical trials and as part of the neurological examination in AIS [171, 178, 179]. NIHSS has been validated for retrospective use [180-183], serial assessments for gauging the change in neurological impairments [178], and for remote evaluation in telemedicine programs [184, 185]. The NIHSS scores range from 0 to 42, and a score greater than 25 indicates critical medical condition. Stroke severity measured using NIHSS scale may be useful in making informed decisions when discussing or explaining the bleeding
risk associated with thrombolytic therapy for an individual patient or family [186]. Moreover, NIHSS score is one of the eligibility criteria to determine whether a patient is a suitable candidate for intravenous thrombolysis with Alteplase between 3 and 4.5 hours of symptom onset [187]. Notably, there was no minimum NIHSS requirement in the European Cooperative Acute Stroke Study III (ECASS III) trial; however, patients with NIHSS score >25 were excluded from the trial [188]. A significant shortcoming of the NIHSS is its inability to record all stroke-related impairments, particularly with infarction involving the vertebrobasilar circulation [189, 190]. Modified or shortened versions of the NIHSS (mNIHSS or sNIHSS), too, have the same shortcoming [191, 192].

*Disability Scales:* Barthel Index (BI) and Functional Independence Measure (FIM) are two most frequently used stroke disability scales. The BI measures ten basic aspects of self-care and physical dependency [193, 194], and based on a systematic review and meta-analysis it has excellent inter-rater reliability for standard administration [195]. A score of 100 on BI is a normal score, and scores less than 100 indicate increasing disability. A score of BI<40 corresponds to severe dependency, and a score greater than 60 (and less than 100) corresponds to assisted independence [193]. Interestingly, studies have shown a moderate correlation between BI scores and radiologic infarct size [196-199], and there is limited evidence that BI can predict outcome after stroke [196, 200, 201], except in the setting of acute stroke where its predictive ability is significantly reduced especially within the first 72 hours of stroke onset [190, 202]. Also, BI is relatively insensitive to change in function at the extreme ends of the scale [194, 203, 204]. The FIM assesses the disability of the patient in 13 aspects of motor function and five aspects of cognitive function [205, 206], and is commonly used for: gauging the functional improvement during rehabilitation therapy or in a clinical trial for studying the efficacy of intervention in post-stroke settings [205, 207]. Instrumental activities of daily living (IADL) scales bridge the gap between disability and handicap [208].

*Handicap Scales:* With regards to assessment of impairment, scales such as modified Rankin Scale (mRS) [209, 210], Craig Handicap Assessment and Reporting Technique (CHART), Rankin Focussed Assessment, and Oxford Handicap Scale (OHS) are available [211, 212]. The most widely used among these is the mRS which
is a seven-grade scale to measure functional independence [209, 213]. It is often used in interventional trials as a global measure of the post-stroke functional status [179, 214, 215]. For interventions on reperfusion therapy in acute ischemic stroke, mRS at 90 days is commonly used as an important measure of functional outcome after stroke [179]. The mRS show moderate interrater reliability [216], close correlation with BI scores [217-219], and moderate correlation with the volume of cerebral infarction [197, 199, 220].

1.2.2. Stroke subtype classification system
Clinically, differential diagnosis of lesion caused by a thrombus or an embolus remains a challenging problem. An ideal stroke classification system should be convenient to use, rational, reliable (good to excellent interobserver agreement), evidence-based, and adaptable to incorporate emerging information [221]. Various etiologic classification systems are available to classify the ischemic stroke into different subtypes using (a) causative, and (b) phenotypic subtyping [222, 223]. Causative classification subtype strokes by characterising only the most likely cause(s) of stroke. The most widely used causative classification system is the Trial of Organon in Acute Stroke (TOAST) [223, 224]. The TOAST classification system divides ischemic stroke into five subtypes according to the pathophysiological mechanisms recognised as the cause of the ischemic stroke [225-227]: (a) atherothrombotic (30 % of ischemic strokes, mostly emboli from the bifurcation of the carotid artery), (b) cardioembolic (25-30% of ischemic strokes, mostly due to the atrial fibrillation), (c) small-vessel occlusion (25% of ischemic stroke, leading to lacunar infarcts), (d) other determined causes, and (e) undetermined causes (cryptogenic strokes) [19, 225]. Other causative subclassification systems have been developed including SSS-TOAST [228], Causative Classification System (CCS), Spanish Society of Neurology's Cerebrovascular Disease Study Group (GEECV/SEN) [229] and Chinese ischemic stroke subclassification (CISS) to improve the diagnostic accuracy of TOAST. SSS-TOAST is an evidence-based modification of the TOAST criteria where each TOAST subtype is subdivided into “evident”, “probable”, or “possible” depending upon the weight of the diagnostic evidence as determined by the predefined clinical and imaging criteria [228]. CCS is an automated version of SSS-TOAST devised to improve its usefulness and diagnostic accuracy for stroke subtype. The CCS has a good inter-
rater reliability [230], and consists of a web-based questionnaire style classification scheme available online (https://ccs.mgh.harvard.edu/ccs_title.php) [231].

Stroke phenotyping classification system subtype stroke based on the etiology and the presence of all underlying diseases. The A-S-C-O (Atherosclerosis, Small-vessel disease, Cardiac source, Other cause) is a phenotype-based classification system [223, 224, 232]. A recent study on a large cohort of ischemic stroke patients showed, for admission NIHSS and acute infarct volume, CCS generated more discrete etiologic subtypes with higher inter- and intra- category variability than either TOAST or ASCO [222]. However, CCS, TOAST and ASCO demonstrated similar discriminative accuracy for predicting 90-day mortality.
1.3. NEUROIMAGING IN ACUTE STROKE

Stroke is defined as a focal or global disturbance of cerebral function due to the interruption of blood flow, causing cerebral ischemia or infarction with variable neurological deficits. Imaging techniques including computed tomography (CT) and magnetic resonance imaging (MRI) can identify irreversible ischemic tissue (infarct core) and tissue at risk of death (penumbra) but potentially salvageable. Imaging also enables exclusion of stroke mimics and haemorrhage in acute stroke settings. Neuroimaging in acute stroke is not only crucial for the assessment of the patency of the culprit vessel, perfusion status, and status of ischemic tissue but is also instrumental in guiding appropriate treatment in acute stroke patients. Intravenous thrombolysis, or endovascular intra-arterial thrombolysis, with or without mechanical thrombectomy if appropriate, are the only approved treatments for acute ischemic stroke (AIS). The identification of precise stroke-subtype using advanced multimodal neuroimaging can guide clinicians to choose and prescribe optimal therapy to stroke patients. In this section, we review the state of the art neuroimaging techniques used in diagnosis, prognosis, and driving treatment in AIS.

1.3.1. Goals of neuroimaging in stroke

The aim of neuroimaging in acute stroke patients has five dimensions (see Table 3.1): (i) **parenchyma**: to assess early signs of acute stroke [233], rule out haemorrhage, and differentiate stroke mimics [234, 235] (due to migraine, tumor, seizure, and toxic metabolic disturbances [236-240]), (ii) **pipes**: to identify intravascular thrombi, stenosis, or occlusion of major intracranial and extracranial arteries, (iii) **perfusion**: to assess cerebral perfusion parameters such as cerebral blood flow (CBF), cerebral blood volume (CBV), and mean transit time (MTT) [241], (iv) **penumbra**: to identify and estimate the volume of tissue at risk of dying due to ischemia, and eventually (iv) **profiling**: to select patients for optimal benefit from appropriate intravenous and/or endovascular reperfusion therapy.

<table>
<thead>
<tr>
<th>Goals of acute stroke neuroimaging</th>
<th>Imaging modality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parenchyma</td>
<td>NCCT/DWI/GRE</td>
</tr>
<tr>
<td>To assess early signs of acute stroke, and rule out</td>
<td></td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Haemorrhage and stroke mimics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pipes</td>
</tr>
<tr>
<td>Perfusion</td>
</tr>
<tr>
<td>Penumbra</td>
</tr>
<tr>
<td>Profiling</td>
</tr>
</tbody>
</table>

Table. 3.1. 5 Ps (parenchyma, pipes, perfusion, penumbra, and profiling) of acute stroke imaging (modified from Rowley HA (2001) [233]).

Abbreviations: NCCT = Non-contrast computed tomography, CTA = CT angiography, DWI = diffusion weighted imaging, PWI = perfusion weighted imaging, MRA = magnetic resonance angiography; GRE = gradient recalled echo

1.3.2. Neuroimaging techniques for stroke diagnostics and treatment

CT and MRI are the two most widely used imaging techniques in the diagnosis of acute stroke [242-255]. Time is a crucial factor in acute ischemic stroke management (AIS). Therefore, the American Health Association (AHA)/American Stroke Association (ASA) has made specific time-bound recommendations for acute stroke imaging [242]. Non-contrast CT (NCCT) or MRI should be completed within 25 minutes of the arrival of the AIS patient to hospital. Another (up to) 25 minutes is to be spent on evaluation by a neuroradiologist or stroke neurologist. Non-contrast CT is used to detect early signs of ischemia and exclude haemorrhage [241, 256]. Evaluation of the intracranial and extracranial vascular system may help elucidate the underlying cause of the stroke [246]. This information is crucial to prevent future stroke recurrence or other cardiovascular complications. Noninvasive vascular tests routinely undertaken include MRA, CTA, and carotid ultrasound. An invasive vascular imaging test such as cerebral
angiography, or digital subtraction angiography (DSA), may be used if the risk of stroke or death is minimal. Access to DSA is limited as the procedure is labour- and time-intensive (extra time taken to get into catheterization angiography lab), and require specialist interventional neuroradiologist and nursing staff. A comprehensive list of stroke imaging-based diagnostic tests is provided in Table 3.2.

<table>
<thead>
<tr>
<th>Modality</th>
<th>Diagnostic Technique</th>
<th>Function and significant findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>NCCT</td>
<td>Detection of haemorrhage [257, 258] and early ischemic changes [259] High-density parenchymal contrast staining (&gt;40 HU) on NCCT after DSA and recanalization therapy associated with, temporary loss of BBB integrity, BBB disruption [260-262], increased the likelihood to develop infarction [263], and increased risk of haemorrhage [262, 264, 265].</td>
</tr>
<tr>
<td>CTP</td>
<td></td>
<td>Delineation and quantification of penumbra [266-268]</td>
</tr>
<tr>
<td>Conventional Cerebral arteriography</td>
<td>DSA</td>
<td>Detection of occlusion and lesions, especially of carotid artery [277-279] Interventional neuroradiological procedure [263]</td>
</tr>
<tr>
<td>Sequence</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>T2WI</td>
<td>Detection of oedema, tumour, infarction and haemorrhage. Prediction of parenchymal hematoma [292]</td>
<td></td>
</tr>
</tbody>
</table>
| T2* Gradient Echo (GRE) or SWI | Detection of ICH [293]  
Highly sensitive to ischemia [294, 295]  
Quantification of iron content [296]  
Quantification of oxygen saturation [297, 298]  
Detection of hemorrhagic transformation [299] |
| FLAIR    | Detection of oedema, tumour and periventricular lesion [292]  
Hyperintense vessel sign associated with larger PWI lesion volumes and DWI-PWI mismatch [300]  
Time of onset of acute stroke [301, 302] |
| ADC      | Low signal intensity [303]  
Estimation of lesion age [304] |
| DWI      | Gold standard for acute stroke [303-307]  
TIA assessment [308-310]  
High signal intensity for ischemia [311] |
| PWI-DWI Mismatch | Delineation and quantification of penumbra [312-314] |
| ASL      | Detection of perfusion deficits [315]  
Prediction of mismatch [316]  
Prediction of early reperfusion [317]  
Detection of late-stage (24 hour) perilesional hyperfusion [2, 8, 318]  
Prediction of clinical outcome [315, 319] |
| MRA      | Detection of occlusion [320]  
ICH hematoma expansion  
Recanalisation [321]  
Detection of cervical artery dissection [322] |
| TOF-MRA  | 2D TOF venography sensitive to slow flow [323]  
3D TOF MRA is sensitive to high flow [324, 325] |
| MRS      | Detection of lactate and reduction of N-acetyl aspartate (NAA) compared to contralateral hemisphere [326]. The longitudinal decrease in NAA after the stroke useful for assessment of recovery post-stroke, and efficacy of rehabilitation paradigm [327-329]. |
| fMRI (mainly for recovery phase) | Functional deficit or motor recovery quantification [330-335]  
Guiding recovery strategies for rehabilitation phase [336-342] |
<table>
<thead>
<tr>
<th>Procedure</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>DTI</td>
<td>Prognostic indicator of long-term functional outcome, motor recovery [338, 341, 343-350]</td>
</tr>
<tr>
<td></td>
<td>Delineation and quantification of white matter involvement [343, 344, 346, 349]</td>
</tr>
<tr>
<td>Nuclear imaging</td>
<td>PET                                                                                                                                             Detection of mismatch or penumbra [312, 351-356]</td>
</tr>
<tr>
<td></td>
<td>Stroke pathogenesis [355, 357-359]</td>
</tr>
<tr>
<td></td>
<td>Response to treatment [356]</td>
</tr>
<tr>
<td></td>
<td>Collateralization pattern [360]</td>
</tr>
<tr>
<td></td>
<td>Early postischemic hyperperfusion [9, 361-363]</td>
</tr>
<tr>
<td>18F-FDG PET</td>
<td>Evaluation of glucose metabolism in stroke [352, 354, 355, 364-366]</td>
</tr>
<tr>
<td>SPECT</td>
<td>Presence of collateral flow [367]</td>
</tr>
<tr>
<td></td>
<td>Reperfusion assessment[368-370]</td>
</tr>
<tr>
<td></td>
<td>Stroke pathogenesis and outcome [367, 370-377]</td>
</tr>
<tr>
<td></td>
<td>Cerebral blood flow measurements [368, 377, 378]</td>
</tr>
<tr>
<td></td>
<td>Response to thrombolytic therapy [368, 369, 372, 374, 375, 378]</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>Carotid Ultrasound                                                                                                                             Detection of stenosis [379, 380]</td>
</tr>
<tr>
<td></td>
<td>CIMT Ultrasound*                                                                                                                               Detection and quantification of stenosis [381-389]</td>
</tr>
<tr>
<td></td>
<td>Sonothrombolysis [390]</td>
</tr>
<tr>
<td></td>
<td>Quantification of atherosclerosis and stroke risk [387]</td>
</tr>
<tr>
<td></td>
<td>Surrogate marker of treatment efficacy [391, 392]</td>
</tr>
<tr>
<td>TCD Ultrasound</td>
<td>Sonothrombolysis [393, 394]</td>
</tr>
<tr>
<td>TTE</td>
<td>Detection of cardiac sources of stroke [395-399]</td>
</tr>
<tr>
<td>(for cardiac diagnosis</td>
<td>Estimation of thromboembolic risk of chronic or paroxysmal AF [397], left atria thrombi [396], left atria enlargement[396], stenosis or regurgitation of mitral valve [398], left ventricular dysfunction (due to hypertrophy, pericarditis, pulmonary embolus, aortic stenosis)</td>
</tr>
<tr>
<td>only)</td>
<td>TEE (for cardiac diagnosis only)                                                                                                                Detection of cardioembolic source [400, 401].</td>
</tr>
<tr>
<td></td>
<td>Estimation of thromboembolic risk of chronic or paroxysmal AF, left atria thrombi, left atria enlargement, stenosis or regurgitation of mitral valve [402, 403]</td>
</tr>
<tr>
<td>ECG</td>
<td>Heart monitoring with Holter and cardiac telemetry                                                                                               Determination of cardiovascular risk factors such as cardiac arrhythmia, paroxysmal AF [404].</td>
</tr>
</tbody>
</table>
Table 3.2. List of diagnostic imaging options available for acute ischemic stroke.

Abbreviations: BBB = blood brain barrier; TTE = transthoracic echocardiogram; TEE = transesophageal echocardiogram; ECG = electrocardiogram; TCD = trans-cranial doppler; DSA = digital subtraction angiography; PET = positron emission tomography; SPECT = single positron emission computed tomography; TOF = Time of flight; MRA = magnetic resonance angiography; MRS = magnetic resonance spectroscopy; ASL = arterial spin labelling; DWI = diffusion weighted imaging; ADC = apparent diffusion coefficient; GRE = Gradient echo; T1WI = T1 weighted imaging; T2WI = T2 weighted imaging; CTP = computed tomography perfusion imaging; CTA = CT angiography; PET = positron emission tomography; SPECT = single-photon emission computed tomography.

* CIMT refers to carotid intima media thickness measured using non-invasive ultrasound to detect plaques, both hard (calcified) and soft, in the carotid arteries [383], and quantify thickness of the carotid artery wall and lumen [381-389]. It is used as a biomarker for atherosclerosis [387], surrogate marker to test the efficacy of treatment for ischemic stroke [391, 392], and as a plaque reducing therapeutic intervention [390].

1.3.3. Computed Tomography in Acute Stroke Imaging

CT is used widely in hospitals due to its widespread availability, the speed of imaging, cost-effectiveness and accessibility, and ease of use in the emergency department [250, 405-410]. Next, we will discuss specific CT modalities used in diagnosis and therapeutic decision making in AIS.

1.3.3.1. Non-contrast CT (NCCT)

Non-contrast CT is highly accurate for identifying acute intracerebral haemorrhage and subarachnoid haemorrhage [257, 258], but is insensitive for detecting acute ischemia. Familiar early signs of infarction on NCCT include: (a) hypodensity or hypoattenuation of brain tissue, (b) loss of gray and white matter interface/distinction within 3 hours of stroke onset (50-70%) [411] characterised by the obscuration of the lentiform nucleus (or blurring of the basal ganglia), and loss of the insular ribbon sign, (c) hyperdense middle cerebral artery (MCA) sign (35-50%), (d) gyral swelling and loss of cortical sulcal effacement (12-24h), and (e) haemorrhagic transformation (HT) (15-45%). Figure 3.1 shows an NCCT image of a patient with left middle cerebral artery (MCA) infarct.
Figure 3.1. Non-contrast CT (NCCT) demonstrates effacement of the sulci and blurring of the normal grey/white matter differentiation in the left frontoparietal region.

These early signs may be associated with poor prognosis in AIS. A systematic review of early ischemic changes (EIC) observed on CT found increased risk of poor outcome in patients who showed EIC [412]. Loss of the insular ribbon sign and obscuration of the lentiform nucleus are frequently seen on early NCCT [411]. Loss of insular ribbon sign is due to swelling of the insular cortex and demonstrates high sensitivity for detection of cerebral ischemia because of insular cortex lying distal to collateral supply from both anterior or posterior cerebral arteries [413]. Hyper-dense vessel sign on NCCT has high specificity for irreversible ischemic damage if detected within 6 hours of symptom onset, but low sensitivity for acute cerebral ischemia [414]. It is caused by the hyperattenuation of a large vessel or by acute thrombus present in the vessel. The hyperdense dot sign is linked to the occlusion of MCA branches in Sylvain fissure (16-17%). Parenchymal hypodensity on NCCT is of prognostic significance as hypodensity in one-third of the MCA territory in the acute phase is associated with large infarcts [415-417]. Moreover, substantial hypoattenuation may increase risk of HT after thrombolysis by eight-fold [418, 419]. Extensive hypoattenuation has been used in excluding patients who are more likely to have poor prognosis after thrombolytic treatment [420]. Haemorrhagic transformation can be parenchymal or may manifest as petechial infarcts, and are typically observed between 24-48 h. It is important to rule out hyperdense vessel mimics caused by polycythaemia, diffuse cerebral oedema, and microcalcification in the vessel wall. Moreover, parenchymal
hypodensity can appear on CT due to astrocytoma, encephalitis and other inflammatory conditions, and contusions.

Some of the shortcomings of NCCT are (a) large inter-observer variability [421], (b) poor sensitivity [422], and (c) early ischemic changes seen in only 15-60% of patients [259, 422]. Despite the limitations of NCCT, it has high specificity for large ischemic anterior circulation infarction within 6h after onset [415-417, 423, 424], and to rule out haemorrhage. Hypodensities detected on NCCT within an acute ICH may predict hematoma expansion [425, 426], poor outcome, and increased mortality [427, 428]. High-density parenchymal contrast staining (>40 HU) on NCCT after digital subtraction angiography (DSA) and recanalization therapy were found to be associated with transient loss of blood-brain barrier (BBB) integrity [260-262], increased likelihood to develop infarction [263], and increased risk of hemorrhage [262, 264, 265].

In the backdrop of diagnostic deficiencies of NCCT, standardised, sensitive methods such as the Alberta Stroke program early CT score (ASPECTS) have been developed to reliably assess EIC in brain parenchyma on CT and identify patients who are unlikely to make an independent recovery despite reperfusion therapy [424, 429]. Barber PA et al. demonstrated that the baseline ASPECTS is inversely correlated with the NIHSS, and predicts symptomatic intracerebral haemorrhage (sICH) and 90 days functional outcome [429]. Another study showed substantial Inter-observer and intra-observer reliability [430]. ASPECTS has also been used with diffusion-weighted imaging (DWI) MRI scans with comparable sensitivity to that of CT-based ASPECTS for detecting early infarction [431]. AIS patients, treated with tissue plasminogen activator (tPA), with baseline CT ASPECTS and baseline DWI ASPECTS of 8-10 were 1.9 (95% CI 1.1 to 3.1, P=0.002) and 1.4 (95% CI 1.0 to 1.9, P=0.10) times more likely to have a favourable outcome at 90 days, respectively [431]. Authors concluded that CT is a better neuroimaging modality than MRI for AIS treatment given its accessibility and rapid scan time [431, 432]. However, these findings were contradicted by later studies [306, 433, 434], which showed greater accuracy for DWI to identify acute ischemia and higher sensitivity for detecting hyperacute infarction greater than one-third of MCA territory [434]. Moreover, unlike CT, acute DWI lesion volume showed strong correlation with final infarct volume [306]. More recently, ASPECTS has been applied to computed tomography angiography (CTA) source images (CTA-SI) and CT
perfusion (CTP) [435-438]. CTA-SI ASPECTS score showed greater predictive ability for final infarct size than the NCCT ASPECTS score [436-438]. Furthermore, CTP based ASPECTS demonstrated superior accuracy at predicting final clinical outcome and final infarct volume than NCCT or CTA-SI ASPECTS [435].

Thin slice reconstruction of CT using 1.25 mm to 2.5 mm slices have shown potential for rapid, and highly sensitive diagnostic yield by rapid identification of hyperdense MCA sign, and evaluation of length and volume of thrombotic clot [439-441]. Using quick clot-size measurement after thin slice NCCT, authors demonstrated no recanalization success for clot size > 8 mm [440]. Thin slice reconstruction might be useful to indicate and quantify extent of vascular obliteration [439].

1.3.3.2. CT Perfusion

CT perfusion (CTP) provides information regarding hemodynamic repercussions of stroke due to a possible vascular occlusion. CTP is used to assess ischemic core and tissue at risk of death, or penumbra, to select patients who are most likely to benefit from reperfusion therapy. CTP qualitatively and quantitatively measures the following haemodynamic parameters following a bolus infusion of contrast media, based on estimated contrast media passage and kinetics: cerebral blood volume (CBV), cerebral blood flow (CBF), mean transit time (MTT), and time to peak (TTP) [442].

CBV ranges from 4 to 6 mL/100g and corresponds to the volume of blood per unit of cerebral tissue. CBF refers to the volume of flow per unit of brain tissue per minute, and normal CBF ranges from 50 to 60 ml/100g/min. Also, average CBF values in grey matter and white matter are 75 and 45 mL/100g/min, respectively. Time to peak (TTP) is the time taken to reach the maximum contrast concentration within a region of interest (ROI) since the start of contrast administration. Time to maximum (Tmax) represents the TTP after deconvolution. MTT is the difference in time between the arterial inflow and venous outflow (normal 4 seconds). Perfusion maps corresponding to each CTP parameter are reconstructed.

For processing of CTP datasets, first, an ROI is chosen over an input artery (e.g. anterior cerebral artery) and an output vein (e.g. sagittal sinus) to obtain arterial input functions (AIF) and venous output functions (VOF), respectively. Some post-
processing software packages automatically select the AIF and VOF, while others require the user to select these manually. Once selected, perfusion maps are derived. AIF and VOF curves are generated; following which, deconvolution analysis of AIF and tissue time-attenuation curves give an MTT estimate. CBV is calculated from the following equation: 

\[ CBV = \frac{AUC_{\text{parenchymal pixel}}}{AUC_{\text{arterial pixel}}} \]

where AUC is the area under the curve. Finally, CBF is calculated as per the equation: 

\[ CBF = \frac{CBV}{MTT} \]

Along with these parameters, using whole brain CTP with helical or new generation multi-detector dynamic CT scanners, CTA raw data sets of early contrast-enhanced whole brain images, called CTA-source imaging (CTA-SI) can also be acquired. CTA-SI can provide a quick estimate of perfusion deficits following stroke due to improved contrast enhancement, covers the whole-brain in one image unlike CTP, and may not require administration of a second bolus of contrast. CTP scans can be acquired immediately post-NCCT acquisition and need 5 to 10 minutes of additional scanning time. Moreover, an additional CTA dataset can be acquired along with CTP using separate contrasts.

Low absolute CBV values and prolonged MTT have been suggested to most accurately describe infarct core and ischemic penumbra, respectively [443]. The mismatch between these parameters describe an area at risk of infarction [244]. Wintermark et al. proposed an absolute CBV of less than 2 mL/100g to define infarct core, and relative MTT increase of >145% to define penumbra [244]. CBF threshold of less than 25 mL/100g/min has also been suggested to describe areas of infarct [444]. In an analysis of 30 patients with acute stroke, Murphy et al. demonstrated CBF \( \times \) CBV as the best predictor for differentiating core infarct and penumbra, better than either of these thresholds alone [444]. MTT is highly sensitive to perfusion deficits; however, it tends to overestimate the ischemic penumbra [445]. Penumbra or tissue at risk of infarction or reversible ischemia can be visualised on regional CBF (rCBF) map. In addition, for infarct core or irreversible ischemia, regional CBV (rCBV) displays high specificity [446]. Good correlation has been found between CTP and MR diffusion and perfusion imaging [447-449]. However, there is no consensus on standardised thresholds of CTP parameters for guiding therapy in AIS [450]. Notably, another study found a good correlation between CTP parameters such as CBV ASPECTS and MTT ASPECTS with favourable collateral grades and larger penumbra in patients with anterior circulation stroke treated with IV-rtPA [451].
CTP has been used to predict infarct growth, and clinical outcome in AIS patients treated with reperfusion therapy [435, 444, 451-458]. CTP is very sensitive for detecting supratentorial infarctions (95% accuracy) but is not suitable for lacunar strokes [459, 460]. Overall, CTP using advanced CT scanners have high sensitivity and specificity for detecting infarction [461]; and has demonstrated the potential for therapeutic decision making, more so, when MRI is not available or contraindicated [449].

1.3.3.3. CT ANGIOGRAPHY (CTA)

Once an infarction is diagnosed, the next step is to evaluate the intracranial vasculature to determine which vessel is involved by performing CT angiography (CTA). Figure 3.2 shows the presence of an occlusion on CTA.

![Figure 3.2. Three-dimensional volume-rendered image from spiral computed tomography angiography (CTA). The white arrow points at the presence of a left M2 occlusion.](image)

CTA is used to detect and quantify: (a) occlusions [269-271, 462], (b) stenosis [463-465], (c) status of reperfusion or recanalization to determine the efficacy of reperfusion therapy[2-4], (d) cortical venous flow dynamics [3, 276], (e) hematoma expansion in intracerebral haemorrhage (ICH) [272, 273], and (f) status and impact of collaterals [2-4, 105, 107, 274, 275, 466-471]. Moreover, CTA has been shown to detect intracranial stenosis, thrombi or intravascular malformations reliably, with accuracy comparable to
DSA and MRA [472, 473]. Additionally, CTA has high sensitivity (92-100%) and specificity (82-100%) for large artery occlusion making it a reliable and proficient alternative to conventional digital subtraction angiography (DSA), which is considered the gold standard for neuroangiography [472, 474-476].

The predictive value of CTA in predicting clinical outcome has been studied [406, 477]; however, its value for predicting treatment response remains insufficiently known [478]. Patients with large vessel occlusion (proximal MCA or significant thrombus), identified using CTA, may be poor candidates for intravenous (IV) thrombolysis; and, such patients, instead, may be appropriate for endovascular reperfusion (intra-arterial (IA) or mechanical thrombectomy (MT)/thrombolysis) [443, 479, 480]. Much of the success (vis a vis recanalization efficacy) achieved during the MR CLEAN, SWIFT PRIME, EXTEND-IA, ESCAPE, REVASCAT, THERAPY and THRACE endovascular trials has been attributed to the selection of large vessel occlusion cases with CTA/MRA, along with the use of second-generation thrombectomy devices [480-484]. The Interventional Management of Stroke (IMS) III trial demonstrated utility of CTA in pre-surgical planning as patients receiving pre-treatment CTA received expedited treatment due to reduced groin access to thrombectomy time [485]. Contrast extravasation, also called clot sign, on CTA has been found to be an independent predictor of hematoma expansion in ICH [272, 486-490]. Patients with ICH presenting within 3h on symptom onset are at increased risk of hematoma expansion [272, 489]. CTA can thus be useful in guiding therapies in ICH [487].

CTA has also been used to assess collateral status in AIS [2, 3, 105, 107, 268, 468, 470, 491-499], with several studies reporting the independent association of baseline collateralization on CTA with final infarct volume [107, 467, 499, 500] and clinical outcomes [106, 107, 457, 467, 470, 499, 501] in AIS patients treated with IA therapy. A combination of ASPECTS and CTA-based collateral score have demonstrated an improved predictive value over either single parameter alone [501]. Moreover, a collateral assessment with dynamic CTA is a better predictor of 3 months clinical outcome than single-phase conventional CTA [499, 502].

Location of the clot (on CTA) or the site of occlusion is a crucial determinant of outcome with reperfusion therapy in AIS [4, 503-507]. Proximal clots are associated
with poorer clinical outcomes than distal clots [507]. Clot burden score (CBS) is a qualitative tool, based on the presence or absence of contrast opacification on CTA, to evaluate the extent of intracranial thrombus burden in patients with anterior circulation AIS [508-513]. CBS may be of prognostic significance in patients treated with thrombolytic therapy [456, 457, 514]. Other clot characteristics [515], such as clot density (in Housefield unit (HU)) [512, 516-519], thrombus volume, location of the clot [520], and length of the thrombus [516, 521], have been used in endovascular trials to predict stroke subtype [517], revascularization efficacy [511, 517-519, 521] and clinical outcome [511, 516, 521] following endovascular reperfusion therapy in AIS with large vessel occlusion [522].

The relatively recent advent of helical and multidetector CT scanners have enabled high-quality, fast, and non-invasive angiographic imaging of both extracranial and intracranial arteries [246, 423, 474, 476, 523, 524]. Images are obtained, from the origin of the aortic arch vessels to the circle of Willis, using a single bolus intravenous infusion of 50 mL of iodinated contrast media. This enables acquisition of both cerebral and cervical CTA datasets with single contrast bolus. Raw CTA datasets can be analysed using appropriate reconstruction algorithms to produce a variety of image types, maximum intensity pixel (MIP) or volume rendering (VR) reconstruction, or directly on raw axial CTA source images (CTA-SI). CTA-SI can be used to extract information on collateral status along with vessel patency assessment indirectly [525, 526]. CTA-SI is also more sensitive to EIC than NCCT because the perfused and poorly perfused brain areas are much better differentiated on CTA-SI [527]. Moreover, CTA-SI has been demonstrated to be a stronger predictor of final infarct volume and clinical outcomes than NCCT [436-438, 528]. CTA-SI does not need post-processing; therefore, images are rapidly available for examination. Overall, CTA provides valuable information on vessel patency and may be useful to guide therapy in AIS [271, 457, 474, 502, 510, 525, 529].

### 1.3.3.4. Multimodal CT in Stroke

A comprehensive CT based mismatch assessment is presented in Figure 3.3. A combination of a CT-based integral imaging routine including NCCT (for detection of haemorrhage, stroke or swelling), CTP (for infarct or penumbra detection), and CTA
(for assessment of occlusion, stenosis, and collateral patency) is increasingly being incorporated in stroke care facilities. The entire multimodal CT protocol adds 10-15 minutes to the existing protocol and has demonstrated additional benefit in therapeutic decision-making [449, 526]. Another multimodal CT protocol includes NCCT followed by CTA-SI [251, 423]. CTA can indicate infarcted and penumbral tissues and can help spot the occlusion or stenosis. Raw CTA-SI data then can be processed using arterial reconstructions within 15-20 minutes. Finally, CTP can be obtained using a contrast bolus, and perfusion maps are generated. Mismatch of CBV vs. CBF or CBV vs. MTT can be utilised for selecting patients who are more likely to recanalize with the appropriate reperfusion therapy.

Figure 3.3. CT Perfusion (CTP) imaging in a 78-year old stroke patient. CTP (coronal 5 mm average registered obtained on a CT helical scanner) indicates presence of penumbra and demonstrated region of increased mean transit time (MTT), as well as reduced cerebral blood flow (CBF) and blood volume (CBV) in the right MCA cortex. CTP haemodynamic maps were obtained from CTP raw images. After motion correction, an arterial input function and venous output function is automatically selected. The mismatch ration for the patient was 112, with core being 0.9 ml (1%), and total volume being 100.6 ml (99%). A variable delay-time map based on various DT thresholds: DT+ 2 (204.3ml), DT +3 (100.6 ml), DT + 4 (35.3 ml), DT+ 6 (6.17 ml), DT+8 (4.52 ml), DT +10 (3.93 ml). The standard DT was set at 0.7 second.
Endovascular trials including EXTEND-IA and SWIFT PRIME used multimodal CT imaging along with an automated perfusion analysis software called RAPID (iSchemaView, Inc) to process the CTP dataset. CTA is used to identify patients with large vessel occlusion. In the EXTEND-IA trial, investigators reported that patients with large vessel occlusion and penumbra on CTP were more likely to have a greater recanalization, early neurological recovery, and better long-term functional outcome [530]. Furthermore, other studies have demonstrated a benefit for physiologic imaging-guided patient selection using a CTP mismatch beyond 4.5, and up to 6 h [531] [532].

### 1.3.4. MRI IN STROKE IMAGING

MRI has a better sensitivity for cerebral ischemia than CT [533]. Hyperintensity on MR indicates irreversible ischemic brain damage. The use of diffusion and perfusion weighted imaging (DWI-PWI) has contributed to our enhanced understanding of stroke pathophysiology. The goal of multimodal MR imaging is to undertake a comprehensive evaluation of stroke by assessing its tissue status (using DWI), perfusion status (using PWI), and vessel patency (using MRA). For a list of other conventional and cutting-edge MRI sequences used in acute stroke imaging, please refer to the Table 3.2.

Gradient echo (GRE), susceptibility-weighted imaging (SWI), and fluid-attenuated inversion recovery imaging (FLAIR) are important stroke MRI sequences. T2-weighted and FLAIR exhibit improved contrast over conventional T1 sequences. GRE is highly sensitive for detecting acute ischemia [294, 295]. Moreover, GRE is also very sensitive in detection of ICH. Kidwell et al. demonstrated comparable sensitivity for acute haemorrhage with GRE and NCCT [534]. Notably, GRE was more accurate than CT for identifying chronic ICH [534]. Another study reported higher sensitivity for GRE than FLAIR and NCCT for HT in hyperacute ischemic stroke [535]. In some cases, HT may be visible on GRE earlier than NCCT. Clinically silent, and/or undetectable (on NCCT), cerebral microbleeds can be identified on GRE. FLAIR sequence can estimate the time of onset of stroke [301, 302]. FLAIR is also useful in differential diagnoses of oedema, tumour and periventricular lesion [292]. SWI can detect HT [299], quantify iron content [296] and estimate oxygen saturation [297, 298] apropos to a specific brain tissue.
T1-weighted imaging (T1WI) is a valuable MRI tool for delineation of grey matter, white matter, and cerebrospinal fluid (CSF). On T1WI, CSF appears hypointense compared to the brain tissue. T1WI is especially suitable for anatomical differentiation between vascular structure and in intracranial plaque characterization [280, 281], and detection of vessel and luminal wall lesions [282-284]. T1WI is sensitive to haemorrhage during the subacute phase [285-287]. It is also used in differential diagnosis to rule out tumours and abscesses. The following sections will discuss specific MR modalities used in acute stroke imaging.

1.3.4.1. Magnetic Resonance Angiography (MRA)

MRA has evolved as an important imaging modality capable of imaging blood flow in arteries, veins and cerebrospinal fluid [323, 325, 536-541]. MRA has been used in the detection of occlusion [320], detection of cervical artery dissection [322], and for evaluation of carotid bifurcation in AIS patients [542-546]. MRA is predominantly performed using two techniques, contrast-enhanced and non-contrast-enhanced MRA [323]. Contrast-enhanced MRA involves administration of Gadolinium (Gd)-based contrast agent. Gadolinium chelate brings about T1 shortening. Among non-contrast enhanced MRA techniques, 3D time of flight MRA (3D TOF-MRA) and phase contrast angiography are available. Time of flight MRA is the most widely used MRA technique. TOF-MRA is susceptible to saturation effects, and flow-simulation by short T1 materials. In contrast, phase contrast MRA exhibit excellent background suppression, depicts information of the flow direction and offers variable velocity encoding [536].

TOF-MRA is particularly beneficial in cranial venography in assessment of patency of the dural sinuses or venous drainage from arteriovenous malformation and describing small to medium-sized aneurysms [536]. Three-dimensional (3D) phase contrast imaging is also useful in depicting small and medium-sized aneurysms, while 2D-phase contrast imaging can reliably assess patency of prominent vascular features.

Diagnostic accuracy of MRA is similar to CTA, DSA, and colour Doppler ultrasound (DUS) in detecting symptomatic carotid stenosis [542, 546]. Both MRA and CTA exhibit similar interobserver variability [546]. CTA/MRA are comparatively better than ultrasound technique in detection and quantification of atherosclerotic calcification due to ultrasound attenuation in calcified deposits [547]. However, in carotid artery stenosis
with extensive calcifications, CTA is preferably used [544]. Sensitivity and specificity of MRA in detecting cervical and intracranial stenosis range from 70% to 100% [420]. There are concerns over overestimation of degrees of stenosis in the ICA using contrast-enhanced MRA in comparison to the DSA [548]. Three-dimensional 3D-TOF MRA does not overestimate carotid stenosis [549]. MRA is also used in determining the efficacy of recanalization after reperfusion therapy in AIS [321, 538].

1.3.4.2. **Diffusion-weighted imaging (DWI)**

DWI is the often considered gold standard for acute stroke detection due to excellent sensitivity for detection of hyperacute ischemic injury [303-307]. Early infarcts that are not easily detected by conventional T1W1, T2W1 MRI sequences for 6 h following stroke can be appreciated on DWI. Moreover, unlike CT, DWI can demonstrate small infarcts for days. DWI is a measure of the magnitude of diffusion or random (Brownian) motion of water molecules. Cerebral ischemia leads to swelling due to inward shift of water resulting in the restricted/reduced diffusion. This appears as increased signal on DWI and low signal on *apparent diffusion coefficient (ADC)*, providing excellent differentiation of cytotoxic oedema. Cytotoxic oedema appears hyperintense and restricted on DWI, leading to high sensitivity to ischemia [311]. It improves the sensitivity of hyperacute stroke to 95%. It is also useful in the assessment of transient ischemic attack (TIA) [308-310]. DWI sequences are helpful in distinguishing between old and new stroke, and to detect irreversible infarct core. However, given the anatomical complexity and location, DWI has reduced sensitivity in detecting brainstem and medulla within 24 hours of symptom onset due to inherent susceptibilities (significant distortions due to pulsatile and respiratory activity). Figure 3.4 shows a DWI image of a right MCA infarct.

The extent of diffusion lesion is associated with poor prognosis in AIS patients treated with reperfusion therapy [550-559]. Also, the volume of pretreatment diffusion lesion is considered a risk factor for hemorrhagic transformation [560]. Stanford age and DWI (SAD) score developed using age, and DWI lesion predicted poor outcome following endovascular reperfusion treatment [561]. Moreover, another study using lower age, lower NIHSS at admission, and smaller pretreatment DWI lesion volume predicted good functional outcome at 90 days in AIS patients treated with IV-tPA [552]. ASPECTS scoring on pre-treatment DWI has been proposed as a reliable surrogate
of DWI lesion volume in predicting outcome [555, 559, 560, 562-568]. Clinical-diffusion mismatch (CDM) determined using DWI ASPECTS was found to be associated with neurological improvement in tPA-treated AIS patients [567]. Additionally, in an interesting study, Yoo AJ et al. reported excellent predictive association of combined acute DWI, MTT and NIHSS with clinical outcomes [568]. However, some concerns over the reliability of ASPECTS-DWI score in patients with malignant profile AIS have been raised [565].

![Figure 3.4. Diffusion-weighted imaging (DWI) showing right middle cerebral artery (MCA) infarct. Irreversible ischemia can be identified as the region of hyperintensity on DWI.](image)

1.3.4.3. Perfusion-weighted imaging (PWI) and the mismatch concept

Stroke MRI is a useful imaging tool to make an approximate estimate on the tissue at risk. PWI techniques are widely used in diagnosis and treatment monitoring of several clinical conditions. Dynamic susceptibility contrast (DSC) and arterial spin labelling (ASL) are the two most commonly used methods of perfusion MRI [569]. DSC, popularly known as perfusion-weighted imaging, is based on the detection of the first passage of an intravascular contrast agent (e.g., gadolinium chelate) [569]. On the other hand, ASL uses magnetically labelled arterial blood water as a diffusible flow tracer [570, 571].

The aim of PWI is to detect abnormal tissue perfusion. PWI can identify areas of hypoperfusion or reversible ischemia. Co-registration of PWI and DWI colour maps with structural imaging can help determine the mismatch volume, and identify the target for thrombolysis. DWI/PWI mismatch is a well-known marker of ischemic
penumbra [572]. Penumbra or mismatch can be used to profile an individual patient or subgroup of patients who are likely to show either poor prognosis and/or augmented risk for haemorrhage (malignant profile) or better outcome following effective reperfusion therapy (target mismatch profile) especially beyond 4.5-hour window [573]. Figure 3.5 illustrates the concept of mismatch. Patients with target mismatch (PWI > DWI) who show complete reperfusion after intravenous or endovascular thrombolysis have smaller final infarct volume on 24 h DWI scan in comparison to those who don’t reperfuse.

![Figure 3.5. PWI-DWI mismatch concept](image)

**Figure 3.5. PWI-DWI mismatch concept.** (a) No target mismatch (PWI ≤ DWI), (b) PWI-DWI mismatch image, and (c) target mismatch (PWI > DWI) are depicted. Baseline DWI and PWI represents the area with irreversible ischemia (ischemic core) and area of hypoperfusion, respectively. Follow-up imaging (at 24 h) shows the final infarct on the DWI. The picture in the middle (b) represents the perfusion-diffusion mismatch or the tissue at risk of infarction. The goal of reperfusion therapy is to salvage the penumbra indicated by the mismatch.

PWI = perfusion-weighted imaging, DWI = diffusion-weighted imaging, IVT = intravenous thrombolysis, EVT = endovascular thrombolysis.

Patients with no target mismatch are not good candidates for reperfusion therapy. Also, patients with malignant profile, empirically defined as a baseline lesion on DWI > 100 mL and/or a lesion PWI of > 100 mL using Tmax delay of >8 seconds, show poor prognosis with reperfusion therapy [574-576]. Some trials have shown the benefit
of a mismatch for reperfusion including DIAS, DIAS 2, DEFUSE, EPITHET, and DEDAS. These trials used a penumbra flow (PF) threshold using time to peak (TTP) maps to define mismatch criteria [577]. There is currently a lack of consensus on a standardised PF threshold for selecting mismatch patients [450].

Poor revascularization is associated with risk of expansion of infarction into the areas of perfusion deficit [578]. Severe perfusion deficits in the PWI/DWI mismatch, defined by TTP delays of greater or equal to 6s, are associated with higher risk of lesion enlargement [579]. On the contrary, substantial reduction in MTT lesion volume (>30%) in 2 hours after tPA administration was found to be associated with excellent long-term clinical outcome [580]. In a longitudinal study of 21 patients with DWI-PWI MRI, Beaulieu et al. found a correlation of large acute PWI and DWI volumes with baseline NIHSS scores, NIHSS scores at the outcome, and final lesion volume [581]. Despite the success of the mismatch approach, discrepancies may arise due to poor co-registration of DWI and PWI images. The volumetric analysis might underestimate the PWI/DWI mismatch in comparison to a more accurate co-registration method [582]. Aetiology of the AIS may influence the evolution of specific DWI/PWI patterns [583]. Also, visual estimation of penumbra is operator dependent and therefore it has been suggested that automated methods (such as RAPID (iSchemaView, Inc), a fully automated image processing program, used in DEFUSE and EPITHET trials) to estimate penumbra volume should be adopted in future clinical trials that use mismatch to identify patients for receiving thrombolytic therapy [584, 585].

In summary, perfusion-diffusion mismatch is an important determinant of the prognosis of AIS. However, pathophysiologically, the mismatch is not specific to the concept of penumbra [312]. Moreover, despite the potential benefits of mismatch for reperfusion therapy, it hasn’t yet moved into routine clinical use.

1.3.4.3.1. Arterial spin labelling (ASL)

Arterial spin labelling (ASL) a quantitative, non-invasive cerebral perfusion measurement technique that does not require contrast administration [571, 586, 587]. Blood water is harnessed as an endogenous contrast agent in ASL. The proton spins of the arterial water molecules moving to the brain are labelled by inverting or saturating the spins of water molecules within the blood vessels feeding the brain [8,
When the labelled spins reach the capillary bed after a time delay, these labelled blood spins are exchanged with unlabelled spins in the tissue water resulting into a perfusion-weighted signal, and hence a "labelled" image is acquired (Fig 3.6) [588]. The signal from a particular voxel in the tagged image represents a sum over both blood and tissue spins [589]. The exchanging process leads to decreased signal in the tissue. A “control” image is also acquired without labelling the arterial water spins (see Fig 3.6). The subtraction of labelled and unlabelled (control) image results in an image of cerebral tissue perfusion or ASL map, where images are proportional to CBF [587, 590]. However, if the time delay until the labelled spins reach the imaged volume is long, as is the case in stroke, the signal decays dramatically resulting in poor signal-to-noise ratio. Therefore, particularly in context of AIS which is characterised by delayed blood perfusion, ASL has a poor signal-to-noise ratio and relatively low spatial resolution [586]. Technical and computational advances have significantly improved the sensitivity of ASL perfusion MRI [591-593].

Several different ASL-MRI approaches based on the labelling technique have been developed: spatially selective ASL (SS-ASL) and velocity selective ASL (VS-ASL) [594, 595]. The SS-ASL labels the arterial blood in a region proximal to the specific region of interest [594]. Two commonly used variants of SS-ASL method are pulsed ASL-MRI (PASL), where the blood with the labelling volume is instantly inverted [596-599] [600], and continuous ASL (CASL), where blood is flowing through a specific plane is inverted continuously [589, 590, 596, 600]. CASL, or flow-driven inversion, uses a low-amplitude, continuous radio frequency (RF) pulse to continuously tag arterial blood as it moves through a thin tagging plane [601]. PASL uses a spatially selective RF pulse to tag blood in a large slab proximal to the imaging slice. In comparison to PASL, signal to noise ratio (SNR) is higher for CASL; however, the ratio of SNR and time is comparable [595]. PASL achieves slightly higher temporal resolution than CASL. Unlike CASL, PASL is acquiescent to simultaneous CBF/BOLD using a presaturation pulse.

VS-ASL employs a velocity selective pulse to tag arterial blood moving above an expected cut-off velocity [595]. The guiding principle behind VS-ASL is: (a) blood accelerates as it enters the veins, and (b) blood slows down (decelerates) as it flows into the capillaries. Unlike SS-ASL, there are no issues of transit delays with VS-ASL. In clinical studies, ASL has been used to assess perfusion in neurodegenerative
diseases, epilepsy, central nervous system neoplasms and vascular malformations [602].

Figure 3.6. Arterial spin labelling (ASL) technique. Schematic of how blood is used as an endogenous contrast agent in ASL. Inflowing arterial blood is tagged by magnetic inversion (step 1), following which tag image is acquired (step 2). The experiment is repeated without the tag (step 3), and the control image is acquired (step 4). Finally, ASL image is generated by subtracting tag image from the control image. The difference in magnetisation between the two images is proportional to the cerebral blood flow (CBF) on the ASL image.

A difficulty with ASL-MRI, however, is that in patients with cerebrovascular disease, especially carotid artery stenosis, the quantification of cerebral blood flow is hampered by the recruitment of additional blood flow through collateral pathways [591, 602-604]. These alternative pathways of blood flow lead to a delayed arrival of labelled blood bolus to brain tissue [571, 586, 589, 605-609]. Given that most ASL-MRI techniques acquire perfusion-weighted images at a fixed time point after the initial labelling of arterial blood, it is possible that the magnetic label will not reach the imaging plane, leading to an underestimation of CBF. By acquiring a series of perfusion-weighted
images at increasing delay times after the initial labelling, it is possible to compensate for such blood transit delays without prior knowledge of the transit times [610].

In comparison to more traditional radionuclide methods such as positron emission tomography (PET), ASL has been shown to have improved spatial and temporal resolution, uses non-ionising radiation, and shows potential for repeat, follow-up, and/or longitudinal studies [592]. Furthermore, ASL offers an alternative to dynamic susceptibility contrast (DSC) MRI amidst growing concerns on toxicity associated with gadolinium-based contrast agents. For stroke imaging, ASL offers several advantages compared with conventional DSC bolus-tracking methods including (a) no need for gadolinium-based contrast, (b) repeatability, and (d) quantification of absolute CBF [596-599]. Therefore, ASL offers a unique platform to track and quantify the evolving perfusion abnormalities associated with stroke. Also, in future, it may be possible to perform real-time monitoring of cerebral perfusion during surgical intervention following a stroke [593]. ASL suffers from intrinsically low SNR and can struggle to produce robust, reliable estimates of perfusion given the complexity of cerebral anatomy and the multifaceted nature of neuropathology [593]. This is changing rapidly given the use of innovative set-up using pulsed ASL with 32-channel phased-array coils and parallel imaging at 3 Tesla [611, 612]. Moreover, novel methods to improve signal to noise ratio of ASL using single-shot 3D imaging are being explored which might improve its reliability to measure perfusion in clinical settings [613]. In addition, 4D non-contrast vessel-selective ASL dynamic angiography can provide valuable information regarding the patency of blood vessels, status of collaterals, functional status of the arteries, and status of peri- and intra-lesional perfusion [614-618]. Moreover, increasing numbers of imaging centres have incorporated ASL sequences for various routine clinical imaging purposes. Ever since the initial studies using ASL perfusion MR imaging technique more than two decades ago [619], the method is increasingly used in many clinical and neuroscientific studies [620-631].

1.3.4.3.2. Dynamic susceptibility contrast-enhanced perfusion-weighted imaging (DSC-PWI)

Dynamic susceptibility contrast-enhanced or dynamic susceptibility contrast (DSC) perfusion-weighted imaging (DSC-PWI) is a commonly used MR perfusion technique; often called bolus-tracking method since the technique records signal at a fast pace
as the first bolus passes. It is based on the magnetic susceptibility induced signal-loss of the blood caused by the passing of a paramagnetic contrast agent (such as commonly used gadolinium compounds/chelates) through a capillary bed on T2* weighted sequence [632]. It has excellent signal to noise ratio and is sensitive to microvascular perfusion. After administration of contrast agent, gadolinium interacts with the water molecules that causes water molecule to emit a stronger signal – thereby creating a hyperintense image. Gadolinium-based contrast agents can be classified as (a) linear (agents at greater risk of gadolinium release causing accumulation in tissues), and (b) macrocyclic agents (agents which are less likely to release gadolinium, and are comparatively more stable).

Recent findings on increased signal intensity in multiple areas of the brain, including the dentate nucleus and/or globus pallidus on unenhanced T1-weighted images, in patients with previous history of multiple gadolinium-based contrast administrations, have ignited vigorous debate over the safety of gadolinium-based contrast agents such as gadolinium chelates [633-637]. In a stunning move, the Pharmacovigilance Risk Assessment Committee (PRAC) of the European Union’s main pharmaceutical regulatory body, European Medical Agency (EMA), issued a recommendation to withdraw four gadolinium-based linear contrast agents (gadopentetic acid (Magnevist, Bayer HealthCare Pharmaceuticals), gadodiamide (Omniscan, GE Healthcare), gadobenic acid (MultiHance, Bracco Inc), and gadoversetamide (Optimark, Guerbet Inc)) after the committee’s review found compelling evidence of accumulation of gadolinium in the brain after months of last gadolinium-contrast administration [638]. The United States Food and Drug Association (FDA) have issued repeated warning regarding the usage of gadolinium-based contrast agents in patients with kidney failures [639].

Studies on long-term effects of gadolinium build-up in the brain tissues are limited. The No symptoms linked to gadolinium accumulation in brain have been reported in clinical population. However, PRAC, in light of limited data, took a rather precautionary approach to suspend these drugs unless until evidence in favour of new benefits in identified patient groups outweigh its risks. Gadolinium accumulation in other organs/tissues have been found to be associated with rare side effects of skin plaques and nephrogenic systemic fibrosis (NSF) (in patients with kidney impairment). Referring physicians need to be sensitised about the long-term retention of
gadolinium, and possible toxic effects. Also, caution ought to be exercised by treating physicians in ordering DSC-MRI serial examinations. Need of the hour is to develop new formulations of contrast agents which would either be refractory to dechelation (gadolinium release), considerably minimise/prevent tissue retention or use non-gadolinium ferromagnetic contrast. Moreover, technological advancements to improve SNR/quality of perfusion imaging using ASL, which doesn’t required external contrast, should be expeditiously pursued towards incorporation into routine acute stroke MRI workup.

1.3.5. MR vs. CT in stroke

In summary, DWI and PWI MRI indicate irreversible ischemia (infarct) and reversible ischemia (penumbra) [640, 641]. For infarct on CTP, the criteria are defined by CBV< 2mL/100g. With regards to penumbra on CTP, the criterion is rMTT > 145%. Infarct core and total infarct size when measured with CT and compared with MR show excellent correlation [642]. Despite the high sensitivity of MRI in detecting cerebral ischemic injury, its usefulness and ubiquity has been constrained due to limited access (fewer scanners), greater costs per patient, incompatibility to MR scanners (due to metal implants, e.g. pacemakers, etc), longer acquisition time, and advanced expertise in handling MRI equipment (vis a vis interpretation of images and varied contrast mechanisms may be challenging to (trainee) stroke neurologists). MR does not require the use of iodinated contrast media, unlike CT, and carries no risk associated with exposure to ionising radiation. However, MR perfusion may use gadolinium agents to estimate CBV. MRI has full brain coverage in comparison to limited coverage on traditional CTs. However, current 320-slice MDCT scanners offer whole brain coverage with a single rotation [533]. In the case of a suspected infratentorial lesion or lacunar infarction, MRI is the preferred modality. However, wide accessibility, round the clock availability of CT in the emergency units, the simplicity of scanning procedure, faster scanning time (<10 minutes), better patient surveillance during the scanning, and low costs, give CT an edge [461, 529].

1.4. Conclusion

Neurovascular imaging in combination with neurological examination is crucial to elucidate the underlying aetiology of stroke, make a quick diagnosis, rule out haemorrhage, estimate the tissue at risk of infarction, exclude stroke mimics, and
select patients who are more likely to recanalise on reperfusion therapy. Both CT and MRI techniques offer valuable information that may be used to diagnose, plan treatment, and prognosticate after AIS event. However, many of these techniques are not yet fully absorbed in routine clinical practice. In addition to neuroimaging based approaches, there is a compelling need for public health-based perspectives to facilitate speedy arrival at, and assessment/treatment-delivery in, hospitals. Looking ahead, physiologic-based neuroimaging may provide better selection of candidates and appropriate therapy, hopefully directing treatment choices among the more marginal cases such as those around the edge of the window. To conclude, imaging in acute stroke has both diagnostic and prognostic dimensions, with the ability to guide treatment for achieving high recanalization, better functional outcome, and minimising the risk of hemorrhagic complications.
"A righteous man falls down seven times and gets up."

- King Solomon, Proverbs, 24:16
Abstract
Following the success of recent endovascular trials, endovascular therapy has emerged as an exciting addition to the arsenal of clinical management of patients with ischemic stroke in acute setting. Patient profiling is an approach to selecting stroke patients based on clearly defined clinical and imaging metrics. It may be useful in individualising treatment decisions and tailoring recovery plans for acute ischemic stroke patients, as well as selecting patients for clinical trials. It is crucial to identify the correct stroke-subtype so clinicians can offer optimal therapy. In this paper, we will explore intravenous and endovascular reperfusion strategies, and the constraints of present time-window based approaches to thrombolytic treatment. We propose that patient profiling using multimodal neuroimaging identifies those who will benefit from reperfusion therapy more accurately than simple time windows, and offers therapy to many who present beyond the current time window but still have salvageable brain tissue.

Keywords: Stroke, Reperfusion therapy, Prognosis, Endovascular therapy, Neuro-interevention, Thrombolytic therapy, Acute stroke imaging, Ischemia, Neuroimaging, Patient selection.
**Introduction**

An overwhelming number of studies and clinical trials confirm the efficacy of thrombolytic therapy, in a given therapeutic window, in improving the clinical outcome and recovery of acute ischemic stroke (AIS) patients [6, 414, 643-645]. The primary therapeutic goal for patients with AIS is the timely restoration of blood flow for salvaging ischemic brain tissue that is not already infarcted [242]. Reperfusion therapy using thrombolysis, including intravenous (IV) recombinant tissue plasminogen activator (rtPA) and endovascular interventions such as mechanical thrombectomy (MT), are the only approved treatments for AIS. Both these treatment options have limitations when used as monotherapies. The only pharmaceutical agent approved for the treatment of AIS is IV-rtPA; however, it is not effective in patients with AIS due to large artery occlusion, where the clot burden is very high. In these patients, MT has proven more effective. Currently, the primary criterion for candidate selection in reperfusion is the time from stroke symptom onset. Reperfusion therapy must be administered within a narrow window time of up to 4.5 hours after symptom onset for IV-rtPA, and up to 6-8 hours for endovascular MT. The restriction on IV-rtPA treatment beyond 4.5 h disqualifies the majority of stroke patients admitted beyond this time-window (around 85 %), thereby drastically limiting the eligible population [573, 646-648]. Feasibility and safety studies indicate that advanced neuroimaging may be performed in the majority of acute stroke patients without delaying treatment [649, 650]. Furthermore, application of advanced neuroimaging in selecting patients based on advanced criteria, for instance taking into account the state of blood flow or perfusion and collateral status, may assist informed decision making in a significant number of patients. Stroke patients presenting beyond the accepted time-window could still benefit from thrombolysis. While this may cause an escalation of health care costs due to the additional imaging requirements [541, 650], this may be offset by lower long-term costs if patients survive with lesser disability and experience better quality of life.

Advanced neuroimaging techniques have already been studied as an aid in appropriate patient selection for thrombolytic therapy who may benefit from treatment beyond the conventional time-based guidelines [573, 646-648, 651, 652]. This can potentially extend the therapeutic time window [647]. Individual patient profiling using clinical and advanced neuroimaging metrics may include: (a) diffusion- and perfusion-
weighed MRI [573, 648, 651, 653, 654]; (b) combined perfusion imaging and diffusion-weighted imaging (DWI) (Diffusion-Perfusion mismatch) [640, 653, 655-661]; (c) use of magnetic resonance angiography (MRA)-diffusion mismatch [537, 541, 640, 653, 662-664]; (d) use of DWI-PWI mismatch in combination with other factors such as age, NIHSS thresholds [564, 665-667], Alberta Stroke Program Early CT Score (ASPECTS) [567, 666, 668, 669]; (e) use of computed tomography angiography (CTA) [270, 271, 529, 670-677], CTA source images [106, 457, 678-682], dynamic perfusion CT (CTP) [241, 252, 444, 477, 683-688], and MRA [538, 551, 663, 664, 689]; (f) use of non-contrast based Arterial Spin Labelling (ASL) perfusion MRI technique [2, 8, 315-317, 570, 690, 691]; (g) clinical-DWI mismatch (CDM) [551, 564, 567, 665, 666, 692]; (h) susceptibility-weighted imaging (SWI) [15, 18, 693-699]; and (i) assessment of baseline arterial collateral health [15, 18, 457, 467, 470, 471, 491-500, 504, 700-722] and cortical venous filling patterns [3, 4, 276, 723]. These additional techniques and surrogate imaging markers provide valuable structural and vascular information regarding the state of the brain parenchyma and the neuro-vasculature, including the state of collaterals.

In this article, we review the literature on the various reperfusion strategies available for AIS patients, and the use of advanced neuroimaging in the selection of appropriate patients for thrombolytic therapy beyond the time window of 4.5 hours after stroke onset, and provide insights on potential applications and limitations of imaging-based approaches in the treatment of AIS.

**Time-based vs. individual patient profiling approach**

The principle ‘time is brain’ relies on adherence to a strict time window for defining salvageable brain tissue [717, 724, 725]. The strongest evidence for this paradigm comes from a meta-analysis of the major randomised control trials (RCTs) for IV-rtPA [643, 644, 726]. Analyses suggested IV-rtPA (Alteplase), when administered within 4.5 hours of stroke onset to patients younger than 80 years of age, significantly improves the overall odds of a good stroke outcome. Benefit follows a continuum, with earlier treatment associated with higher proportional benefits; beyond 4.5 hours, risks of therapy appear to outweigh benefit [727]. However, other studies have demonstrated clinically relevant improvements in functional outcome and health-related quality of life, despite early hazards, for an extended time window of up to 6 hours [728-733]. A
recent long-term follow-up study on the effect of alteplase treatment on survival after ischemic stroke (IST-3) found treatment was associated with significant increase in survival after three years for patients who survived the acute phase, and in a small reduction, though non-significant, in risk of death in long-term survival [730]. In light of concerns about the effect of alteplase on survival, these findings come as a reassurance for clinicians. However, the “time is brain” approach does not account for: (a) the variability of impairment in cerebral blood flow (CBF) between individual patients, (b) the role of the collateral circulation (e.g., by leptomeningeal collaterals in middle cerebral artery (MCA) occlusion), and (c) the variations in determining the therapeutic time window in individual patients [646].

One of the major failings of the time-based approach is that it allows only a minority (1-7%) of eligible patients to receive thrombolytic therapy [734-737], mainly because of late presentation. Neither the size of the infarct nor the overall cerebral blood flow state of the patient is taken into account, both of which carry prognostic information [738-745]; indeed some studies have demonstrated the presence of viable penumbral tissue as late as 12 to 24 hours since stroke onset [746-748]. The conventional time-based approach also does not take into consideration the extent of collateral circulation around the infarct. Clearly, the time-based approach is not sufficiently granular for individual patient level decision-making.

There is a compelling need for the careful selection of appropriate patients for thrombolytic therapy. Individual patient profiling based approach using neuroimaging is an approach to identify common pathophysiological subtypes of ischemic stroke that have prognostic relevance for use in (a) guiding individualised acute intervention strategies, and (b) conducting appropriate recovery intervention strategies. The concept of patient profiling entails the inclusion of certain key metrics known to be predictors of good functional outcomes, in addition to the already laid down protocols and guidelines of thrombolysis, for clinical decision-making. We hypothesise that the individual patient profiling approach will allow: (i) more precise diagnosis, (ii) appropriate patient selection for reperfusion therapy (to select patients who will most likely benefit), (iii) greater numbers of patients as potential candidates by extending the time window, and (iv) identification of patients who are at higher risk of developing hemorrhage or who are unlikely to benefit from thrombolysis. Metrics used for
individual patient profiling may include: (a) volume of salvageable tissue or penumbral volume, (b) location and size of the infarct, (c) site of occlusion and underlying vascular pathology, and (d) collateralization status. For example, studies have shown that patients profiled using such characteristics may potentially show the largest benefit in response to thrombolytic therapy. Key characteristics include (a) moderate or severe persisting neurological deficit for less than 3 to 6 hours [749-754], (b) MCA occlusion, due to better recanalization rates compared with ICA occlusions [755, 756], (c) efficient collateral circulation [2, 472, 495, 496, 500, 700, 709, 710, 712, 715, 755], and (d) Initial CT with no signs of extended infarct as defined by Hacke et al in 1995 [525, 737, 750]. There is also a potential risk to the patients if thrombolytic agents are administered once the spontaneous lysis of the occluding thrombus has already taken place [525]. Therefore, additional techniques of vascular evaluation (using CTA, intra-arterial digital subtraction angiography (DSA), MRA, etc.) to determine site and extent of the vascular occlusion may be very helpful in determining the “tissue salvageability” with thrombolysis [472, 525, 643, 757].

Evolution of reperfusion therapy
Thrombolysis, commonly known as “clot-busting”, is a pharmacological treatment using an infusion of analogs of tissue plasminogen activator (tPA) which leads to the breakdown (lysis) of the culprit blood-clot. Thrombolytic drugs dissolve blood clots by activating a proteolytic enzyme, plasminogen, to plasmin. Fibrin molecules provide the structural scaffold for blood clots, and plasmin cleaves cross-linkages between fibrin molecules. Subsequently, the clot becomes soluble and undergoes further degradation through proteolysis by other enzymes, eventually restoring blood flow. Due to their mechanism of action, thrombolytic drugs are also referred to as "plasminogen activators" or "fibrinolytic drugs". The three major classes of plasminogen activators are (i) tissue plasminogen activator (tPA), (ii) streptokinase (SK), and (iii) urokinase (UK). Thrombolytic drugs differ in the mechanism by which they act on fibrin clots. The sequence that leads to the breakdown of the clot or fibrinolysis by tPA is shown in Figure 1. At first, tPA binds to clot-bound fibrin. This activates fibrin-bound plasminogen from an inactive form to plasmin, the active form. The enzyme plasmin acts on the fibrin mesh, leading to the breakdown of the fibrin scaffold. Subsequently, the dissolution of the fibrin clot produces circulating fibrin fragments called fibrin degradation products. These products prevent the conversion
of fibrinogen to fibrin, which slows down clot formation. The liver and kidney, ultimately clear these products, as well as other proteases. Two serine protease inhibitors, namely plasminogen activator inhibitor-1 and 2 (PAI-1 and PAI-2), endogenously inhibit t-PA and UK. Alpha 2-antiplasmin and alpha 2-macroglobulin also act as inhibitors of plasmin. Factors XI1, XIIa, and Kallikrein stimulate the process of plasmin formation from plasminogen. On the other hand, another factor called thrombin-activatable fibrinolysis inhibitor (TAFI) alters the fibrin to make it more resistant to plasminogen activated by tPA. Efficacy of thrombolytic drugs depends on the size, location, and age of the clot because of the increased density of fibrin cross-linking which make clots more compact and harder to dissolve the older they are.

Some of the known tPA analogs are Alteplase (Activase®; recombinant tissue plasminogen activator (rtPA)), Retaplace (Retavase®) and Tenecteplase (TNK-tPA). Alteplase is the most commonly used thrombolytic drug and is a fibrin-selective analogue of tPA, administered intravenously or intra-arterially. It is the only Food and Drug Administration (FDA)-approved thrombolytic agent for the treatment of AIS. rt-PA causes lysis of fibrin; thereby dissolving the thrombus and resulting in recanalization of the occluded artery [242]. It has a short half-life (~5 min) and is therefore administered as an IV bolus followed by an infusion. Several trials including the NINDS [421] and European Collaborative Acute Stroke Study (ECASS) have demonstrated evidence for the benefit of rt-PA for a select group of AIS patients. In comparison to rtPA, Tenecteplase (TNK-tPA) has a greater binding affinity for fibrin, higher resistance to inactivation by PAI-1, and a longer half-life.

Urokinase, also known as urinary-type plasminogen activator (uPA), is found in urine. Clinically, UK is used as a thrombolytic drug in conditions such as pulmonary embolism, myocardial infarction, and severe deep venous thrombosis (DVT). In comparison to rtPA, SK and UK lack fibrin specificity and bind equally to circulating plasminogen and clot-bound plasminogen. On binding with plasminogen, SK forms a complex that activates plasmin, triggering a proteolytic cascade. The proteolytic cascade leads to thrombolysis (clot degradation in AIS) or extracellular matrix degradation (e.g., tissue degradation causing tissue invasion and metastasis in tumour malignancy) depending upon the physiological environment. Therefore, SK and UK have less favourable adverse event profiles than rtPA. Thrombolytic drugs can be
used as (a) Intravenous, and (b) Intra-arterial. Next, we will discuss the important aspects of these thrombolytic procedures.

**Intravenous thrombolysis**

Table 1 gives a detailed overview of clinical trials that evaluated the “time is brain” paradigm. The selection of patients for IV thrombolysis was based mainly on time since stroke onset. All of these trials used non-contrast CT (NCCT) for assessment of parenchymal injury. The exclusion criteria were the presence of a large infarct occupying more than one-third of the MCA territory or causing severe oedema or mass effect visible on NCCT [646].

The field of acute ischemic treatment using thrombolytic therapy before 1995 was despairing given the high rates of intracerebral haemorrhage in early clinical trials, which changed dramatically after the publication of results of NINDS stroke trial, which showed benefit for IV alteplase within 3 hours of symptom onset [645]. The trial recruited 624 patients who were randomly assigned to treatment with IV alteplase (0.9 mg/kg up to 90 mg; 10 percent as a bolus followed by a 60-minute infusion [758]) or placebo. A significantly greater proportion of patients (38 (alteplase) vs. 21 percent (placebo)) who received alteplase showed a favourable clinical recovery at 90 days after stroke. However, the alteplase group showed a dramatic 10-fold increase in symptomatic intracerebral haemorrhage. There was no significant difference in 90 days mortality between the two groups. Notably, less than 1 percent of patients experienced severe systemic bleeding at three months. A one-year follow-up study showed patients with AIS treated with alteplase within 3 hours after the onset of stroke were more likely to have minimal or no disability in comparison to patients receiving placebo [6, 759].

Subsequent serial observational registries and prospective randomised controlled studies conducted to investigate the safety and efficacy of IV alteplase within the eligible time window showed a time-dependent relationship, with earlier treatment associated with bigger proportional therapeutic benefits [644, 726, 731]. The European Cooperative Acute Stroke Study (ECASS)-III trial showed clear benefit for patients treated between 3 and 4.5 hours after stroke onset [188], which the previous RCTs failed to show because the earlier trials recruited small number of patients in
this time window, and had treatment time windows of up to 6 hours [760-762]. Other studies including the Canadian Alteplase for Stroke Effectiveness Study (CASES) registry [763], and Safe Implementation of Thrombolysis in Stroke-International Stroke Thrombolysis Register (SITS-ISTR) [764] provided further evidence of benefit for the administration of IV-rtPA therapy in the 3-4.5 hour treatment window. These registries, CASES and SITS-ISTR, found comparable rates of mortality, functional independence, and incidence of symptomatic ICH within 24 hours [652]. The extension of the time-window up to 4.5 hours obviously offers an opportunity for treatment for more patients [764]. With regard to the treatment beyond 4.5 and up to 6 hours, three trials including the Alteplase Thrombolysis for Acute Noninterventional Therapy in Ischemic Stroke (ATLANTIS-A for up to 6 hours [762], and ATLANTIS-B for 3 to 5 hours [761]), ECASS-II (for 0-6 hours) [760], and Third Internal Stroke Trial (IST-3) (for 0-6 hours) [731] failed to demonstrate a treatment benefit for rt-PA. However, a recent meta-analysis of 12 trials including 7012 patients, who received alteplase within 6 hours of onset of AIS, found significant improvement in the favourable outcome (mRS 0-1), functional independence (mRS 0-2), and survival rates at the end of final follow-up [732]. On the one hand, this analysis reinforces the need to treat patients as early as possible, though, it also suggested that some patients might benefit from alteplase up to 6 hours after stroke [731]. However, subgroup analysis for the treatment window between 4.5 and 6 hours was not presented. Moreover, no significant trend toward a favourable outcome was found in a subset of patients (n=4971) who received alteplase between 3 and 6 hours. The overall trend towards a beneficial outcome in 0-6 hour window may have been influenced by the dominant trend observed in 0-3 hour window. Pooled analysis of three trials, published in 2004, including the National Institute of Neurological Disorders and Stroke (NINDS) trials (3-h window), ECASS trials (6-h window), and two ATLANTIS trials (6-h and 5-h window), demonstrated strong association between rapid treatment and favourable outcome for patients receiving IV thrombolytic therapy within 3 hour time window [643]. Moreover, the results of two other recent pooled analyses published in 2010 (n=2775 pooled from ECASS, ATLANTIS, NINDS, and EPITHET trials) and 2014 (n=6756 pooled from IST-3, ECASS, EPITHET, ATLANTIS, NINDS) also indicated modest, yet clinically valuable, benefits to a select group in the therapeutic window of three to 4.5 hours [643, 644, 726, 765]. However, risk outweighed benefit beyond 4.5 hours [644, 726, 731]. In light of the emerging evidence and to provide more patients with an opportunity
to receive tPA, acute stroke guidelines for the administration of rtPA following AIS have been revised by both the European Stroke Organization (ESO) [766] and American Heart Association/American Stroke Association (AHA/ASA) [767] by expanding the window of treatment from 3 hours to 4.5 hours. Interestingly, the FDA has not yet approved this extended indication.

Some contraindications limit the use of IV-rtPA in AIS (see Table 2) [768]. Moreover, the narrow time window of 4.5 h along with the multitude of contraindications prevent many patients from receiving treatment; indeed, less than 3 percent of patients presenting with AIS receive IV-rtPA [769]. Other limitations of IV-rtPA are: (a) increased rate of mortality and intracranial bleeding in internal carotid artery occlusion (ICA) [770-773] or other disabling strokes such as those with no detectable residual flow signals [503, 504, 774], (b) low recanalization rate ranging from 13 to 50 percent in large artery occlusion such as the proximal MCA, the ICA, or the basilar artery [462, 503, 505, 753, 768, 775-777], and (c) unresponsiveness to large thrombi (especially when the thrombus length exceeds 8 mm [439, 440, 778, 779], or location is proximal, such as terminal carotid artery occlusion [504, 780]. One study found only 10% and 25% of ICA and proximal MCA occlusions are recanalizable by IV-rtPA [781]. Incomplete recanalization is often observed in patients treated with IV-rtPA. For instance, 70% of patients who received IV-rtPA were found to have angiographically confirmed residual thrombus requiring complimentary intra-arterial treatment such as clot angioplasty [782].

Novel therapies are being currently investigated to overcome the limitations of IV thrombolysis or to extend the time window of treatment, for example: (i) use of alternative fibrinolytic agents such as desmoteplase [783-787], argatroban [788], tenecteplase [762, 789], albumin [790], and plasmin [791], (ii) mixed approaches that involve combination of rtPA and other agents or therapies such as GP IIb/IIIa antagonists [792-794], antiplatelet agents (e.g., acetylsalicylic acid [795-797]), low-molecular-weight heparin [798, 799], and sonothrombolysis [800-803] to enhance microcirculatory flow, reduce residual thrombus, and boost lytic efficacy, (iii) use of non-invasive or minimally invasive methods such ventilator support [804], and pterygopalatine ganglion and petrosal nerve stimulation [805] to augment cerebral blood flow by cerebral vasodilation and alleviate blood flow steal, and (iv)
endovascular procedures such as intra-arterial thrombolysis, stenting, and angioplasty to achieve greater clot manipulation and significantly higher rates of arterial recanalization [780, 806].

**Endovascular treatment**

**Intra-arterial thrombolysis**

Intra-arterial (IA) thrombolysis has emerged as a promising intervention, especially for AIS patients with contraindications for IV-tPA [807-809]. Intra-arterial procedures are performed under direct visualisation; therefore, one can limit the dose of the fibrinolytic agent, mechanically manipulate the clot if required and deliver higher concentrations of the agent to the clot target (local delivery) with reduced systemic effects [810, 811]. IA can also deliver higher recanalization rates. However, IA therapy also has its unique set of challenges and disadvantages. Mechanical manipulation of a clot during IA may also increase the risk of injuring adjacent blood vessels. Moreover, advanced training is required for neuro interventionists or neuroradiologists to gain expertise in IA procedures which can be demanding [808, 812-814]. As such, endovascular treatment is only available in a limited number of specialised stroke centres.

Prolyse in Acute Cerebral Thromboembolism (PROACT) was the first randomized trial (phase II study; n=46) of 6mg recombinant pro-urokinase (rpro-UK) versus placebo undertaken in patients with angiographically documented proximal MCA occlusion to test the safety, recanalization frequency, and clinical efficacy of intra-arterial local infusion of plasminogen activators in AIS patients with symptomatic MCA occlusion of less than 6 hours’ duration [815]. Investigators found a significant association of intra-arterial local rpro-UK infusion with greater frequency of recanalization in acute stroke patients with M1 or M2 occlusions compared with placebo (57.7% vs. 14.3%, P=0.017). However, an increased, though not significant, symptomatic haemorrhage rate was also reported. Subsequently, a phase III, PROACT II study was undertaken, involving fifty-four stroke centres in the United States and Canada, with an increased 9 mg dosage of r-proUK administered over 2 hours infusion while using heparin in low dose (from PROACT I) to improve the recanalization in addition to containing symptomatic brain haemorrhages [816]. Out of 180 randomised patients with AIS, treatment with IA r-proUK within 6 hours of the onset of AIS caused by MCA occlusion was significantly associated with improved clinical outcome at 90 days. The IA r-proUK
group demonstrated significantly higher recanalization rates (66% vs. 18%, P<0.001), and increased favourable independent outcome (60% vs. 18%, P<0.001) than the IA heparin alone. However, higher frequency of early symptomatic ICH was also observed in the intervention group (10% vs. 2%). Overall, the proportion of ICH was relatively higher in PROACT II than previous IV thrombolysis trials [815], perhaps due to greater baseline NIHSS in PROACT II in comparison to other trials. The median baseline stroke severity in PROACT was 17, in contrast to 14 and 11 in the NINDS and ECASS II trials respectively. Contrary to the positive findings of PROACT II, another large open-label trial, SYNTHESIS Expansion, found that endovascular therapy was not superior to standard treatment with IV-rtPA [483]. Disability-free outcome at 90 days was not significantly different between the endovascular treatment and IV-rtPA groups (30.4% vs. 34.8%; adjusted odds ratio= 0.71, 95% CI 0.44-1.14). Another randomized trial, the Middle Cerebral Artery Embolism Local Fibrinolytic Intervention Trial (MELT), conducted in Japan, to investigate the safety and clinical efficacy of intra-arterial urokinase (UK) in patients within 6 hours of onset of stroke with angiographically documented M1 or M2 occlusion, found a trend towards favourable outcome (defined by mRS 0-2) at 90 days, and a substantial increase in likelihood of excellent outcome (defined by mRS 0-1) [817]. However, the primary endpoint (good outcome; mRS 0-2) did not reach statistical significance as the trial was aborted prematurely.

A meta-analysis of five RCTs with 395 AIS patients with MCA occlusion compared IA thrombolysis with control (IV heparin) [818]. The study concluded that IA thrombolysis was significantly associated with substantial increases in recanalization rates and good (odds ratio=2.05; 95% CI, 1.33 to 3.14; P=0.001) and excellent outcomes (odds ratio= 2.14; 95% CI, 1.31 to 3.51; P=0.003) in AIS. Intra-arterial thrombolytic treatment is gaining traction at a number of comprehensive stroke facilities at tertiary hospitals. It is often administered as an off-label therapy within 6 hours of onset of stroke in patients with anterior circulation and up to 12-24 hours after onset in the posterior circulation [808]. As per the guidelines of the AHA issued in 2005, and again in 2013, IA thrombolysis has been recommended in appropriately selected AIS patients with MCA occlusions within six h provided they were not candidates for IV-rtPA (Class I, Level of Evidence: B) [420]. Also, the FDA has not approved IA-proUK.
**Mechanical Thrombectomy (MT)**

MT involves a minimally invasive surgical procedure using a microcatheter and other thrombectomy devices to trap and remove the blood clot from the occluded artery. MT, delivered as a stand-alone treatment or in conjunction with systemic thrombolysis (IV-rtPA or IA thrombolysis), is currently the standard of care for AIS therapy [819]. MT devices can be classified into different subtypes based on their mechanism of action: (a) coil retriever, (b) aspiration, (c) stent-retriever, and (d) mechanical clot disruption, using laser or ultrasound. A comprehensive list of past and current MT devices is given in Table 3. Coil retriever devices and the early Penumbra aspiration system [820, 821] were the first generation of MT devices; they failed to show long-term improvements in clinical outcomes despite good revascularization efficacy (up to 50%) [822]. Coil retrievers such as MERCI [823-826], Phenox [827, 828] and Catch retrievers [829-831] use microcatheters to deliver a coiled wire across the targeted clot in the occluded artery [823-826]. Once the coil is deployed, the neuro interventionist pulls both the coil and the clot towards the catheter leading to the removal of the clot [832]. Aspiration devices such as Penumbra (Penumbra Inc., US) use vacuum aspiration to remove target clot in the occluded artery. The early generation of aspiration devices were often subject to clogging of the aspiration tips; this has been overcome in the later models by the addition of a separator wire with a bulbous tip inside the bore, which can be pushed in and pulled out by the neurointerventionist. The continual back and forth motion cleave the clot detaching it from the lumen. Eventually, the clot is sucked in, without clogging the tip, ahead of the catheter [833]. The second generation of MT devices used self-expanding stents to trap the clot by deploying them in the occluded artery [833]. They were originally conceived for stent-assisted coiling and retraction of aberrant coils dislodged during the endovascular procedures. These self-expanding stents are first lodged across the thrombus within the stent wall, following which, once the clot is entrapped, the stent-clot combination is subsequently retracted back under constant aspiration into the delivery guide microcatheter. Some clinical trials using the new generation stent retrievers, such as Solitaire, Trevo Pro, and ReVive, have yielded recanalization rates as high as 85% in AIS with large vessel occlusion [825, 832-834].
Combined interventions, using both suction embolectomy with large bore catheters and mechanical retrieval using stent retrievers, have shown promise in recent studies [835]. In this technique, aspiration of the clot, a cheaper alternative, is attempted first using large bore microcatheter such as Penumbra MAX systems [836-839]. If the aspiration fails, mechanical retrieval is attempted by inserting the stent retrievers via the aspiration catheter. Using this sequential combination, phenomenal recanalization rates of up to 95% have been achieved [835], compared with stand-alone direct aspiration rates of 78%. Another application of combined approach is the latest generation Penumbra device called Penumbra 3D separator, which incorporates lesional aspiration technology coupled with an advanced stent retriever device, allowing the breakdown of a clot in addition to radial direction using stent struts [832, 840]. Penumbra 3D separators have demonstrated good revascularization of large vessel occlusions and a greater rate of functional independence at 90 days [840, 841]. A detailed comparative overview of successful clinical trials concerning endovascular treatment for AIS along with the imaging and clinical selection criteria, and the outcome measures is presented in Table 4.

Following the success of five multicentre, open-label, randomized controlled endovascular acute stroke trials including MR CLEAN (Multicenter Randomized Clinical Trial of Endovascular Treatment for Acute Ischemic Stroke in the Netherlands) [842], ESCAPE (Endovascular Treatment for Small Core and Anterior Circulation Proximal Occlusion with Emphasis on Minimizing CT to Recanalization Times) [843], SWIFT PRIME (Solitaire With the Intention for Thrombectomy as Primary Endovascular Treatment of Acute Ischemic Stroke) [844], EXTEND-IA (Extending the Time for Thrombolysis in Emergency Neurological Deficits–Intra-arterial) [810], and REVASCAT (Randomized Trial of Revascularization with Solitaire FR Device vs Best Medical Therapy in the Treatment of Acute Stroke Due to Anterior Circulation Large Vessel Occlusion Presenting within Eight Hours of Symptom Onset) [478], it is now accepted that the combined treatment with second-generation stent retriever MT devices and IV-rtPA within 6 hours after stroke onset is superior to standard medical therapy (with IV-rtPA alone) for AIS caused by a proximal large artery occlusion of the anterior circulation administered [481].
Immediately after the announcement of the MR CLEAN results [842], the other four trials were terminated prematurely for the interim analysis. The results of these trials were then published in quick succession in late 2014 and early 2015. The number needed to treat (NNT) for these five trials ranged from a minimum of three (EXTEND-IA) to a maximum of 7.4 (MR CLEAN). These successful trials have revolutionised stroke therapy. However, given that only trained neuro-interventionists can perform the MT procedures, stroke care facilities should expeditiously work on integrating MT with the standard of care, to minimise the time required for imaging and preoperative preparation needed for this therapy [484]. A recent meta-analysis, based on a pooled analysis of 1287 patients, published by the Highly Effective Reperfusion evaluated in Multiple Endovascular Stroke Trials (HERMES) collaboration, showed that endovascular MT added to the best medical therapy more than doubles the odds of a higher rate of (a) functional independence (mRS score at 90 days of 0 to 2) (46% vs. 27 %, odds ratio 2.35, 95% CI 1.85-2.98) [845], and (b) significantly reduced disability (improvement of ≥1 points on the mRS at 90 days) (adjusted OR 2.49, 95% CI 1.76-3.53) compared with best medical therapy alone in AIS patients with large vessel occlusion in anterior circulation. Authors also found that the rates of symptomatic ICH or 90-day mortality were not significantly different between the endovascular MT and control groups [479]. Another recent meta-analysis by the same HERMES collaboration has suggested that the endovascular MT plus medical therapy is beneficial up to 7.3 hours after the onset of stroke [846]. To sum up, these studies make a convincing case for the administration of early thrombectomy using second-generation stent retrievers for limiting post-stroke disability in patients with large vessel occlusion. They also reinforce the importance of early treatment based on an inverse association between time to endovascular reperfusion and better functional outcome [846].

**Clinical trials with first-generation devices**

Prior to MR CLEAN trial, three endovascular trials, using mainly first generation MT devices during 2004 and 2012, including MR RESCUE (Mechanical Retrieval and Recanalization of Stroke Clots Using Embolectomy) [847], the IMS (Interventional Management of Stroke) III [482], and the SYNTHESIS Expansion (Intra-arterial vs. Systemic Thrombolysis for Acute Ischemic Stroke) [483] failed to demonstrate any functional benefit for intra-arterial treatment in AIS. The results of these trials, which
were published in 2013, raised concerns regarding the efficacy of endovascular MT in large vessel occlusion. Potential causes for this lack of effect were suggested including: (a) the three trials used old-generation devices with reduced recanalization efficacy in comparison to the later generation devices which were used in the MR CLEAN trial, and (b) previous trials did not use vessel occlusion as an eligibility criteria because radiological diagnosis of large artery occlusion was missing due to limited availability of CTA at that time [848-850].

**Phase 2 clinical trials with second-generation devices**

The second-generation MT devices, Solitaire flow restoration device and Trevo retriever, are based on stent retrievers, which are very effective in capturing thrombus, and produced excellent vessel recanalization. Two phase 2 clinical trials, SWIFT (Solitaire Flow Restoration Device Versus the Merci Retriever in Patients with Acute Ischemic Stroke) and TREVO 2 (Trevo Versus Merci Retrievers for Thrombectomy Revascularization of large vessel occlusion in Acute Ischemic Stroke), demonstrated that stent retrievers achieved better clinical outcomes by increasing recanalization of large artery occlusions, than the first-generation Merci Retriever and Penumbra System devices [825, 826, 851, 852]. The SWIFT study assigned eligible patients to receive MT treatment with either MERCI device (n=55) or Solitaire stent retriever (n=58). The Solitaire group showed significantly improved recanalization, as defined by a Thrombolysis in Myocardial Infarction (TIMI) Score of two or three (61% vs. 24%, P<0.0001), and better functional outcomes (58% vs. 33%, P<0.0001) than the MERCI group. The TREVO trial produced similar outcomes, with the TREVO device achieving better recanalization (86% vs. 60%, P<0.0001), defined by Thrombolysis in Cerebral Infarction (TICI) score of 2 or 3, and improved long-term clinical outcomes (40% vs. 22%, P<0.013) in comparison to the first generation Merci retriever. No difference in the incidence of secondary intracerebral haemorrhage (sICH) was reported between TREVO and Merci groups.

**Phase 3 clinical trials with second-generation devices**

MR CLEAN was a Dutch endovascular trial that assigned 500 patients to the intervention arm (IAT with standard therapy; n=233) and the control arm (standard therapy; n=267) alone [842]. All patients in the control arm received IV-rtPA. Endovascular procedures were performed using second-generation stent retrievers.
Stent retrievers were used in 81.5% (190/233) of patients who received IA treatment. MR CLEAN included patients presenting within six hours of stroke onset, aged 18 and above, with a minimum NIHSS score of two, and large artery occlusion confirmed on CTA. MR CLEAN trial did not have an upper age threshold for eligibility unlike previous trials, where patients above 80 years were excluded. Moreover, an ASPECTS score was not used in the randomization of patients. Patients who received MT and standard treatment (intervention arm) showed good recanalization (defined by TICI score of 2b or 3) in 59% (115/196) of the patients. This arm also demonstrated improvement in good outcome at 90 days (32.6% vs. 19.1%; OR = 1.67 [95% CI 1.21 to 2.3]), and significant decrease in brain infarct at 24h (49 mL vs 79 mL, P<0.01) in comparison to the standard medical therapy group. The number need to treat (NNT) was 7.1. No significant difference in the occurrence of sICH or death was found in between the two groups.

The ESCAPE trial randomised 315 patients, 150 in control arm and 160 in the MT arm, for an extended therapeutic time window of 0-12 hours [843]. Strict inclusion criteria including NIHSS>5, pre-morbid Barthel index of 90 and above, large artery occlusion confirmed on CTA, ASPECT score of ≥6, and good or moderate leptomeningeal collateral status on CTA was applied. In comparison to previous trials, ESCAPE used CTA, preferably multiphase CTA, to select patients based on the neuroimaging assessment of the site of occlusion and collateral status. The trial was unique given the pioneering enrollment goals, including door to puncture times of less than 60 minutes, and door to recanalization time of fewer than 90 minutes. Given the trial was stopped following the announcement of MR CLEAN results, an interim analysis showed good recanalization rates (72%), defined by TICI score of 2b or 3, and significantly better functional outcomes in the MT group vs. the standard therapy (53% vs. 29%, P<0.001). Concerning safety endpoints, MT showed reduced mortality rate (10% vs. 19%, P=0.04), and comparable sICH rates (3.6% vs. 2.7%, P>0.05) vs. the control group.

The SWIFT-PRIME trial boasted the maximum number (n=39) of recruitment centres spanning the United States and Europe [844]. Between 2012 and 2014, 196 patients presenting within 6 hours of stroke onset were enrolled and assigned to treatment groups: MT (using Solitaire FR stentriever) plus standard therapy, IV-rtPA (n=98), or
standard therapy alone (n=98). For randomization, patients with NIHSS scores between 8 and 30, aged between 18 and 80 years, and a premorbid score of mRS<1 were considered. The SWIFT-PRIME study used neurovascular angiography imaging (CTA/MRA) to identify patients with large artery occlusion prior to randomization. SWIFT PRIME introduced an automated post-processing imaging pipeline software for penumbra profiling (RAPID), although its use was not mandatory or part of the inclusion criteria. Infarct core volume was estimated from CTP using RAPID. A cut-off of infarct core volume of >50 mL on CTP was used to exclude patients. The ischemic core was defined by regional cerebral blood volume (CBV) or delayed time to peak (TTP) of the residual function. Moreover, patients with baseline ASPECTS score of less than six were excluded. The SWIFT PRIME study achieved the highest recanalization (defined by modified TICI score of 2b or 3) rate of 88% for the MT arm in comparison to the other four contemporary endovascular trials. The MT group showed 24.7% improvement in the functional outcome at 90 days (mRS 0-2: MT 60.2% vs standard therapy 35.5%, P<0.001). Efficacy of MT in SWIFT-PRIME (60.2%) was higher than MR CLEAN (32.6%) and ESCAPE (53%) trials. No significant group differences in sICH risk (0% vs 3%, P=0.12), or 90-day mortality (9% vs 12%, P>0.05) were found.

EXTEND-IA was an Australasian trial involving ten centres across Australia and New Zealand that enrolled 70 patients who were randomised to receive IV-rtPA alone, or MT using Solitaire FR stentriever plus IV-rtPA [810]. Like the SWIFT PRIME, EXTEND-IA also required administration of MT and IV-rtPA within 6 and 4.5 hours of onset of stroke symptom, respectively. However, unlike other trials, there was no restriction on stroke severity as a criterion for patient inclusion. Moreover, age or ASPECTS score were not part of criteria for randomization. Pre-morbid functional status of the mRS 0 to 2 was required for inclusion in the study. With regards to neuroimaging selection criteria, CTP for detection of favourable penumbra using RAPID software similar to SWIFT PRIME (CTP core volume less than 70 mL or mismatch ratio greater than 1.2), and CTA for large artery occlusion diagnosis were used to randomise patients. The primary outcome for the EXTEND-IA study was a reduction in the NIHSS score of 8 or higher, a score of 0 or 1 on mRS at 90 days, or reduction in perfusion lesion volume. EXTEND-IA showed the highest improvement in functional outcome at 90 days in comparison to other four-endovascular trials (31.4%).
and recanalization rate (86.2%) comparable to SWIFT PRIME. The number needed to treat (NNT) was the lowest for EXTEND-IA (NNT: 3.2 (EXTEND-IA) vs. 7.1 (MR PRIME) vs. 4.2 (ESCAPE) vs. 4 (SWIFT-PRIME) vs. 6.3 (REVASCAT)). The MT arm showed significantly improved functional outcome compared to the standard therapy arm (70% vs. 40%, P=0.001). The investigators reported no significant differences in risk of sICH and mortality between the two groups.

REVASCAT was a Spanish trial involving four tertiary hospitals that enrolled 206 AIS patients presenting within 8 hours of the symptom onset randomised to receive MT with the Solitaire FR plus the standard medical therapy (n=103) or standard medical therapy including IV-rtPA for eligible patients (n=103) [478]. Clinical inclusion criteria included age between 18 and 80 years, NIHSS of 6 or more, and a pre-treatment mRS score of less or equal to one. Neuroimaging was used to select patients with large vessel occlusion (on CTA or MRA). Moreover, only patients with ASPECTS scores of seven and above on NCCT or six and above on DWI MRI were included. Interestingly, the inclusion criteria were revised to age<85 years and ASPECTS score of eight or above on NCCT after the enrollment of 160 patients. The REVASCAT trial showed 65.7% recanalization for patients in MT arm, and a significant reduction in brain infarction volume at 24 h (16 mL vs. 39 mL, P=0.02) vs. standard medical therapy. No significant differences were noted in the rates of sICH (1.9% vs. 1.9%, P>0.05) or mortality (18.4 vs. 15.5, P=0.06) between the two groups. Patients in the MT arm were more likely to have a better functional outcome at 90 days (43.7% vs. 28.2%; OR 2.1, 95% CI 1.1 to 4). In summary, these five endovascular randomised controlled trials have consistently shown that MT significantly improves reperfusion and functional outcome at 90 days without an increase in mortality compared to patients receiving standard medical therapy. Use of advanced imaging features such as ASPECTS scale or perfusion imaging may assist in selecting patients who are most likely to benefit from a combined approach. Advanced imaging helps to differentiate between salvageable tissue and irreversibly dead core. Unfortunately, selection of patients using advanced imaging excludes many patients who otherwise would have received intra-arterial treatment.

Two new clinical trials, the Trial and Cost-Effectiveness Evaluation of Intra-arterial Thrombectomy in Acute Ischemic Stroke (THRACE) [853] and the Assess the
Penumbra System in the Treatment of Acute Stroke (THERAPY) [854], have addressed these shortcomings by keeping selection criteria to a minimum except for the use of angiographic technique such as CTA or MRA to localise and confirm the arterial collusion. The THRACE trial, conducted across 26 centres in France, included 336 patients, aged 18 to 80 years and NIHSS score ranging between 10 and 25, presenting within 5 hours on symptom onset with moderate to a severe stroke caused by the large artery occlusion of the anterior circulation (radiologically confirmed on CTA), out of which 195 received IVT, and 141 received combined IVT and MT treatment, without a selection based on advanced imaging-based criteria. Combined IV-rtPA and MT provided a higher rate of functional outcome at 90 days (54.2% vs. 42.1%). No significant differences in mortality and sICH risks were noted between the MT and control arms. The THERAPY trial, undertaken across four centres in the United States, selected patients with AIS presenting within 4.5 h of symptom onset who have evidence of large clot burden (clot length≥8mm) in the anterior circulation [854]. CTA was used to identify patients with large vessel occlusion. Patients with NIHSS score of eight and above and age between 18 and 85 years were included for randomization. Patients were then treated with both combined IV-rtPA and IAT with the Penumbra aspiration system (a new technique of aspiration thrombectomy) or with the IV-rtPA alone. The results showed a positive trend, though not significant, of aspiration thrombectomy towards better functional outcomes (38% vs. 30.4%). Interestingly, the intervention arm showed a considerable reduction (11.9%) in mortality (mortality in intervention arm vs. control arm: 12% vs. 23.9%). No significant difference in sICH risk was observed (10.9% vs. 11.3%).

To sum up, MT has a number of advantages over systemic thrombolysis [819]: (a) MT yields higher rates of revascularization, and reduced rates of long-term functional outcome, in a select group of AIS patients with large vessel occlusion, compared to IVT alone [812, 813, 855-857], (b) MT uses an extended therapeutic time-window up to 8 hours, beyond the 4.5 hour restricted time window applicable to IVT, from the onset of stroke [808, 858-860], (c) MT presents a viable alternative to patients with large vessel occlusion who respond poorly, vis a vis poor recanalization rates and risk of haemorrhage, with systemic therapy [860], or those who have contraindications to the use of systemic thrombolysis, and (d) MT is efficient in dissolving clots which are resistant to enzymatic degradation, such as old and large clots with hardened fibrin.
and cross-linked thrombi containing calcium and cholesterol crystals, which have poor recanalization yields with systemic thrombolysis [825, 859, 861].

**Combined intravenous and endovascular therapy: a multimodal reperfusion therapy (MMRT) approach**

The idea behind combining intravenous and endovascular approaches, also called bridge therapy [862-867] or multimodal reperfusion therapy (MMRT), is to take advantage of best of both approaches by allowing fast and early access to IV-rtPA within the first 4.5 hours of stroke onset, and superior recanalization rates even for delayed time windows beyond 4.5 hours using endovascular therapy [868]. Notably, the Interventional Management of Stroke (IMS) I [869] and II [870] trials were conducted to investigate the feasibility and safety of combined interventions: low dose, 0.6 mg/kg, IV-rtPA followed by intra-arterial rtPA within 3 hours since stroke onset, to recanalization of AIS. The bridge IV and IA therapy were not significantly different from the IV-rtPA alone as both yielded similar proportions of ICH, rates of mortality and MRS at 90 days. The subsequent IMS III trial also found no additional benefit of bridge therapy compared with IV-rtPA alone [871]. One of the important factors that may have played a role in these results is the time delay (approximately 32 minutes) between the IV-rtPA and initiation of intra-arterial therapy [482, 869]. Unlike the neutral results of IMS III trial, a recent meta-analysis reported a significantly strong association of combined intravenous-IA thrombolysis over IV fibrinolysis alone with favourable outcome, reduced mortality, and improved recanalization rates [872].

The Stent-Assisted Recanalization in Acute Ischemic Stroke (SARIS) trial, conducted to investigate the safety of intracranial stent deployment within 8 hours of stroke onset, demonstrated expeditious recanalization, and favourable outcomes at 30- and 180 days clinical follow-up [873, 874]. Stent deployment averts arterial reocclusion and thrombus reformation in cases with partial embolectomy or arterial stenosis. The utility of self-expanding stents (SES) has been explored. The SES yields dramatically high recanalization rate of up to 90% by a combination of balloon angioplasty and stent implantation [873]. Undoubtedly, the bridge endovascular therapy using MT, intracranial stent deployment, and IV thrombolysis allows an extended time window, and therefore a higher proportion of revascularisations and improved clinical outcomes in AIS patients with large artery occlusion of anterior circulation [652].
A number of MMRT approaches including combined IV-rtPA and IA-tPA [862-864, 866, 867, 875], IV-tPA followed by multimodal endovascular therapy [876], combined IA thrombolytics and glycoprotein IIb/IIIa inhibitors [877], IA administration of microbubbles and continuous 2-MHz ultrasound insonation [865], IA-tPA followed by stenting[878, 879] or angioplasty or both [716, 880], IA urokinase and mechanical clot disruption following failed IV-tPA [881], MT using balloon angioplasty and adjuvant systemic thrombolysis (IV-tPA, IA urokinase, both IV-tPA and IA-urokinase, and IV and/or eptifibatide) [882], MT (MERCI retrieval, angioplasty/stent) with or without adjunctive IA-tPA/Urokinase [883], MT using clot retrievers and angioplasty with intracranial or extracranial stenting [877], have demonstrated considerably improved recanalization, reperfusion and clinical outcomes [716, 866, 876, 877, 880, 884, 885]. Gupta et al (2011) reported significantly higher recanalization rates for multimodal therapy (MT using intracranial stenting in conjunction with IV/IA thrombolysis) (74% [435/584]) in comparison to pharmacologic treatment only (61% [160/264]), or MT only (63% [173/274]) in a large retrospective cohort of 1122 AIS patients involving the anterior circulation who received IAT within 8 hours of stroke onset [879].

Drawing from the success of these studies, an endovascular MMRT approach using pharmacological thrombolytics (IA lytic drugs), in conjunction with MT using mechanical devices such as clot retrievers, angioplasty with stenting, aspiration devices, and stentriever is being increasingly adopted as the treatment of choice for stroke due to large vessel occlusion [879, 884]. Endovascular MMRT or bridge therapy offers a safe alternative for AIS patients, with large intracranial vessel occlusion, who fail to reperfuse with systemic thrombolytic drugs.

**Role of collaterals in penumbral sustenance and recanalization**

Recanalization is positively associated with favourable clinical outcome and increased survival rates in acute ischemic stroke [505, 660, 777, 886-889]. A meta-analysis of 53 studies encompassing 2066 patients reported strong association of recanalization with the good functional outcome (OR 4.43, 95% CI 3.32 to 5.91), and reduced mortality (OR 0.24, 95% CI 0.16 to 0.35) at three months in AIS [872]. The rate of recanalization classified based on intervention: spontaneous, IV fibrinolytic, intra-arterial fibrinolytic, combined IV and intra-arterial thrombolysis or MT was 24.1, 46.2,
63.2, 67.5, 83.6 percent, respectively. Early recanalization is associated with rapid clinical improvement in some patients [890]. However, despite early recanalization some patients, who are otherwise unresponsive to treatment over short-time follow-up, may show delayed recovery or favourable long-term outcome suggesting the possibility of an “ischemic stunning” or “stunned brain” syndrome [891]. Recanalization enables restoration of cerebral blood flow to the hypoperfused brain region surrounding the infarcted ‘core’. This area of the brain with reversible ischemia surrounding the core is called ischemic penumbra [892]. Animal studies have indicated that considerable salvage of penumbral tissue is possible on the restoration of blood flow to the hypoperfused brain area, even after 24 h, irrespective of time of reperfusion [893, 894]. From a pathophysiological standpoint, the survival of penumbra, independent of time, for up to 48 hours has been reported using a ligand that selectively binds to hypoxic but viable tissue ([18]F)fluoromisonidazole), and positron emission tomography (PET) on consecutive patients presenting within 48 h of AIS [895]. As expected, the penumbra reduces over time; it is observed in 90-100% of stroke patients in the first three h after stroke onset [896], 75-80% of patients 6h after stroke onset, and approximately 33% of patients 18 h after stroke onset [897].

Despite the success of endovascular procedures, a number of patient-specific factors may determine the response to treatment such as (a) collateral circulation [715, 898, 899], (b) site of vessel occlusion [782], (c) onset to angiographic reperfusion time [900-902], (d) hyperglycaemia [903-905], and (c) location of cerebral ischemia [898, 906]. In the case of cerebral ischemia, collaterals compensate for the sudden drop in CBF in the hypoperfused area by the maintenance of a continual blood supply to ischemic penumbra. For recanalization to translate into positive outcomes, adequate collaterals must delay the infarction of tissue until recanalization is achieved [907]. Collaterals can sustain the penumbra even in the absence of reperfusion or recanalization. According to a meta-analysis, good baseline collaterals were associated with favourable outcome at 90 days, decrease in the risk of symptomatic ICH, and decrease in risk of death at 90 days in patients with AIS receiving endovascular treatment [899]. Good baseline collaterals have been found to be associated with 24 hours perilesional hyperperfusion [2], good clinical outcome [470, 493, 700, 718, 899], lower rates of symptomatic ICH and mortality [899], improved radiologic outcome [493], cortical infarct volume [491], good reperfusion [701], and stroke severity [701].
On the other hand, delayed-cortical vein filling was independently associated with reduced baseline collateral status in AIS [2]. Collateral grading is used to determine recanalization rate after endovascular reperfusion therapy [495, 715]. Collateral pathophysiology may have predictive value, and patent collaterals may help boost reperfusion [908]. Some scales and grading tools are available for reliable and quick assessment of the patency of collaterals by visual examination of multiphase CTA [470].

**Patient profiling using neuromaging-based approaches**

Time is a crucial factor in stroke management. Therefore, access to neuroimaging becomes an important factor as it can rule out haemorrhage or tumour during emergency scanning work-up (using NCCT). Also, depending on whether the patient is suitable for systemic thrombolysis (with IV-rtPA) or not, he or she may be administered an alternative treatment regimen such as endovascular MT as a standalone intervention or in conjunction with IV-rtPA or IA-rtPA. The two commonly used imaging modalities are CT and MRI [909-911]. The overall goal of imaging-based approaches is to: (i) extend the restricted time window to allow treatment of more patients, (ii) better tailor the intervention, systemic thrombolysis or MT, and (iii) identify a subgroup of patients who are more likely to benefit from the appropriate reperfusion therapy, and (d) monitor the therapeutic response to the reperfusion modality.

At the moment, CT remains the preferred imaging modality for the screening and treatment of stroke patients, mainly due to its ubiquity, although, with the greater availability of advanced MRI scanners, this may change in the future. NCCT is very efficient in detecting bleed due to haemorrhage but has limited power to show early ischemic changes such as loss of gray-white matter distinction, cortical swelling, and parenchymal hypo-attenuation or sign of arterial occlusion [243, 534, 912-914]. NCCT has a high degree of inter-rater variability for assessment of new ischemic changes [256, 412], and poor accuracy in the diagnosis of hyperacute AIS [256, 433, 915, 916]. Early ischemic changes such as parenchymal hypoattenuation and isolated focal swelling on NCCT may be indicative of infarct core and penumbral tissue respectively [686, 917]. MRI techniques such as diffusion-weighted imaging and perfusion-weighted imaging are very sensitive to early pathological changes of ischemic infarction and subtle brain oedema [485, 522, 918, 919]. The ability to detect ischemia
or morphological abnormalities much sooner than the CT gives MRI a decisive edge. However, conventional MRI techniques have a disadvantage of being very insensitive to haemorrhage [243, 534, 912-914]. However, a recent study reported similar detection accuracy for acute haemorrhage with gradient echo (GRE) MRI or NCCT [534]. For chronic ICH, GRE was more sensitive than CT [534]. Notably, GRE showed higher detection accuracy for HT in AIS than either FLAIR or NCCT [535]. GRE may identify HT even before NCCT. Cerebral microbleeds that go undetected on NCCT can be easily identified on GRE.

CT is by far the most robust modality in neuroradiological haemorrhage validation. CT can be used for the first-hand detection of other symptomatically similar presentations such as a tumour, subdural or epidural hematoma, subarachnoid haemorrhage, and intraparenchymal haemorrhage. One of the main requirements in all thrombolysis trials is to rule out haemorrhage because thrombolytic therapy may inflict fatal bleeding in stroke patients [421, 645, 920, 921]. Also, the ECASS trial demonstrated that early signs of significant/severe cerebral infarction, such as sulcal effacement, mass effect, oedema, and loss of insular ribbon, show strong association with an increased risk of bleeding/intracerebral haemorrhage in patients who receive thrombolytic therapy [414, 922, 923]. Therefore, the presence of these early signs should also be carefully observed during initial head CT examination and should be taken as a contraindication to thrombolytic therapy as suggested by guidelines issued by the American Academy of Neurology and American Health Association [909-911, 924]. Some guidelines have incorporated these more subtle signs into recommendations against thrombolytics when present. However, these CT criteria were not included in the NINDS trial for making decisions on whether to select the patient for thrombolysis [645, 749].

As per the recommendations by the American Heart Association (AHA)/American Stroke Association (ASA), either non-contrast CT or MRI should be completed within 25 min of patient arrival at the hospital, with a further 20 minutes for interpretation by a skilled stroke physician [909, 910]. Other techniques such as PET, cranial Doppler, and Xenon CT are still in their experimental phase [918, 925-929].

The future of penumbra imaging lies in multimodal CT or MRI techniques. The CT perfusion (CTP) based mismatch concept is shown in Figure 2. Multimodal techniques
provide a rapid, reliable and comprehensive assessment. Some of the metrics that can be studied using multimodal imaging are: (i) imaging validation of ischemic injury, and estimation of its size, (ii) status of vascular perfusion, (iii) presence and site of occlusion in the intra-extra cranial vessels, (iv) health of the collateral flow, and (v) exclusion of intracranial haemorrhage. This is achieved by an imaging routine that combines parenchymal, penumbral, and vascular imaging [574, 930]. Furthermore, this enables improved selection of patients who will benefit from reperfusion therapies [541, 574]. All five successful endovascular MT trials used CTA to select patients with larger artery occlusion stroke. Furthermore, CTA was also used in selecting patients with good collaterals in the ESCAPE trial [843]. ESCAPE and EXTEND-IA trials also included CTP enabled lesion volume for selection of patients with small core [810, 843]. Also, NCCT was used to exclude patients with ICH, and to calculate the ASPECTS score. The phenomenal success of these trials could mainly be attributed to the use of second-generation stentrieverers, and application of CT/MR or DSA to include patients with intracranial occlusion of the MCA (M1 or M2), distal intracranial ICA, or anterior cerebral artery (ACA) (A1 or A2).

Previous trials using MR or CT diffusion-perfusion mismatch or penumbral imaging such as EPITHET, DIAS-1, DIAS-II, DEDAS, and MR RESCUE failed to demonstrate clinical benefit. From the clinical standpoint, multimodal imaging is also a useful strategy to determine the age of the stroke in those with an unknown time of onset (commonly known as “wake-up” strokes). The time required for running the multimodal CT sequence is <10 min, whereas for multimodal MRI it is closer to 15–30 min [931-933]. A list of the various thrombolytic trials/studies stratified by imaging modality is given in Table 5.

Prognostic Neuroimaging and Clinical Scales
With the number of thrombolytic clinical trials mushrooming over the last decade or so, it is imperative to have standardised protocols so that the clinical and neuroimaging evaluation can be replicated at different hospitals, as well as in research labs. To this end, neuroimaging scores have been developed to assist physicians and researchers make standardised therapeutic decisions and prognostic predictions. A list of various standardised scales used to predict clinical outcome of reperfusion therapy after AIS is given in Table 6.
Alberta Stroke Programme Early CT Score (ASPECTS) is one such scale developed for CT imaging [429, 744, 934]. An illustration to explain ASPECTS scoring system is shown in Figure 3. ASPECTS is a standardised and validated 10-point scoring scale developed to quantify the extent of early ischaemic changes in the MCA territory on non-contrast CT [429]. The ASPECTS scoring system is based on evaluating specific structures on two axial CT slices. One limitation of ASPECTS is that it is highly dependent on the imaging plane. Furthermore, many other surrogate-scoring systems have been developed using ASPECTS. One such scoring scale is the posterior circulation ASPECTS scale, which is a predictive scale for quantifying posterior circulation changes [935]. In combination with specific clinical markers, ASPECTS has been used to predict response to both intravenous [936] and intra-arterial therapies. Moreover, it has also been used in many other applications, such as in combination with (1) quick symptomatic intracranial haemorrhage risk assessment before thrombolytic therapy [937], (2) magnetic resonance diffusion-weighted imaging [938], and for (3) scoring of cerebral blood volume [453]. The ASPECTS score is also used as a standardised scoring scale in some clinical trials. Two such trials are Interventional Management of Stroke 3 (IMS III) trial [482] and the Solitaire FR Thrombectomy for Acute Revascularisation (STAR) trial (NCT01327989) (ClinicalTrials.gov) [939].

Other examples of such scoring paradigms are the newly designed ABC/2 method [940-942], the Boston Acute Stroke Imaging Scale (BASIS) [943-945] and the 1/3rd MCA territory method (hypoattenuation in less than one-third of the MCA) [946]. The “ABC/2” method has been used to rapidly and accurately calculate the volume of infarcted tissue. A and B are calculated on the axial DWI slice with the largest hypoperfused region. C is computed from the number and thickness of axial CT slices the abnormality appears on (slice thickness multiplied by the number of axial slices). The volume of the infarcted tissue is given by the formula: volume = [(a×b×c)/2]. On an average, a large MCA infarction, covering one-third of the MCA territory, between 70 and 100 cm³ is associated with poor clinical outcome.

Along with neurological scores, measurement of neurological deficits is essential. NIHSS is the most widely used system, being reproducible and validated and providing
a quantitative assessment of stroke severity [947, 948]. NIHSS is used in clinical trials as well as in routine clinical practice to quantify stroke severity. The NIHSS score can also monitor stroke progression, recovery rate and response to thrombolytic therapy or rehabilitative intervention such as transcranial direct current stimulation (tDCS). Initial stroke severity is a strong predictor of both short and long-term outcome (including mortality) in acute ischaemic stroke patients. For NIHSS >20, patients have only a 4–16% chance of good outcome at one year, whereas in patients with NIHSS<10 the chance increases to 60–70% [5-7]. In light of these findings, NIHSS should be a significant covariate in the selection of patients suitable for an appropriate thrombolytic therapy, or in overall stroke outcome assessment.

**Limitations and Unresolved Questions of Endovascular Reperfusion Therapy**

Despite the substantial advantages of MT, this is only offered to a limited number (5-10%) of AIS patients due to procedural issues [857]. Currently, vascular imaging such as CTA or MTA is not routinely performed. The treating physician needs to make a rapid decision based on the vascular imaging (whether or not angiography shows a large vessel occlusion), assessment of ischemic penumbra (based on CT or MR perfusion imaging), time since the onset of stroke symptoms, along with other baseline clinical factors and previous history (such as significant past trauma, haemorrhage or stroke) [822, 853, 949]. The need for advanced imaging, especially CTA, implies that only a few select AIS patients would be suitable for MT. MT with current devices is not well suited for AIS with occlusions in distal locations and with penetrator occlusions due to difficulty in navigating with the catheter, increased risk of intraprocedural vessel perforation, and a higher risk of mortality [833, 950]. Also, patients with in situ intracranial atherothrombi (in situ atherosclerotic plaque with supervening thrombosis), may be more suited for balloon angioplasty and stenting over MT [833]. MT yields superior recanalization rates with occlusions of cardioembolic origin [951] or proximal aortocervical arterial source [833]. Delayed treatment may lead to further shrinking of salvageable penumbral tissue due to absence or insufficient reperfusion. MT is a highly specialised interventional procedure; as such, it is strongly recommended that MT be performed in a comprehensive stroke unit with a well-equipped neuro intervention suite by an interventional neuroradiologist or an endovascular specialist with experience in the procedure, or a credentialed neuro interventionist along with a team of stroke neurologists, and nursing staff [952]. Lack
of experienced practitioners (such as credentialed neuro interventionist, endovascular specialists) [952], along with logistic difficulties such as prehospital delays (e.g., prolonged transfer time to rural hospitals), and interfacility transfers, make MT quite unlikely to be widely implemented. A comprehensive national and international stroke care policy need to be adopted to address the logistical and other systemic deficiencies in the present healthcare system.

In addition to the limitations of present reperfusion therapies in AIS settings, a number of unresolved questions or issues need to be addressed.

**Extending the time window of MT beyond 6 hours**
Evidence for efficacy and safety of endovascular reperfusion beyond 6h is still insufficient given that no randomised trial has used this as an inclusion criterion. However, a recent meta-analysis has indicated the benefit of endovascular MT plus medical therapy up to 7.3 hours after the symptom onset [846]. Some trials including the premise of the Diffusion and Perfusion Imaging Evaluation for Understanding Stroke Evolution (DEFUSE 3), the DWI or CTP Assessment With Clinical Mismatch in the Triage of Wake-Up and Late Presenting Strokes Undergoing Neurointervention (DAWN), the Perfusion Imaging Selection of Ischemic Stroke Patients for Endovascular Therapy (POSITIVE) and Imaging-Guided Patient Selection for Interventional Revascularization Therapy (START) trials seek to extend the time to endovascular reperfusion. DEFUSE 3 selects AIS patients using penumbral mismatch on CTP or MRI who may benefit from MT between 6 hours and 16 hours post onset [953]. Similarly, the goal of the POSITIVE trial is to use appropriate imaging selection to improve stroke-related disability, and functional outcome in AIS patients treated with MT presenting between 6 to 12 hours who are either ineligible for or refractory to IV-tPA treatment [954]. DAWN, is presently recruiting patients in which treatment with Trevo MT is initiated within 6-24 h after stroke onset [955].

**General anaesthesia vs. conscious sedation**
The type of anaesthesia, whether general anaesthesia (GA) or conscious sedation, has implications for outcomes in AIS patients during and after endovascular treatment [956]. This is a subject of ongoing debate given that several retrospective studies have hypothesised that GA may be associated with periprocedural hypotension that may
cause poorer clinical outcome [956-958]. A consensus is still missing over the anaesthetic management of AIS patients during IAT [959]. The MR CLEAN pre-trial study group found that non-GA was significantly associated with good clinical outcomes in AIS patients undergoing IAT [959]. Three randomized trials, Sedation vs Intubation for Endovascular Stroke Treatment (SIESTA) [960], the General or Local Anaesthesia in Intra-arterial Therapy (GOLIATH) [961], and the Sedation Versus General Anaesthesia for Endovascular Therapy in Acute Stroke (ANSTROKE) [962], are investigating the impact of anaesthesia type on neurological outcome in IAT, aiming to investigate whether conscious sedation is the optimal anaesthesiologic management modality in endovascular stroke therapy [848] [963].

**Posterior circulation stroke**

AIS in the posterior circulation is associated with poor prognosis with standard medical therapy (IV-tPA) [964, 965]. Basilar artery occlusion (BAO) is a form of posterior circulation stroke that results in higher rates of poor functional outcome and mortality if not recanalized [964, 966]. A systematic analysis of 420 BAO patients treated with IVT (76) and IAT (344) found that without recanalization only 2% patients were likely to have good outcome [966]. Recanalization was more common in IAT-treated patients than those who received IVT (65% (225/344) vs. 53% (40/76). The success of recanalization of acute BAO following IVT depends on thrombus length [779]. Another analysis on 592 BAO patients drawn from a prospective register also challenged the notion of unequivocal superiority of IAT over IV-tPA in BAO patients [967]. Interestingly, bridge therapies combining IAT using modern MT devices and IV-tPA have yielded good recanalization and improved survival rates for acute BAO [968-970]. Based on these findings, a multicentre randomised control trial, Basilar Artery International Cooperation Study (BASICS), was conceived, and is currently underway, to evaluate the efficacy of IAT plus standard medical treatment versus standard therapy alone in patients with acute BAO stroke [971, 972]. Recent studies have shown MT with the Solitaire FR device is associated with high recanalization rates and favourable outcome [973, 974].

**Minimising the delay to endovascular reperfusion**

Direct Transfer to an Endovascular Centre Compared to Transfer to the Closest Stroke Centre in Acute Stroke Patients With Suspected Large Vessel Occlusion (RACECAT)
is a prospective, randomised controlled trial which aims to evaluate the effectiveness of direct transfer to an endovascular stroke centre based on identification of suspected large vessel occlusion in AIS patients using a prehospital screening tool, rapid artery occlusion evaluation (RACE) scale, in comparison to the transfer to the closest local stroke centre [975]. The motivation behind this trial is to minimise the time to endovascular reperfusion. Triaging of AIS patients for their eligibility to IAT requires CT for determination of the presence of large artery occlusion. However, it may be possible to select patients based on non-imaging scales that can be easily applied without extensive training. Staff with emergency medical services (EMS) could be trained to use these scales (e.g., NIHSS, RACE) in screening AIS patients for their eligibility to receive reperfusion therapy.

In addition to the above issues, other treatment options such as the use of antithrombotic medications along with reperfusion therapy are also being explored. A phase III trial, Multi-Arm Optimisation of Stroke Thrombolysis [976], is investigating the efficacy of IV delivery of Argatroban and Eptifibatide in combination with rtPA in AIS. This strategy may be extended to patients who undergo MT. Some trials are currently underway or are in the planning phase to address the issues that limit the efficacy of endovascular reperfusion. A comprehensive list of ongoing and future trials is shown in Table 7.

**Conclusions, Discussions, and Future Recommendations**

The only approved treatment for acute ischemic stroke is reperfusion therapy using systemic thrombolysis or endovascular mechanical thrombectomy. The recent success of endovascular trials has revolutionised the way large artery occlusion stroke patients are managed. AHA/ASA has accordingly updated its guidelines for stroke care. The recommendation is to use MT with stent retriever in combination with standard therapy (IV-rtPA) in AIS patients, aged ≥18 years; baseline ASPECTS score ≥6, baseline NIHSS score≥6 with angiographically confirmed large vessel occlusion, who present within six h of symptom onset (Level 1A evidence) [475, 977]. The workflow algorithm detailing the standard of care for IV-tPA and mechanical thrombectomy in AIS patients is shown in Figure 4.
Endovascular therapy also provides an alternative to systemic thrombolysis for patients who fail to reperfuse with IV-rtPA or those who are not eligible for IVT due to restricted time-window, or those with “wake-up” stroke, or contraindications [879, 884, 965]. However, due to a shortage of neurointerventionists or endovascular specialists experienced in the procedure [952], procedural complexity, high costs, pre-hospital delays, interfacility transfers, and a limited number of institutions that offer endovascular treatment, only a select number of patients will likely be offered this therapy [978]. Future efforts to shorten the endovascular procedural time, build infrastructure to provide MT, and increase access to endovascular facilities are required [768]. Comprehensive stroke centres with access to IV and IA techniques, trained stroke neurointerventionists, imaging-guided treatment workflow, and access to advanced neurosurgical support will pave the way for high-end stroke care delivery. Meanwhile, given the present limitations, careful selection of patients is crucial to maximise the gain for appropriate patients. It has been suggested that patients with angiographically confirmed proximal large vessel occlusion with a viable penumbra on MRI and NIHSS>18, within 8 and 24 hours of stroke onset in the anterior and posterior circulation respectively, should be given endovascular MMRT [884]. Other authors recommend a lower threshold of NIHSS ≥ 7 on the addition of advanced neuroimaging parameters including ASPECTS ≥ 6 or 7, and angiographically confirmed large vessel occlusion with moderate-to-good collaterals on multiphase CT to select candidates for endovascular MT using stent retriever [530]. With regards to MRI led patient selection based on infarct volume, endovascular therapy is also preferred for large artery occluded AIS patients with small infarct defined by early DWI volume< 70 mL, and an accessible proximal occlusion. Endovascular MMRT or standalone MT is not suitable with distal occlusion or with lacunar stroke. High revascularization yields obtained using (a) MMRT or combinatorial approaches that include IA/IV thrombolytic agents, stenting, angioplasty, aspiration, clot retrieval, (b) second generation MT devices such as Solitaire and Trevo, (c) combined MT interventions using suction embolectomy with large bore microcatheter such as Penumbra MAX [836-839] and stent retrievers, and (d) with latest generation Penumbra 3D separator [832, 840, 841] are encouraging.

The patient selection or individual profiling of appropriate AIS patients for reperfusion therapy can be improved by a combined approach that includes both clinical and neuroimaging metrics. The present time-window based approach to select patients for
IV-rtPA have shown good outcomes for a time window of 3-4.5 hour after stroke onset. However, this approach excludes a large number of AIS patients who do not meet the time-based criteria and also does not take into account baseline collateral status that apparently plays a crucial role in the sustenance of penumbra beyond the time-window. Outside the current time-window, advanced neuroimaging can provide vascular, perfusion and anatomical information on the state of the brain parenchyma and of the neuro-vasculature [979]. The success of endovascular trials including MR CLEAN has been, to some extent, attributed to the use of neurovascular imaging such as MRA/CTA, in addition to the use of second-generation MT devices, to select AIS patients with large artery occlusion in the anterior circulation. DWI-MRI is superior to NCCT for detection of focal ischemia and differential diagnosis of stroke mimics. However, previous trials did not show clinical benefit for MRI-based approach to select patients for IV-rtPA. Therefore, MRI or neurovascular imaging should be used only if it does not delay IV-rtPA therapy to eligible candidates with AIS who present within 4.5-hour time-window. Advanced imaging techniques such as CTA, CTA-source imaging [681], or CTP, and the use of multiphase multidetector CT scanners [494, 673, 700], can give additional insights on collateral health [2, 492, 495-497, 674, 710, 712], cortical venous pattern and dynamics [3, 4], and robust estimates of infarct core [252, 654, 686, 704, 980]. A novel biomarker identified on dynamic CTA imaging in AIS, delayed cortical vein filling in late venous phase, has been reported to be associated with poor collaterals and with poor angiographic status at 24 hours [2, 3]. Moreover, non-invasive ASL-MRI can be used to detect early peri-lesional hyperperfusion in AIS patients, which is a predictor of salvageable tissue and clinical outcome [2, 8, 9, 363]. The pressing need now is to find physiologic-imaging based metrics that can identify those who will benefit from reperfusion therapy despite being outside the usual time window; such metrics include: the DWI-PWI mismatch, ASPECTS score, final infarct volume [220, 436, 550, 745, 981-989], the volume of surrounding hypoperfused tissue [845, 979, 990, 991], the status of the collateral circulation [106, 470, 492, 701, 705, 907, 992], drainage pattern or flow dynamics of cortical veins [3, 4], clot burden score [508], and location (and extent) of the clot [504, 774, 993-997]. However, these measures need further validation in larger cohorts or clinical trials. Future trials should move towards the automated user-independent platform, for computation of imaging variables and their correlation with clinical outcomes, such as infarct core volume estimation, and hypoperfused tissue volume.
estimation at varying values of time-to-peak thresholds [979]. It is also important to standardise and quantify imaging analysis, by using scores such as ASPECTS [710], to predict clinical outcome.

Future trials are needed that combine rational imaging (ASPECTS score, final infarct core volume, collateral status, peri-lesional perfusion status, cortical venous flow patterns, and site of occlusion) and clinical (such as severity of neurological deficit or baseline stroke severity, pre-morbid functional status) variables to select candidates for reperfusion therapy. Such studies will provide further validation and rationalisation of this approach by refining and stratifying the selection criteria for individual patients appropriate for reperfusion strategy [530, 646]. In conclusion, both IV thrombolysis and endovascular treatment have been incorporated into the standard of care in stroke therapy. However, further research, exploring bridge therapy or MMRT in addition to advanced imaging-based approaches to select appropriate patients, may widen the time-window for patient selection and would contribute immensely to early thrombolytic strategies, better recanalization rates, and improved clinical outcomes. The identification of precise stroke-subtype using advanced neuroimaging parameters and clinical measures can guide clinicians to choose and prescribe optimal therapy to the stroke patients.
List of Figures

Figure 1. The illustration of the fibrinolytic mechanisms: (a) tissue plasminogen activator (tPA) causes breakdown of the clot, and (b) detailed mechanism of fibrinolysis. Green arrow denotes activation/stimulation, and the red arrow indicates inhibition.

\[ tPA = \text{tissue plasminogen activator}; \ UK = \text{Urokinase}; \ PAI = \text{plasminogen activator inhibitor}. \]
Figure 2. The Penumbra Mismatch Concept.

An acute left-hemispheric ischemic infarct is shown. CTP imaging show increased CBV, decreased CBF, and prolonged MTT over the left posterior MCA territory indicating a mismatch, or tissue at risk of infarction (penumbra). Mean arterial enhancement on MIP reconstruction shows occlusion of the left MCA territory. Mismatch ratio, which is defined as the ratio of total volume (44.7 ml) divided by the core volume (11 ml), was 4.1. The total penumbra was calculated to be 33.7 ml constituting 75% of the total infarct. The core accounted for 25% of the overall infarct volume. A band map based on variable delay time (DT) is also shown. Normal DT was 0.2 second. The volumes corresponding to the DT+2, DT+3, DT+4, DT+6, DT+8, and DT+10 were 64.5 ml, 44.7 ml, 33 ml, 11.2 ml, 3.18 ml, and 1.19 ml, respectively. The CTP images were processed using MiStar® (Apollo Medical Imaging, Melbourne, Australia) software using an automatic selection of arterial input function (AIF), and venous output function (VOF).

CTP = perfusion computed tomography, MCA = middle cerebral artery, CBV = regional cerebral blood volume, rCBF = regional cerebral blood flow, MTT = mean transit time, MIP = maximum intensity projection.
Figure 3. ASPECTS scoring on NCCT, and depiction of ischemia on CTP.
A patient with acute right MCA stroke is shown. The ASPECTS scoring is based on an assessment of ten distinct regions of the MCA distribution. Two axial slices, one at the level of basal ganglia and thalamus, and other at the level of the centrum semi-oval, are used. One point is assigned to each of the regions: M1, M2, M3, M4, M5, M6, I, C, L, and IC. One point is deducted from the total score (of 10) on each side for region affected by cerebral ischemia. A score of 7 or less on ASPECTS score is associated with increased risk of neurological deterioration, poor clinical outcomes, and mortality [998]. (b-e) CTP images (5 mm axial average registered) showing increased (b) rCBV over the right MCA territory, (c) prolonged TTP, (d) decreased rCBF, and (e) prolonged MTT indicating a significant hypoperfused region, potentially viable tissue (penumbra).

ASPECTS = Alberta stroke program early CT score, NCCT = non-contrast computed tomography, CTP = perfusion computed tomography, MCA = middle cerebral artery, I = insula, C = caudate, L = lentiform nucleus, IC = internal capsule, rCBV = regional cerebral blood volume, TTP = time to peak, rCBF = regional cerebral blood flow, MTT = mean transit time.
Figure 4. Workflow diagram detailing the algorithm to select AIS patients with or without large artery occlusion for IV-tPA, MT, or IV-tPA followed by MT.

CT = computed tomography; MRI = magnetic resonance imaging; IV-tPA = intravenous trans-plasminogen activator; ICU = intensive care unit; CTA = CT angiography; MRA = MR angiography; LVO = large vessel occlusion; NIHSS = National Institutes of Health Stroke Scale; ICA = internal carotid artery
List of Tables
Table 1. List of thrombolytic trials in acute ischemic stroke based on time-window.

<table>
<thead>
<tr>
<th>Trial (n)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TIME WINDOW 0-3 HOURS</strong></td>
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</tbody>
</table>
| NINDS [645, 727]   | Time window: 0-3 h, 3-6 h  
Endpoints: A favourable outcome was defined as recovery with minimal or no deficit three months after treatment using four outcome measures: the BI ≥ 95, mRS seven ≤ 1, Glasgow Outcome Scale 8 of 1, and NIHSS score ≤ 1.  
Results: Treatment with tPA within 3 hours of the onset led to the improved clinical outcome at three months, and increase in the incidence of symptomatic ICH. |
| SITS-MOST [999]    | Time window: 0-3 h  
Endpoints: Primary outcomes were symptomatic (a deterioration in NIHSS score of ≥4) ICH type 2 within 24 h and mortality at three months. Functional independence (defined by an mRS score of 0–2 at 90 days) was a secondary outcome.  
Results: Treatment with Alteplase is safe and effective when used within three h of stroke onset. |
| TESPI [798, 1000]  | Time window: 0-3 h  
Endpoints: Primary endpoint for efficacy was the disability at day 90, dichotomized as a favourable outcome (mRS 0-2) or unfavourable outcome (mRS 3-6) [798]. The end-point for safety is symptomatic ICH radiologically confirmed on the 22-36 h post-treatment scan combined with neurological deterioration leading to an increase of ≥1 point/s on the NIHSS scale.  
Results: Higher mortality in patients aged >80 years than younger patients treated with IV-rtPA. No significant differences in symptomatic ICH nor for a favourable outcome. Thrombolytic therapy should not be a priori denied for appropriately selected >80-year old patients [1000]. |
| SITS-NEW [1001]    | Time window: 0-3 h  
Endpoints: Primary endpoints were symptomatic (deterioration in NIHSS score ≥4 or death within the first 24 h) intracerebral haemorrhage type 2 22-36 h after the thrombolysis, and mortality at 90 days follow-up. The secondary outcome was functional independence (mRS 0-2) at three-months [1001].  
Results: IV alteplase is safe and efficient in the treatment of ischaemic stroke in Asian population in congruence to the observations of SITS trials done on European population. |
| **TIME WINDOW 3-4.5 HOURS**                                                                 |                                                                                                                                                           |
| ECASS-III [421]    | Time window: 3-4.5 h  
Endpoints: Primary outcome was a disability at three months, assessed by the mRS as either favourable (score of 0 or 1) or unfavourable (score of 2 to 6). Secondary end-points included |
combined BI and RS, Scandinavian Stroke Scale (SSS) at 90 days, and 30-day mortality. Tertiary outcomes included early neurologic recovery (SSS) and duration of in-hospital stay.

Results: Significant benefit of IV rt-PA when administered up to 4.5 hours. Symptomatic ICH is significantly more likely with alteplase than with placebo. No difference in mortality between the groups.

### CASES [763] (n=1112)

**Time window:** 3-4.5 h, 0-3 h  
Endpoints: The primary endpoints were mRS at 90-days, mortality and symptomatic ICH. An mRS 0-1 (no symptoms at all or no significant disability despite symptoms, able to carry out all usual duties and activities) at 90 days was defined as a favourable outcome.

Results: IV alteplase is efficacious in treating AIS patients in 3-4.5 h window; however, there is a tendency towards increased risk of symptomatic ICH in the later time window.

### SITS-ISTR [764] (n=23942)

**Time window:** 3-4.5 h  
Endpoints: Primary endpoints were functional independence at three months, and incidence of symptomatic ICH.

Results: Safety and the functional outcome less favourable after three h.

### TIME WINDOW 4.5-6 HOURS

#### ATLANTIS-B [761] (n=613)

**Time window:** 3-5 h  
Endpoints: Primary efficacy endpoint was an excellent neurologic recovery at day 90 (NIHSS ≤ 1); Secondary endpoints included remarkable recovery on functional outcome measures (BI, mRS, and Glasgow Outcome Scale) at days 30 and 90. Serious adverse events such as symptomatic ICH were also assessed.

Results: No significant rt-PA benefit on the 90-day efficacy endpoints in patients treated between 3 and 5 hours. Significant increase in the risk of symptomatic ICH with treatment using IV-rtPA.

#### ECASS-II [760] (n=800)

**Time window:** 0-3 h and 3-6 h  
Endpoints: The primary outcome was the mRS at 90 days, dichotomised for favourable (score 0-1) and unfavourable (score 2-6) outcome.

Results: Trend towards benefit for alteplase, though not statistically significant. Increased risk of symptomatic ICH (8.6% alteplase-group vs. 3.4% placebo-group).

#### ATLANTIS-A [762] (n=142)

**Time window:** 0-6 h  
Endpoints: Primary efficacy endpoints were the number of patients with a decrease of ≥ points on the NIHSS scale at 24 hours and day 30, along with infarct volume at day 30. Secondary outcomes included mortality and functional status on the BI and mRS scales at days 30 and 90.

Results: Significantly higher proportion of 4-point NIHSS improvement at 24 hours for rtPA groups (40%) vs. placebo (21%). The trend reversed at 30 days with more improvement observed in placebo (75%) vs. rtPA patients (60%). rtPA is strongly correlated with an increased risk of ICH, especially in patients treated between 5 and 6 hours after onset.

#### IST-3 [731]

**Time window:** 0-6 h
Endpoints: Primary outcome was the proportion of patients alive and independent, as defined by an Oxford Handicap Score (OHS) of 0-2 at six months. Symptomatic ICH recorded at seven days, and at six months.

Results: Higher proportion of symptomatic ICH at seven days in the rtPA group (7%) vs. control group (1%), and deaths at seven days in rtPA (11%) vs. control group (7%). A similar number of fatalities in either rtPA or control groups (27% each). Despite early hazards, IV alteplase improved functional outcome.

IV = Intravenous; rtPA = recombinant tissue plasminogen activator; NINDS = National Institute of Neurological Disorders and Stroke; mRS = Modified Rankin Score; BI = Barthel Index; SITS-MOST = Safe Implementation of Thrombolysis in Stroke-Monitoring Study; NIHSS = National Institute of Health Stroke Scale; ICH = Intracerebral haemorrhage; TESPI = Thrombolysis in Elderly Stroke Patients in Italy; SITS-NEW = Safe Implementation of Thrombolysis in Stroke-Non-European Union World; ECASS = European Cooperative Acute Stroke Study; ATLANTIS = The Alteplase ThromboLysis for Acute Noninterventional Therapy in Ischemic Stroke; CASES = Canadian Alteplase for Stroke Effectiveness Study; IST-3 = third international stroke trial.
Table 2. Contraindications for intravenous recombinant tissue plasminogen activator (rtPA) in acute ischemic stroke.

<table>
<thead>
<tr>
<th>Contraindications applicable to use of intravenous rtPA in acute ischemic stroke (AIS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset of stroke symptoms more than 4.5 hours.</td>
</tr>
<tr>
<td>History of stroke or significant head trauma in previous three months</td>
</tr>
<tr>
<td>Previous intracranial haemorrhage.</td>
</tr>
<tr>
<td>Symptoms are suggestive of subarachnoid haemorrhage.</td>
</tr>
<tr>
<td>Prolonged blood pressure elevation (systolic ≥185 mmHg or diastolic ≥110 mmHg).</td>
</tr>
<tr>
<td>Hypoglycemia (serum glucose &lt; 50 mg/dL (&lt;2.8 mmol/L)).</td>
</tr>
<tr>
<td>Active internal bleeding, acute bleeding diathesis, including platelet count &lt;100, 000/mm³, current anticoagulant use with an INR &gt; 1.7, or PT &gt; 15 s.</td>
</tr>
<tr>
<td>Heparin use within 48 hours with an abnormally elevated aPTT.</td>
</tr>
<tr>
<td>Arterial puncture at noncompressible site in previous seven days.</td>
</tr>
<tr>
<td>History of gastrointestinal tract haemorrhage within 21 days.</td>
</tr>
<tr>
<td>The recent history of major surgery intracranial or intraspinal surgery within 14 days.</td>
</tr>
<tr>
<td>Previous history of a previous aneurysm, arteriovenous malformation, or intracranial neoplasm.</td>
</tr>
<tr>
<td>Current use of a direct thrombin inhibitor or direct factor Xa inhibitors with an evidence of anticoagulation effect by laboratory tests such as aPTT, INR&lt; ECT, TT, or relevant factor Xa activity assays.</td>
</tr>
<tr>
<td>Early ischemic changes are visible on CT in more than one-third of MCA artery vascular territory consistent with irreversible injury or evidence of haemorrhage on CT scan.</td>
</tr>
</tbody>
</table>

Additional criteria applicable for IV-rtPA between 3 to 4.5 h: patient older than 80 years, severe stroke (baseline NIHSS >25), no prior history of diabetes mellitus and AIS (both), and not currently on any oral anticoagulants regardless of INR.

CT = computed tomography; INR = international normalised ratio; IV-rtPA = intravenous recombinant tissue plasminogen activator; MCA = middle cerebral artery; NIHSS = National Institute of Health Stroke Scale; PT = prothrombin time; aPTT = activated partial thromboplastin time; ECT = ecarin clotting time.
Table 3. List of mechanical thrombectomy (MT) devices.

<table>
<thead>
<tr>
<th>MT device</th>
<th>Vendor</th>
<th>Mechanism of action</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Merci Clot Retriever</td>
<td>Concentric Medical</td>
<td>Coil retriever</td>
<td>[823-826]</td>
</tr>
<tr>
<td>Phenox</td>
<td>Phenox, Bochum, Germany</td>
<td>Coil retriever/Aspiration</td>
<td>[827, 828]</td>
</tr>
<tr>
<td>Catch</td>
<td>Balt, Montmorency, France</td>
<td>Coil retriever</td>
<td>[829-831]</td>
</tr>
<tr>
<td>Distal Access Catheter (DAC)</td>
<td>DAC; Concentric Medical, US</td>
<td>Coil retriever</td>
<td>[855]</td>
</tr>
<tr>
<td>Early Penumbra</td>
<td>Penumbra Inc., US</td>
<td>Aspiration</td>
<td>[820, 821, 1002-1004]</td>
</tr>
<tr>
<td>AngioJet</td>
<td>Possis Medical, MN, USA</td>
<td>Aspiration/Rheolytic thrombectomy*</td>
<td>[1005-1008]</td>
</tr>
<tr>
<td>EKOS Primo</td>
<td>EKOS, Bothell, WA</td>
<td>Ultrasound-based on mechanical clot disruption</td>
<td>[1009]</td>
</tr>
<tr>
<td>Neuroform</td>
<td>Stryker Neurovascular, US</td>
<td>Stent Retriever</td>
<td>[1010, 1011]</td>
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<tr>
<td>Enterprise</td>
<td>Codman, Raynham, MA, US</td>
<td>Stent Retriever</td>
<td>[1012-1014]</td>
</tr>
<tr>
<td>Solitaire</td>
<td>Covidien/Medtronic, Dublin, Ireland</td>
<td>Stent retriever</td>
<td>[508, 852, 973, 1015-1020]</td>
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<tr>
<td>Trevo</td>
<td>Stryker, Kalamazoo, Michigan, US</td>
<td>Stent Retriever</td>
<td>[824, 1021-1025]</td>
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<tr>
<td>ReVive™</td>
<td>Micrus Endovascular, CA, US</td>
<td>Stent Retriever</td>
<td>[1026, 1027]</td>
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<tr>
<td>APERIO</td>
<td>Acandis, Pfzorheim, Germany</td>
<td>Stent Retriever</td>
<td>[1027-1029]</td>
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<td>Embotrap Revascularization system</td>
<td>Neuravi, Ireland</td>
<td>Stent Retriever</td>
<td>[1030]</td>
</tr>
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<td>pREset</td>
<td>Phenox, Bochum, Germany</td>
<td>Stent Retriever</td>
<td>[1031]</td>
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<tr>
<td>The Mindframe Capture LP</td>
<td>Medtronic, Minneapolis, Minnesota, USA</td>
<td>Stent Retriever</td>
<td>[1032, 1033]</td>
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<tr>
<td>ERIC</td>
<td>MicroVention, CA, US</td>
<td>Stent Retriever</td>
<td>[1034]</td>
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<tr>
<td>SOFIA</td>
<td>MicroVention, CA, US</td>
<td>Stent Retriever</td>
<td>[1035-1037]</td>
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<td>Penumbra ACE 64</td>
<td>Penumbra Inc., California, US</td>
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<td>[837, 839, 1039, 1040]</td>
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<td>Penumbra 3D separator</td>
<td>Penumbra Inc., California, US</td>
<td>Aspiration</td>
<td>[840, 841]</td>
</tr>
<tr>
<td>Product</td>
<td>Manufacturer, Location</td>
<td>Method</td>
<td>Reference</td>
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<tr>
<td>---------------------------------</td>
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<tr>
<td>LaTIS Neurolaser laser</td>
<td>Latis Inc., Minneapolis, Minn</td>
<td>Laser recanalisation based mechanical clot disruption</td>
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<tr>
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<td>EndoVasix, Belmont, CA</td>
<td>Laser recanalisation based mechanical clot disruption</td>
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<td>MicroLysUS catheter</td>
<td>EKOS, Bothell, WA, US</td>
<td>Ultrasound-based mechanical clot disruption</td>
<td>[1042]</td>
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<td>Wingspan</td>
<td>Stryker Neurovascular, Fremont, CA, US</td>
<td>Ultrasound-based mechanical clot disruption</td>
<td>[1043-1050]</td>
</tr>
</tbody>
</table>

ERIC = Embolus Retriever with Interlinked Cage; SOFIA = Soft Torqueable Catheter Optimized For Intracranial Access

*Rheolytic thrombectomy refers to the mechanical procedure of removing thrombus using multiple high-velocity, high-pressure saline jets of saline from the tip of a catheter using an AngioJet system [1051].
Table 4. Comparison of baseline characteristics and outcome measures of the recent endovascular trials [480, 768, 949].

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</thead>
<tbody>
<tr>
<td>Region</td>
<td>Nethe rlands</td>
<td>United States, Canada, South Korea, Ireland, United Kingdom</td>
<td>Austral ia and New Zealan d</td>
<td>United States and Europe</td>
<td>Spain</td>
<td>Unite d State s</td>
<td>France</td>
</tr>
<tr>
<td>Number of centres</td>
<td>16</td>
<td>22</td>
<td>10</td>
<td>39</td>
<td>4</td>
<td>4</td>
<td>26</td>
</tr>
<tr>
<td>Number of patients; n (CG/IA)</td>
<td>500 (267/233)</td>
<td>315 (150/165)</td>
<td>70 (35/35)</td>
<td>196 (98/98)</td>
<td>206 (103/103)</td>
<td>108 (54/54)</td>
<td>412 (208/204)</td>
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<tr>
<td>BASELINE CHARACT ERISTICS</td>
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<tr>
<td>Age Range</td>
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<td>≥ 18</td>
<td>≥ 18</td>
<td>18 - 80 years</td>
<td>18 - 80 years</td>
<td>18-85</td>
<td>18-80</td>
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<tr>
<td>NIHSS Range</td>
<td>≥ 2</td>
<td>&gt; 5</td>
<td>N.R.</td>
<td>8-29</td>
<td>≥ 6</td>
<td>≥8</td>
<td>10-25</td>
</tr>
<tr>
<td>Control group</td>
<td>Standard medical therapy (+/- IV tPA)</td>
<td>Standard medical therapy (+/- IV tPA)</td>
<td>IV-tPA only</td>
<td>IV-tPA only</td>
<td>Standard medical therapy (+/- IV tPA)</td>
<td>IV-tPA only</td>
<td>IV-tPA only</td>
</tr>
<tr>
<td>Intervention group</td>
<td>IAT</td>
<td>IAT</td>
<td>ET with Solitai re FR stentri ever</td>
<td>ET with Solitaire FR stentriever</td>
<td>ET with Solitaire FR stentriever</td>
<td>ET with Penumbra aspiration system</td>
<td>Endovascular MT</td>
</tr>
<tr>
<td>Intervention using Stent</td>
<td>81.5%</td>
<td>86.1%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>0%</td>
<td>N.R.</td>
</tr>
<tr>
<td><strong>Time window</strong></td>
<td><strong>0-6 hours</strong></td>
<td><strong>0-12 hours</strong></td>
<td><strong>0-6 hours</strong></td>
<td><strong>0-8 hours</strong></td>
<td><strong>0-4.5 hours</strong></td>
<td><strong>0-5 hours</strong></td>
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</tr>
<tr>
<td><strong>Neurologic inclusion criteria</strong></td>
<td>N.A.</td>
<td>Barthel Index of ≥90</td>
<td>mRS scores of 0-2</td>
<td>mRS scores of 0-1</td>
<td>mRS scores of 0-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Neuroimaging techniques</strong></td>
<td>CT/CTA</td>
<td>CT/CTA/CTA Multiphasic (for collaterals)</td>
<td>CT/CTA/CTP (for mismatch)</td>
<td>CT/CTA/MRA/MRP/CTP (for infarct core)</td>
<td>CT/CTA (MRA/DSA)</td>
<td>CT/CTA</td>
<td></td>
</tr>
<tr>
<td><strong>Large artery occlusion</strong></td>
<td>CTA</td>
<td>CTA</td>
<td>CTA or MRA</td>
<td>CTA or MRA</td>
<td>CTA or MRA</td>
<td>CTA or MRA</td>
<td></td>
</tr>
<tr>
<td><strong>Affected arteries</strong></td>
<td>TICA, M1, M2, A1, A2</td>
<td>TICA, M1</td>
<td>TICA, M1, M2</td>
<td>TICA, M1, M2</td>
<td>TICA, M1</td>
<td>MCA, ICA, M1, M2, TB, M2</td>
<td></td>
</tr>
<tr>
<td><strong>Infarct core/perfusion</strong></td>
<td>N.R.</td>
<td>NCCT, CBV or CBF ASPECTS ≥ 6</td>
<td>Core&lt;sup&gt;vy&lt;/sup&gt; &lt;70 ml (&gt;1.8)&lt;sup&gt;vy&lt;/sup&gt;</td>
<td>Core&lt;sup&gt;vp&lt;/sup&gt; &lt;50 ml (&gt;1.2)&lt;sup&gt;vp&lt;/sup&gt;</td>
<td>NCCT ASPECTS ≥ 7</td>
<td>Clot length ≥8 mm</td>
<td></td>
</tr>
<tr>
<td><strong>Collateral status</strong></td>
<td>N.R.</td>
<td>Good/Moderate</td>
<td>N.R.</td>
<td>N.R.</td>
<td>N.R.</td>
<td>N.R.</td>
<td></td>
</tr>
<tr>
<td><strong>Median stroke onset to groin puncture</strong></td>
<td>260 min</td>
<td>241 min</td>
<td>210 min</td>
<td>224 min</td>
<td>269 min</td>
<td>226 min</td>
<td></td>
</tr>
<tr>
<td><strong>Baseline NIHSS [Median (IQR)]; CG vs IA</strong></td>
<td>18 (14–22) vs 17 (14–21)</td>
<td>17 (12–20) vs 16 (13–20)</td>
<td>13 (9–19) vs 17 (13–20)</td>
<td>17 (13–19) vs 17 (13–20)</td>
<td>17 (12–19) vs 17 (14–20)</td>
<td>N.R.</td>
<td></td>
</tr>
<tr>
<td><strong>Median ASPECTS (%); CG/IA</strong></td>
<td>9/9</td>
<td>9/9</td>
<td>NR/NR</td>
<td>9/9</td>
<td>8/7</td>
<td>N.R.</td>
<td></td>
</tr>
<tr>
<td><strong>Patients Receiving IV-rtPA (%) ; CG/IA</strong></td>
<td>91/87</td>
<td>79/73</td>
<td>100/100</td>
<td>100/100</td>
<td>78/68</td>
<td>100/100</td>
<td></td>
</tr>
<tr>
<td>STUDY OUTCOMES</td>
<td>Shift in mRS at 90 days</td>
<td>Shift in mRS at 90 days</td>
<td>Reduct ion in perfusion lesion volume ; NIHSS reduction ≥ 8 points or mRS score of 0-1 at day 3</td>
<td>Distribution of mRS at 90 days; % mRS 0-2 at 90 days</td>
<td>Shift in mRS at 90 days</td>
<td>Shift in mRS at 90 days</td>
<td>Shift in mRS at 90 days</td>
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<tr>
<td>Primary Outcomes</td>
<td>Shift in mRS at 90 days</td>
<td>Shift in mRS at 90 days</td>
<td>Reduct ion in perfusion lesion volume ; NIHSS reduction ≥ 8 points or mRS score of 0-1 at day 3</td>
<td>Distribution of mRS at 90 days; % mRS 0-2 at 90 days</td>
<td>Shift in mRS at 90 days</td>
<td>Shift in mRS at 90 days</td>
<td>Shift in mRS at 90 days</td>
</tr>
<tr>
<td>mRS (0-2) at 90 days %; CG vs IA</td>
<td>19.1 vs 32.6, P&lt;0.05</td>
<td>29.3 vs 53, P&lt;0.001</td>
<td>40 vs 70, P=0.001</td>
<td>35.5 vs 60.2, P&lt;0.001</td>
<td>28.2 vs 43.7</td>
<td>30.4 vs 38</td>
<td>42.1 vs 54.2</td>
</tr>
<tr>
<td>Improveme nt in mRS 0-2 at 90 days</td>
<td>13.5%</td>
<td>23.7%</td>
<td>31.4%</td>
<td>24.7%</td>
<td>15.5%</td>
<td>7.6%</td>
<td>12.1%</td>
</tr>
<tr>
<td>sICH risk (%); CG vs IA, P</td>
<td>6.4 vs 7.7, P&gt;0.05</td>
<td>2.7 vs 3.6, P&gt;0.05</td>
<td>5.7 vs 0, P&gt;0.05</td>
<td>3.1 vs 0, P=0.12</td>
<td>1.9 vs 1.9, P&gt;0.05</td>
<td>11.3 vs 10.9</td>
<td>2 vs 2, P=0.71</td>
</tr>
<tr>
<td>Parenchymal Hematoma Risk (%); CG/IA</td>
<td>6 vs 6</td>
<td>2.0 vs 4.8</td>
<td>8.6 vs 11.4</td>
<td>N.R. vs N.R.</td>
<td>5.8 vs 5.8</td>
<td>N.A.</td>
<td>9.45 vs 13.8, P=0.53</td>
</tr>
<tr>
<td>Mortality (%); CG vs IA, P</td>
<td>22.1 vs 21, P&gt;0.05</td>
<td>19 vs 10.4, P=0.04</td>
<td>20 vs 8.6, P&gt;0.05</td>
<td>12.4 vs 9.2, P&gt;0.05</td>
<td>15.5 vs 18.4, P=0.06</td>
<td>23.9 vs 12</td>
<td>13 vs 12; P=0.70</td>
</tr>
<tr>
<td>Decrease in mortality at 90 days</td>
<td>1.1%</td>
<td>8.6</td>
<td>11.4%</td>
<td>3.2%</td>
<td>-2.9%</td>
<td>11.9%</td>
<td>1%</td>
</tr>
<tr>
<td>Complete recanalization rates</td>
<td></td>
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</tr>
<tr>
<td>mTICI Score 2b/3</td>
<td>58.7%</td>
<td>72.4%</td>
<td>86.2%</td>
<td>88.0%</td>
<td>65.7%</td>
<td>N.R.</td>
<td>N.R.</td>
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<tr>
<td>Complete recanalization based on neuroimaging 24-27 h later; CG vs IA</td>
<td>68/20 7 (33%)&lt;sup&gt;a&lt;/sup&gt; vs N.A. vs 141/35 (75%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15/35 (43%)&lt;sup&gt;a&lt;/sup&gt; vs N.A. vs 33/35 (94%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21/52 (40%)&lt;sup&gt;b&lt;/sup&gt; vs N.A. vs 53/64 (83%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>N.A. vs N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>Brain infarction volume at 24 h&lt;sup&gt;c&lt;/sup&gt; (mean, 95% CI); CG vs IA, P&lt;sub&gt;0.01&lt;/sub&gt;</td>
<td>79 mL (34-125) vs 49 mL (22-96), P&lt;sub&gt;0.01&lt;/sub&gt;</td>
<td>N.A. vs N.A.</td>
<td>N.A. vs N.A.</td>
<td>35 mL (0-407) vs 32 mL (0-503), P=0.09</td>
<td>39 mL (12-87) vs 16 mL (8-59), P=0.02</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>NNT</td>
<td>7.1</td>
<td>4.2</td>
<td>3.2</td>
<td>4.0</td>
<td>6.3</td>
<td>13.2</td>
<td>8.3</td>
</tr>
</tbody>
</table>

IQR = Interquartile range; TICA = terminal internal carotid artery (Carotid T/L); M1 and M2 = branches of the MCA; A1 and A2 = branches of the ACA; mTICI = modified Thrombolysis in Cerebral Infarction; mRS = modified Rankin Scale; N.R. = not required; N.S. = not significant; N.A. = not available; CTA = computed tomography angiography; NCCT = non-contrast computed tomography; CBV = cerebral blood volume; CBF = cerebral blood flow; MRA = magnetic resonance angiography; ASPECTS = Alberta Stroke Program Early CT score; CG = control group; IA = Intervention arm; IAT = Intra-arterial therapy; TB = upper third of the basilar artery; MCA = middle cerebral artery; M2 = insular portion of the MCA; M1 = Proximal portion of the MCA; ET = Endovascular Thrombectomy; MT = Mechanical thrombectomy; ESCAPE = Endovascular Treatment for Small Core and Anterior Circulation Proximal Occlusion With Emphasis on Minimizing CT to Recanalization Times; EXTEND-IA = Extending the Time for Thrombolysis in Emergency Neurological Deficits—Intra-Arterial; MR CLEAN = Multicentre Randomized Clinical Trial of Endovascular Treatment for Acute Ischemic Stroke in the Netherlands; NIHSS = National Institutes of Health Stroke Scale; REVASCAT = Randomized Trial of Revascularization with Solitaire FR Device Versus Best Medical Therapy in the Treatment of Acute Stroke Due to Anterior Circulation LVO Presenting within Eight Hours of Symptom Onset; SWIFT PRIME = Solitaire with the Intention for Thrombectomy as Primary Endovascular Treatment; NNT = number needed to treat for benefit (mRS score 0-2); THRACE: Mechanical thrombectomy after intravenous alteplase versus alteplase alone after stroke.

*Target mismatch ratio; ^ Sum of median of parameters; ¶The ischemic core was assessed by MRI or CT; “The ischemic core was defined by regional cerebral blood flow on CT perfusion or diffusion-weighted imaging; *Common odds ratio; Recanalization shown in brain CTA/MRA at 24 h; Reperfusion shown in brain CT perfusion/MR perfusion at 27 h; Brain infarction volume at 24 h after treatment measured with CT in MR CLEAN trial and with CT or MRI in SWIFT PRIME and REVASCAT trials.
Table 5. List of various imaging techniques, including non-contrast computed technology (NCCT), CT or MR-based diffusion-perfusion mismatch, acute lesion volume determination, and angiography, used in the selection of acute ischemic stroke (AIS) patients for reperfusion therapy, diagnostics and monitoring of treatment response [646].

<table>
<thead>
<tr>
<th>Imaging modality</th>
<th>Treatment</th>
<th>References</th>
<th>Significance</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCCT</td>
<td>IV-tPA</td>
<td>NINDS [645, 727], TESPI [798, 1000], SITS-MOST [999], SITS-NEW [1001], ECASS-III [421], ECASS-II [760], CASES [763], SITS-ISTR [764], ATLANTIS-B [761], IST-3 [731], ATLANTIS-A [762]</td>
<td>Rule out subdural or epidural hematoma, subarachnoid haemorrhage, and intraparenchymal haemorrhage. Excluding large infarct (&gt; 1/3 of MCA territory) from tPA therapy. Rule out tumour.</td>
<td>Lacks sensitivity to detect early ischemic changes. Poor inter-observer reliability. Poor accuracy in hyperacute settings. NCCT criteria along with time-window restriction deem only &lt;10% patients eligible for IV rt-PA. Clinical benefit for patients treated with IV-tPA up to 3-4.5 h time window. tPA not beneficial beyond 4.5 h, and associated with increased risk of intracerebral bleeding.</td>
</tr>
<tr>
<td>IV ReopPro (Abciximab) and Retavase (Retepase)</td>
<td>ReoPro and Retavase to Treat Acute Stroke (ROSIE-CT) [1052]</td>
<td>NCCT used to exclude patients with extensive infarctions (&gt;1/3 of MCA territory) or haemorrhage, or other radiological factor associated with risk of haemorrhage with thrombolytic therapy. Study halted.</td>
<td></td>
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<tr>
<td>IAT</td>
<td>IMA I &amp; II (0-3 h) † [869]</td>
<td>NCCT used to exclude patients with</td>
<td></td>
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</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Table</th>
<th>Study</th>
<th>Extensive Infarctions (&gt;1/3 of MCA territory) or with significant oedema and mass effect.</th>
<th>MRI-based PWI-DWI Mismatch-based selection is safer and potentially more effective than NCCT selection. The time window for tPA based on mismatch may be extended up to six h. Patients with PWI-DWI mismatch can be treated up to six h with similar or better results vs NCCT selection &lt;3h.</th>
<th>No significant difference in reperfusion, infarct growth or clinical outcome between MRI mismatch and non-mismatch, for IV-tPA and placebo.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROACT II (0-6h) [816], MERCI(3-8h) [812], Multi-MERCI(3-8h) [855], Penumbra Pivotal(3-8h) [820]</td>
<td>extensive infarctions (&gt;1/3 of MCA territory) or with significant oedema and mass effect.</td>
<td>20% DWI-PWI mismatch not found optimal in a posthoc analysis. DIAS-2 and EPITHET failed to demonstrate clinical benefit for mismatch patients. Mismatch approach poor discriminator for identifying IAT candidates with a favourable collateral status.</td>
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<tr>
<td>Study</td>
<td>Description</td>
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<tr>
<td>START-EXTEND (3-9h)</td>
<td>Not completed. Based on MRI PWI-DWI mismatch.</td>
<td></td>
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<tr>
<td>ITAIS-II (3-9h) [1057]</td>
<td>Based on CTP-CTAS mismatch. Trial not completed.</td>
<td></td>
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<tr>
<td>ITAIS-III (3-9h)</td>
<td>Based on CTP-CTAS mismatch. Trial not completed. Primary outcome: Day 90 mRS</td>
<td></td>
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<tr>
<td><strong>IV Desmoteplase</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>DIAS [785] and DEDAS [784] (3-9 h)</td>
<td>IV Desmoteplase is safe and efficient up to 9h in patients with MR mismatch.</td>
<td></td>
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<tr>
<td>Pooled data from DIAS, DEDAS and DIAS-2</td>
<td>IV Desmoteplase is beneficial in patients with large minimum baseline mismatch volume (MMV) (MMV&gt;60 mL). Ineffective in patients with small MMV, MMV&lt;60 mL.</td>
<td></td>
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<tr>
<td>DIAS 2 [786] and DIAS 3 [783]</td>
<td>IV Desmoteplase is safe up to 9h. No improvement in functional outcome for patients with a mismatch.</td>
<td></td>
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<tr>
<td>Treatment</td>
<td>Timeframes (h)</td>
<td>Results</td>
<td></td>
<td></td>
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<td>---------------------------------</td>
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<td>-------------------------------------------------------------------------</td>
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<td></td>
</tr>
<tr>
<td>DEFUSE</td>
<td>(3-6h) [574]</td>
<td>More favourable clinical response to early reperfusion in patients with mismatch than non-mismatch patients.</td>
<td></td>
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</tr>
<tr>
<td>Abciximab with Reteplase</td>
<td>ROSIE (3-24h)</td>
<td>Based on MR DWI-PWI mismatch. Results awaited.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pooled analysis</td>
<td>EPITHET-DEFUSE pooled dataset [1058]</td>
<td>Mismatch patients treated with alteplase significantly associated with increased reperfusion and decreased infarct volume.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meta-analysis of DIAS, DIAS II, DEDAS, DEFUSE, and EPITHET trials [641]</td>
<td>No treatment required</td>
<td>No clinical benefit with IV thrombolysis in mismatch patients beyond three h and up to 9 h.</td>
<td></td>
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<tr>
<td>No treatment required</td>
<td>1000Plus (0-24h) [1059]</td>
<td>Based on MTT-DWI mismatch. The primary outcome is infarct growth at day 5-7. Trial not completed.</td>
<td></td>
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<tr>
<td>IAT</td>
<td>MR RESCUE (0-8h) [847]</td>
<td>Mechanical Embolectomy using Merci Retriever and Penumbra</td>
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<tr>
<td>Study</td>
<td>Description</td>
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<tr>
<td>No benefit for mismatch patients treated with endovascular therapy. Embolectomy was not found to be superior to standard care.</td>
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<tr>
<td>DEFUSE II (0-12h) [658]</td>
<td>Based on imaging criteria MR PWI-DWI ratio&gt;1.8; Tmax with a threshold of 6s. DWI must be &lt;70mL and Tmax must be &lt;100 mL Mismatch patients who had early reperfusion with IAT had more favourable outcome.</td>
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<tr>
<td>Meta-analysis based on pooled data from DEFUSE studies [656]</td>
<td>In mismatch patients, the degree of reperfusion strongly correlated with both favourable clinical response and good functional outcome. Reperfusion of ≥90% of the perfusion lesion recommended goal for reperfusion therapies.</td>
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<tr>
<td>Advanced imaging using Infarct core volumes clinical diffusion mismatch, or combined DWI/MTT/NIHSS thresholds MRA-DWI Mismatch</td>
<td><strong>IV-tPA</strong></td>
<td><strong>Ebinger M et al (2009)</strong> (3-6h) [1060]</td>
<td>Based on Clinical Diffusion Mismatch (CDM). No increased benefit from tPA with CDM. No difference in reperfusion in CDM and non-CDM patients.</td>
<td>Patients with large pre-treatment DWI lesions do poorly despite reperfusion. Patients with small DWI lesions do well depending largely upon reperfusion. DWI Lesion volume &gt;70 cm³ correlates strongly with poor clinical outcomes with or without therapy. An ADC threshold of 600 ×10⁻⁶ mm²/s is used for ischemic core calculation, and correlates well with clinical outcomes [658].</td>
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<td></td>
<td><strong>Deguchi I et al (2011)</strong>[1063]</td>
<td>MRA-DWI mismatch (MDM) predicts diffusion perfusion mismatch (DPM) at higher specificity and sensitivity than CDM.</td>
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<td></td>
<td><strong>Tei H et al (2007), Davalos A et al (2004), and Tei H et al (2011) [666, 669, 692]</strong></td>
<td>CDM defined by NIHSS≥8 and DWI-ASPECTS≥8 strongly associated with DPM or penumbra or tissue at risk of infarction.</td>
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<tr>
<td></td>
<td><strong>Noguiera RG et al (2017) [980]</strong></td>
<td>CDM better discriminates infarct growth than mean transit time (MTT)-DWI mismatch.</td>
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<tr>
<td><strong>Carerra E et al (2011) [1064]</strong></td>
<td>Adding MTT (PWI) may improve infarct prediction, even within DWI lesions.</td>
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<tr>
<td><strong>MT</strong></td>
<td>Infarct core volume estimated from CTP using automated software RAPID. Cut-off of the core-volume &gt; 50 mL to exclude patients. Ischemic core defined by regional CBV or delayed TTP of the residual function.</td>
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<tr>
<td><strong>EXTEND-IA [810]</strong></td>
<td>Cut-off of the core volume to exclude patients &gt; 70 mL. Ischemic core defined by regional CBF less than 30% of that in the normal tissue.</td>
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<tr>
<td><strong>START (0-8h) [1065]</strong></td>
<td>Intervention using Penumbra MT device. Imaging criteria: NCCT plus CTASI, CTP or DWI.</td>
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<tr>
<td><strong>CTA/MRA Desmoteplase DIAS-IV (3-9h)[1066]</strong></td>
<td>The study stopped recruitment as the result of DIAS 3</td>
<td>CTA/MRA used successfully in five successful endovascular trials for the selection of</td>
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</table>
indicates that the study is unlikely to reach its primary endpoint (good outcome defined by mRS 0-2) with the current protocol.

patients with large artery occlusion.

Angiography is also useful in baseline collateral status assessment.

Multiphase CTA is recommended for collaterals as it allows time-resolved angiography of brain vasculature from the base of the skull to the vertex in three phases after contrast injection. This is achieved by moving the CT gantry three times every 8 seconds apart.

Multiphase CTA is associated with better clinical outcomes than NCCT, single phase CTA, and CTP.

Multiphase CTA adds just 20 seconds to the single phase CTA.

| MT | MR CLEAN [842], ESCAPE [843], SWIFT PRIME [844], EXTEND-IA [810], and REVASCAT [478] | CTA or MRA was used to select patients with large artery occlusion. |
ESCAPE [843]  |  Collateral assessment using CTA for patient selection. Patients with poor or no collaterals in >50% of the MCA territory were excluded.

¶IMS I and II received combined IV and IA tPA bridging therapy.

IV-rtPA = Intravenous recombinant tissue plasminogen activator; DIAS = Desmoteplase in Acute Ischemic Stroke; DEFUSE = Diffusion and perfusion imaging Evaluation For Understanding Stroke Evolution; DEDAS = Dose Escalation of Desmoteplase for Acute Ischemic Stroke; EPITHET = Echoplanar Imaging Thrombolysis Evaluation Trial, MR RESCUE = Recanalization of Stroke Clots Using Embolectomy, ROSIE = ReoPro Retavase Reperfusion of Stroke Safety Study: Imaging Evaluation; NCCT = Non-contrast computed tomography; CTA = Computed tomographic angiography; MRA = MR angiography; CTP = CT Perfusion, tPA = tissue plasminogen activator; MCA = Middle cerebral artery; TTP = Time to Peak; CBV = Cerebral blood volume; CBF = Cerebral blood flow; CDM = Clinical diffusion mismatch, PDM = Perfusion diffusion mismatch, NIHSS = National Institute of Health Stroke Severity Scale; PROACT II: Prolyse in Acute Cerebral Thromboembolism II; PWI = Perfusion-weighted imaging; CTASI = Computed tomography angiographic source image; IA = Intra-arterial; MR RESCUE: MR and Recanalization of Stroke Clots Using Embolectomy; PWI = Perfusion-weighted imaging; MT = Mechanical thrombectomy.
Table 6. Prognostic scales used in predicting clinical outcome in AIS.

<table>
<thead>
<tr>
<th>Standardised scoring system</th>
<th>Utility</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIHSS</td>
<td>Significant predictor of long-term clinical outcome, and mortality.</td>
<td>[765, 1069]</td>
</tr>
<tr>
<td>ABC/2 method</td>
<td>Quick and reliable tool to calculate CT or MR diffusion-perfusion mismatch. Volume of core and penumbra volume can be computed from CTP using the formula, mismatch percentage= 1-CBV/MTT x 100%.</td>
<td>[942]</td>
</tr>
<tr>
<td>BASIS</td>
<td>Middle cerebral artery-BASIS (M1-BASIS) correlate well with the clinical severity of stroke; may predict prognosis in AIS patients treated with thrombolyosis.</td>
<td>[1070]</td>
</tr>
<tr>
<td>One-third of MCA territory method</td>
<td>Detects significant early ischemic changes in a higher proportion of these early CT scans.</td>
<td>[748, 1068, 1071]</td>
</tr>
<tr>
<td>Miteff Scale</td>
<td>For grading of baseline collateral status.</td>
<td>[3, 4, 470]</td>
</tr>
<tr>
<td>ASITN/SIR Scale</td>
<td>For grading of baseline collateral status.</td>
<td>[495, 705, 1072, 1073]</td>
</tr>
<tr>
<td>Christoforidis Scale</td>
<td>For grading baseline collateral status.</td>
<td>[467, 1073, 1074]</td>
</tr>
<tr>
<td>ASPECTS-Collateral Scale</td>
<td>For grading baseline collateral status.</td>
<td>[471, 526, 1067, 1073]</td>
</tr>
<tr>
<td>PRECISE Score</td>
<td>For grading baseline collateral status.</td>
<td>[16, 707]</td>
</tr>
<tr>
<td>THRIVE</td>
<td>Predicts risk of symptomatic ICH, and mortality after IV-rtPA.</td>
<td>[1075-1077]</td>
</tr>
<tr>
<td>iScore</td>
<td>Predicts risk of early neurological deterioration after AIS, response to tPA therapy.</td>
<td>[1078-1080]</td>
</tr>
<tr>
<td>SPAN-100</td>
<td>Aid in estimating the clinical response, functional outcome, risk of haemorrhagic complications after tPA therapy for AIS.</td>
<td>[1081-1085]</td>
</tr>
<tr>
<td>SPAN-100 with imaging</td>
<td>Addition of clot burden score (CBS) and cerebral blood volume (CBV) improved the predictive ability of SPAN-100.</td>
<td>[1086]</td>
</tr>
<tr>
<td>Method</td>
<td>Description</td>
<td>Reference(s)</td>
</tr>
<tr>
<td>-------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>SAD</td>
<td>Predicts poor outcome following endovascular treatment.</td>
<td>[552]</td>
</tr>
<tr>
<td>Stroke-TPI</td>
<td>Prediction of clinical outcome after IV-rtPA for AIS.</td>
<td>[1087, 1088]</td>
</tr>
<tr>
<td>DRAGON</td>
<td>Prediction of clinical outcome of AIS.</td>
<td>[1089, 1090]</td>
</tr>
<tr>
<td>ASTRAL</td>
<td>Prediction of long-term outcomes in AIS.</td>
<td>[1089-1092]</td>
</tr>
<tr>
<td>PRS</td>
<td>Prediction of sICH risk after treatment.</td>
<td>[1087]</td>
</tr>
<tr>
<td>HAT</td>
<td>Prediction of sICH risk after treatment.</td>
<td>[1087]</td>
</tr>
<tr>
<td>SEDAN</td>
<td>Prediction of risk of sICH, mortality after treatment, and in-hospital mortality.</td>
<td>[1087, 1093-1095]</td>
</tr>
<tr>
<td>SITS-ICH</td>
<td>Predicting risk of sICH in AIS.</td>
<td>[1096-1098]</td>
</tr>
<tr>
<td>TURN</td>
<td>Prediction of sICH after IV-tPA.</td>
<td>[1087, 1088, 1099]</td>
</tr>
</tbody>
</table>

NIHSS = national institute of health stroke scale; THRIVE: totalled health risks in vascular events score; BASIS = Boston Acute Stroke Imaging Scale; ASITN/SIR = American Society of Interventional and Therapeutic Neuroradiology/Society of Interventional Radiology; sICH = symptomatic intracerebral haemorrhage defined according to the NINDS criteria; STROKE-TPI = stroke-thrombolytic predictive instrument; PRECISE = prognostic evaluation based on cortical vein score difference in stroke; ASPECTS = the Alberta Stroke Program Early CT Score; ICH = Intra-cerebral haemorrhage; AIS = acute ischemic stroke; tPA = tissue plasminogen activator; SAD = Stanford age and early diffusion-weighted imaging (DWI) volume score; SITS = Safe Implementation of Thrombolysis in Stroke.
Table 7. List of ongoing and upcoming trials aimed to address the issues concerning the endovascular treatment of acute ischemic stroke.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Time window</th>
<th>Purpose</th>
<th>Inclusion criteria</th>
<th>Outcome measure</th>
</tr>
</thead>
</table>
| RACECAT  | 0-8h        | Triage of the acute LVO on direct transfer to EVT-SC bypassing LSC vs. transfer to the LSC according to the current stroke protocol. | a. Premorbid mRS 0-2  
b. Age ≥18  
c. Suspected LVO AIS identified by a RACE scale score > 4 evaluated by EMS professional  
d. Time to arrival at EVT-SC <7 h from symptom onset | a. mRS at 90 days (shift analysis)  
b. Mortality at 90 days  
c. Mortality in hemorrhagic stroke patients  
d. Clinical deterioration (≥ 8 points on the NIHSS)  
e. Clinical benefit of direct vs. local transfer  
f. Dramatic early favourable response (NIHSS improvement ≥ 8 or NIHSS score of 0-2 at 24 hours) |
| DEFUSE   | 6-16h       | Benefit of carefully selected patients with target mismatch and MCA (M1 segment) or ICA occlusion using CT/MR within 6-16 h treated with MT plus standard | a. Age 18-90 years  
b. Baseline NIHSSS is ≥ 6  
c. Time to endovascular treatment since symptom onset = 6-16 h  
d. Premorbid mRS 0-2  
e. ICA or MCA-M1 occlusion by MRA or CTA and | a. Distribution of mRS scores at 90 days  
b. Proportion of patients with mRS 0-2  
c. Infarct growth within 24 h  
d. Reperfusion rates at 24 h  
e. Ischemic lesion growth at 24 h |
| DAWN [955] | 6-24h MT using the Trevo Retriever with medical management | Therapy vs. standard therapy alone. | Target mismatch profile on CTP or MRI (ischemic core volume <70 mL, mismatch ratio \( \geq 1.8 \), and mismatch volume \( \geq 15 \) mL)
  
  f. ASPECT on NCCT \( \geq 6 \)
  
  g. No evidence of tumour, mass effect with midline shift, ICA or aortic dissection
  
  h. No occlusions in multiple vascular territories |

|   | a. Subjects with failed IV-tPA or contraindicated for IV-tPA
  
  b. Age \( \geq 18 \)
  
  c. Baseline NIHSS \( \geq 10 \)
  
  d. Can be randomised within 6-24 h on stroke onset
  
  e. Pre-stroke mRS 0 or 1
  
  f. <1/3 MCA territory involved, as evidenced by CT or MRI
  
  g. Intracranial ICA and/or MCA-M1 occlusion on MRA/CTA |

|   | a. Weighted mRS at 90 days
  
  b. Mortality at 90 days
  
  c. Good functional outcome (mRS 0-2)
  
  d. Revascularization rate at 24 h on CTA/MRA
  
  e. Neurological deterioration defined by an increase in the NIHSS score at 5-7 days or at discharge. |
h. CIM defined on MR-DWI or CTP-rCBF maps: (a) 0-<21 cc core infarct and NIHSS ≥ 10 (and age ≥ 80 years old), (b) 0-<31 cc core infarct and NIHSS ≥ 10 (and age < 80 years old), or (c) 31 cc to <51 cc core infarct and NIHSS ≥ 20 (and age < 80 years old)

i. ICH or differential diagnosis on CT/MRI

| POSITIVE [954] | 6-12h | To determine the safety and efficacy of IAT in AIS patients Ineligible for or refractory to IV-rtPA as selected by physiologic imaging | a. Age≥18  
  b. NIHSS ≥8 at the time of neuroimaging  
  c. Time to the groin puncture 6-12 h  
  d. Large vessel proximal occlusion (distal ICA through MCA M1 bifurcation)  
  e. Patients who have had IV-tPA without improvement in symptoms  
  f. Pre-stroke morbidity mRS 0-1  
  g. Presence of large penumbra | a. 90-day mRS  
  b. Good functional outcome mRS 0-2 at 90 days  
  c. Mortality at 30 and 90 days  
  d. ICH with neurological deterioration (NIHSS worsening > 4) within 24 h  
  e. Arterial revascularization measured by TICI 2b or 3 following MT |
<table>
<thead>
<tr>
<th>ENDOSTROKE [1100]</th>
<th>NR</th>
<th>Predictors of the good or poor clinical outcome following MT in AIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>h. No evidence of SAH or ICH or mass effect with midline shift</td>
<td></td>
<td></td>
</tr>
<tr>
<td>i. &lt;1/3 MCA territory involved, as evidenced by baseline CT or ASPECTS of &gt;7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>START [1101]</th>
<th>0-8h</th>
<th>Efficacy of the Penumbra System in AIS with a known core infarct volume at admission presenting within 8h of onset To study the correlation between infarct-volume and functional outcome at 90 days in</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Age ≥ 18 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Proximal arterial vessel occlusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. No evidence of venous occlusion</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Predicators</th>
<th>a. mRS at 90 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>b. Complete recanalization defined by TIMI grade two or 3.</td>
<td></td>
</tr>
<tr>
<td>c. Periprocedural complication rate (sICH defined by ECASS PH1 and PH2, SAH and thromboembolic events).</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>START [1101]</th>
<th>0-8h</th>
<th>Efficacy of the Penumbra System in AIS with a known core infarct volume at admission presenting within 8h of onset To study the correlation between infarct-volume and functional outcome at 90 days in</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Age 18 to 85 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. NIHSS ≥10 at admission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Evidence of proximal large vessel occlusion (supra clinoid segment of ICA through the M1 segment of MCA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. Patients presenting within 8 h, and those within 3 h must be ineligible or</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Predicators</th>
<th>a. Good functional outcome mRS 0-2 at 90 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>b. Recanalization assessment using TIMI and mTICI immediately after MT</td>
<td></td>
</tr>
<tr>
<td>c. Periprocedural serious events</td>
<td></td>
</tr>
<tr>
<td>d. Good neurological recovery (NIHSS ≥ 10) at discharge</td>
<td></td>
</tr>
<tr>
<td>e. Incidence of sICH and asymptomatic haemorrhage</td>
<td></td>
</tr>
<tr>
<td>MT treated patients</td>
<td>refractory to IV-rtPA</td>
</tr>
<tr>
<td>---------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>e. Core infarct volume assessed by CTP, CTA or DWI scans within 60 minutes to arterial puncture.</td>
<td></td>
</tr>
<tr>
<td>f. No history of stroke within 3 months</td>
<td></td>
</tr>
<tr>
<td>g. No evidence of mass effect with midline shift or ICH on NCCT</td>
<td></td>
</tr>
<tr>
<td>h. No evidence of arterial stenosis proximal to the occlusion that could prevent thrombectomy</td>
<td></td>
</tr>
<tr>
<td>i. No evidence of preexisting arterial injury</td>
<td></td>
</tr>
<tr>
<td>j. Life expectancy &lt; 90 days</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EASI [1102] 0-5h To evaluate the efficacy of IV-rtPA vs combined (MT plus IV-rtPA) treatment in AIS</th>
<th>a. Age ≥18</th>
<th>a. Favourable clinical outcome (mRS 0-2 at 90 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>b. NIHSS ≥8</td>
<td>b. sICH on CT at 24 h</td>
<td></td>
</tr>
<tr>
<td>c. Onset to treatment less than 5 hours or symptom/imaging mismatch</td>
<td>c. Infarct evolution on CT between pre-treatment and 24h using the ASPECT score</td>
<td></td>
</tr>
<tr>
<td>d. Occlusion of MCA (m1 or M2), supraclinoid ICA or basilar trunk</td>
<td>d. Recanalization using TICI</td>
<td></td>
</tr>
<tr>
<td>e. No evidence of</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| BASICS [971, 972] | 0-6h | Efficacy and safety of IAT plus standard medical therapy vs. standard medical alone in patients with an acute symptomatic basilar artery occlusion (BAO) | hemisphere hemorrhagic transformation of the infarcted territory | a. Symptoms of BAO stroke 
b. BAO confirmed by CTA or MRA 
c. Age ≥18 
d. NIHSS ≥8 at the time of neuroimaging 
e. IAT initiated within 6 h of onset of symptom 
f. Premorbid score of 0-2 
g. No ICH or mass effect on CT | a. Favourable outcome mRS 0-2 
b. Excellent outcome mRS 0-3 
c. Recanalization at 24 ± 6 h on CTA 
d. Volume of infarction on NCCT and CTA source images at 24 ± 6 h 
e. sICH at 24 ± 6 h on CTA 
f. Mortality at 90 days 
g. NIHSS pre, and port IV-tPA and at 24 h 
h. EQ-5D – Quality of life at 90 and 120 days |
| SIESTA [960, 1103] | NR | Efficacy of conscious sedation vs. general anaesthesia during IAT. Update: No advantage for the use of conscious sedation recently | scale after thrombectomy e. Procedural complication within 3 months f. ICH on NCCT at 24h | a. Age ≥18 years 
b. Acute stroke in anterior circulation 
c. ICA or MCA occlusion on CTA 
d. No evidence of ICH | a. Higher NIHSS of >10 at 24 h 
b. NIHSS improvement 
c. mRS at 90 days 
d. Mortality before discharge or at 90 days 
e. Duration of hospital stay 
f. Recanalization status on TICI |
<table>
<thead>
<tr>
<th>Study</th>
<th>NR</th>
<th>Efficacy of general vs. local anaesthesia during IAT</th>
<th>Periinterventional complications</th>
</tr>
</thead>
</table>
| GOLIATH [961] | NR | Efficacy of general vs. local anaesthesia during IAT | a. Growth of DWI lesion (48-72 h)
   b. mRS score at 90 days
   c. Blood pressure during intervention (1-2 h)
   d. Time from arrival to the groin puncture and recanalization (1-2 h) |
| ANSTROKE [962] | NR | To study the efficacy of general anaesthesia vs sedation technique during embolectomy for AIS stroke (systolic pressure 140-180 mmHg) | a. 90 day mRS
b. Change in NIHSS score compared to admission (Day 3, 7 and 90)
c. Degree of recanalization and reperfusion (1 day after embolectomy)
d. Periprocedural complications
e. Infarction magnitude
f. CT day 1 including CTP
g. MR on day 3 (2-4) and 3 months
h. Brain markers (GFAP, Tau, S-100B) before, 2, 24, 48, 72 hours and 3 months after the embolectomy |
| MOST [976] | NR | Phase III trial to explore the efficacy of IV delivery of antithrombotic medications Argatroban and Eptifibatide in combination with rtPA in AIS. | NIHSS>6 |

- **a. mRS at 90 days**
- **b. longitudinal model relating 30 day mRS to 90 day mRS**

RACECAT = Direct Transfer to an Endovascular Centre Compared to Transfer to the Closest Stroke Centre in Acute Stroke Patients With Suspected Large Vessel Occlusion; RACE scale = Rapid Arterial occlusion Evaluation; mRS = modified Rankin score; EMS = emergency medical service; LVO = large vessel occlusion; AIS = acute ischemic stroke; LSC = local stroke centre; EVT-LSC = Endovascular stroke centre; DEFUSE-3: Endovascular Therapy Following Imaging Evaluation for Ischemic Stroke 3; DAWN = Trevo and Medical Management Versus Medical Management Alone in Wake Up and Late Presenting Strokes; BASICS = Basilar Artery International Cooperation Study; IAT = intra-arterial therapy; IV-rtPA = intra venous tissue plasminogen activator; NCCT = non-contrast computed tomography; CTA= CT angiography; sICH = symptomatic intracranial haemorrhage; SAH = sub-arachnoid haemorrhage; BAO = basilar artery occlusion; SIESTA = Sedation vs. Intubation for...
Endovascular Stroke Treatment; GOLIATH = The General or Local Anaesthesia in Intra-arterial Therapy; MOST = The Multi-Arm Optimization of Stroke Thrombolysis; NR = not required; ANSTROKE = Sedation Versus General Anaesthesia for Endovascular Therapy in Acute Stroke - Impact on Neurological Outcome; ENDOSTROKE = International Multicenter Registry for Mechanical Recanalization Procedures in Acute Stroke; TIMI = Thrombolysis in Myocardial Infarction; START = Imaging Guided Patient Selection for Interventional Revascularization Therapy; EASI = Endovascular Acute Stroke Intervention Trial.
“I think of life as a good book. The further you get into it, the more it begins to make sense.”

- Harold S. Kushner
CHAPTER 3: PROGNOSTICATION OF ACUTE ISCHEMIC STROKE USING BASELINE STROKE SEVERITY

3.1. Contributions


75% conception and design of research
75% experimental procedures
75% analyses and interpretation of the findings
75% writing of the paper and critical appraisal of the content

Prof Christopher Levi

13/02/2017

Signed: Date:

A/Prof Peter Stanwell

17/02/2017

Signed: Date:

Prof John Attia

15/02/2017

Signed: Date:
Louise-Anne Jordan

Signed: 13/02/2017
Date:

Gemma H. Kitsos

Signed: 16/02/2017
Date:

Rhonda Walker

Signed: 15/02/2017
Date:

Faculty Assistant Dean (Research Training)

Prof Robert Callister

Signed: 17/2/17
Date:
The Influence of Initial Stroke Severity on Mortality, Overall Functional Outcome and In-Hospital Placement at 90 days Following Acute Ischemic Stroke: A Tertiary Hospital Stroke Register Study

Sonu Bhaskar¹,², Peter Stanwell², Andrew Bivard¹, Neil Spratt¹, Rhonda Walker³, Gemma H. Kitsos¹, Mark W. Parsons¹, Malcolm Evans¹, Louise Jordan¹, Michael Nilsson²,⁵, John Attia⁴, Christopher Levi¹

¹Department of Neurology, John Hunter Hospital, University of Newcastle, Australia, ²Centre for Translational Neuroscience and Mental Health, and Hunter Medical Research Institute (HMRI), University of Newcastle, Australia, ³Cardiac and Stroke Outcomes Unit, Hunter New England Local Health District, Australia, ⁴Centre for Clinical Epidemiology and Biostatistics, Hunter Medical Research Institute, University of Newcastle, Australia, ⁵Department of Clinical Neuroscience, Sahlgrenska University Hospital, University of Gothenburg, Sweden

Corresponding author: Dr. Sonu Bhaskar, Department of Neurology, John Hunter Hospital, Lookout Road, New Lambton NSW 2305, Australia
E-mail: Sonu.Bhaskar@uon.edu.au

Submission: 15-02-2016
Review completed: 16-04-2016
Accepted: 26-04-2016
ABSTRACT

Background and Purpose: Epidemiological studies on the extent of the interaction and/or influence of stroke severity on clinical outcomes are important. The aim of the present study was to investigate the putative (and degree of) impact of initial stroke severity in predicting the overall functional outcome, in-hospital placement, and mortality in acute ischemic stroke (AIS) in comparison with age, admission to the stroke, unit and thrombolytic treatment.

Materials and Methods: The John Hunter Hospital acute stroke register was used to collect a retrospective cohort of AIS patients being assessed for reperfusion therapy and admitted between January 2006 and December 2013. Univariate and multivariate logistic regression and receiver operating characteristics analyses were used to assess associations with functional outcome, in-hospital placement, and mortality at 90 days.

Results: 608 AIS patients with complete datasets were included in the study. On univariate analysis, initial stroke severity showed the strongest independent association to the risk of death within 90 days (OR = 1.15; P < 0.001; 95% CI = [1.11, 1.18]); age was a less significant independent influence (OR = 1.02; P = 0.049; 95% CI = [1.00, 1.03]). Multivariate logistic regression analysis demonstrated that initial stroke severity independently predicted 90 days mortality (OR = 1.16; 95% CI = [1.12, 1.2]; P < 0.0001) and unfavourable outcome (OR = 1.16; 95% CI = [1.13, 1.2]; P < 0.0001). Higher National Institute of Health Stroke Scale at admission was significantly associated with longer in-hospital placement (P < 0.0001).

Conclusions: In this acute stroke cohort, initial stroke severity had a major impact on the likelihood of death following an AIS and appears to be the dominant influence on the overall stroke outcome and in-hospital placement.

Key words: Age, hospital assessment, mortality, NIHSS, prognosis, stroke, stroke severity
Introduction

Stroke is a leading cause of death and disability worldwide [24]. According to the Australian Institute of Health and Welfare (2012), Stroke is Australia’s second biggest cause of mortality after coronary heart disease and a leading cause of disability [1104]. More than 65% of stroke survivors also suffer a disability that impedes their ability to carry out daily living activities unassisted [1105]. Understanding of factors contributing to the progression of stroke and/or mortality may have an important impact on future stroke trials and patient management. The National Institute of Health Stroke Scale (NIHSS) is a commonly used stroke impairment scale and is well validated across many hospitals across the globe [1106]. NIHSS sums the scores from individual elements of the neurological examination to provide an overall stroke impairment score [1107]. NIHSS has been used as initial stroke severity assessment for a variety of purposes including prediction of progression of acute stroke [1108] and patient outcomes [568, 1106].

Predicting the clinical course of patients with acute stroke continues to be a prognostic challenge for stroke physicians [1109]. Early risk stratification of acute stroke patients has contributed important clinical estimates of mortality risk using reliable and simple prognostic models [1052, 1110]. At present, prognostic models of mortality are used in the economic and performance evaluation of stroke care centres. However, these models often lack appropriate case-mix adjustment of initial stroke severity. Initial severity of stroke and age are both recognized to influence the likelihood of an unfavourable functional outcome and/or mortality following an acute stroke. However, the extent of the interaction is uncertain. Therefore, epidemiological studies on the extent of the interaction and/or influence of stroke severity and age on mortality and overall functional outcomes are important. Performance evaluation and report cards on hospitals and physicians are increasingly utilized to judge, evaluate, and/or compare health-care provider performance in terms of various “outcome-determinants,” including patient outcomes (such as mortality) and incurred costs [1052, 1111-1126]. Such analyses may use the available demographic data, including age, but not include relevant clinical data such as stroke severity. National stroke care guidelines now recommend the preferential triage of acute stroke to specialized tertiary care stroke units [977, 1127]. This results in specialized tertiary stroke units
receiving different case-mixes compared to non-stroke unit hospitals. For instance, large hospital or university health care centres are more likely to encounter more severe cases including those that come as referrals from local small hospitals with severe morbidity. Based on the clinical experience, it may well be argued that there is anecdotal evidence that these facilities often receive patients who have greater morbidity, are in advanced stages of their illness, and are more likely to have severe comorbid health conditions.

The purpose of this study was to investigate the putative (and degree of) impact of stroke severity in predicting the overall functional outcome, in-hospital placement, and death at 90 days, in comparison with age, admission to the stroke unit, and thrombolytic treatment. Our underlying hypothesis is that the NIHSS will be the dominant clinical determinant of stroke prognosis.

**Materials and Methods**

The John Hunter Hospital (JHH) is a tertiary referral hospital for the Hunter New England Local Health District (HNELHD). JHH acts as the regional thrombolysis referral centre servicing the great Newcastle, Hunter and Manning region within HNELHD. JHH acute stroke register was used to collect a retrospective cohort of acute stroke patients, who were being assessed for potential suitability for reperfusion therapy, admitted between January 2006 and December 2013. The JHH acute stroke registry collects all patients presenting with acute stroke being evaluated for potential suitability for reperfusion therapy. The register prospectively documents covariates including age and baseline stroke severity (measured using the NIHSS) and 90-day functional outcome, as well as the patient demographics, medical comorbidities, risk factors, complications, treatment and diagnostic procedures. Patients with intracerebral haemorrhage and subarachnoid haemorrhage were excluded from the final dataset. The database was then linked, to obtain 90 days mortality status, to HNELHD’s Cardiac and Stroke Outcomes Unit database. HNELHD’s Cardiac and Stroke Outcomes Unit tracks 90 days stroke mortality using medical records coding of all HNE stroke separations using International Statistical Classification of Diseases and Related Health Problems (ICD)-10 (Figure 1). For the cohort identified through the JHH acute stroke register mortality was tracked using the following criteria:
1. ICD10 code of I63 (cerebral infarction) or I64 (stroke, not specified as haemorrhage or infarction) in any of the first five diagnoses on discharge from any HNE hospital in a period of care.

2. Admitted to JHH as part of a period of hospital care that occurred between 1 January 2006 and 31 December 2013.

3. First admission to any hospital in the period of care classified as an emergency admission.

NIHSS score >17 was classified as severe strokes, NIHSS <8 corresponded to a mild stroke, and NIHSS score of 8-16 was grouped in moderate stroke category, as used in other studies [419, 1128]. A score of 0-2 represented a good or favourable outcome and a score of 3-6 a poor or unfavourable outcome. In-hospital placement or length of stay was defined as the total duration of stay in the hospital during the various stages of the acute stage and post-acute rehabilitation treatment.

**Statistical analysis**

All statistical analysis was performed using STATA (Version 10, 2001; College Station, TX, USA). The principal analysis was the use of logistic regression to determine the independent predictors of 90-days mortality. The predictors of interest were gender, age, baseline stroke severity measured by NIHSS, and thrombolytic treatment. Each of these predictors was examined using a simple logistic regression model, and a selection of those with $P < 0.10$ were included in subsequent multiple logistic regression models. These models differed in terms of the particular predictors included. Univariate and multivariate logistic regression were used to assess associations with 90-day mortality. Finally, receiver–operator characteristic (ROC) curve was used to plot baseline stroke severity and age each in relation to 90 days mortality and unfavourable outcome in order to investigate the extent of interactions of age and stroke severity with stroke mortality and overall outcome. We quantified the accuracy and independent effect of stroke severity and age by calculating the area under the curves (AUC) and odds ratio (based on the regression analysis). A $P$-value of $\leq 0.05$ was considered significant.

Logistic regression analysis was also performed to study the influence of stroke severity and age on the level of dependency or overall stroke outcome (modified
Rankin score (mRS) at 90 days and in-hospital placement. The influence of stroke severity and age (as predictor variables) on in-hospital placement (or length of stay post-stroke) was studied using multivariate regression analysis. In addition, we performed correlation to study the extent of association of stroke severity on admission and age with the functional outcome (measured by mRS) at 90 days using pairwise Pearson’s correlation coefficient ($r$). Significance level of the correlation coefficients for each variable was also tested.

**Results**

From the initial 957 patients entered in the register, 201 patients were excluded because of incomplete data on potentially important covariates. The exclusion and inclusion algorithm is shown in Figure 1. Overall, out of 756 patients who were discharged with ICD-10 diagnosis, 608 patients with complete datasets were included in the study after the exclusion of patients with intracerebral and subarachnoid haemorrhages. Dataset of 486 patients with available day 90 mRS scores were used to study the association of stroke severity and other covariates with the overall functional outcome. For the study on association of stroke severity and other covariates with in-hospital placement and mortality, the available dataset on 588 patients was used.

The demography of the population studied is shown in detail in Table 1. The average age of the study population was 75.3 (SD = 12.94, min = 24, max = 98) years. In addition, the average in-hospital placement or length of stay was 17.5 days (SD = 22.57, min = 1 day, max = 153 days). Females constituted 48% ($n = 292$) of the study population, and 81.9% of the admitted patients were 65 years or older. The distribution of patients based on their age category and stroke severity profile is shown in Figure 2. Patients aged 65 and above recorded moderate to severe stroke severity scores of 8 and above (70.7%). Patients with NIHSS score of 17 and above accounted for 33.84% of the overall population. Thrombolytic treatment was given to 53.5% of patients. Patients aged 75 and above accounted for 60% of the overall study population. A total of 126 patients (126/608 = 20.7%) were dead at the end of the follow-up (90 days). Furthermore, 46% of the patients showed good functional clinical outcome at 90 days. A majority of our patients (89%) were admitted to the specialized acute stroke unit.
Prediction of stroke mortality, favourable, and unfavourable outcome at 90 days

Univariate regression analysis showed that the stroke severity at admission, measured with NIHSS, was positively associated with 90-days mortality (OR = 1.15; 95% CI = [1.11, 1.18]; \( P < 0.001 \)) and unfavourable outcome (OR = 1.14; 95% CI = [1.10, 1.17]; \( P \leq 0.0001 \)) in this study population. Age was less significantly associated with 90-days mortality (OR = 1.02; 95% CI = [1.00, 1.02]; \( P = 0.049 \)) and unfavourable outcome (OR = 1.03; 95% CI = [1.01, 1.04]; \( P \leq 0.0001 \)) [Table 2]. Patients admitted to the stroke unit showed a positive association with 90 days favourable outcome (OR = 2.85; 95% CI = [1.44, 5.61]; \( P = 0.003 \)) and were negatively associated with unfavourable outcome (OR = 0.35; 95% CI = [0.18, 0.69]; \( P = 0.003 \)) and mortality (OR = 0.26; 95% CI = [0.15, 0.44]; \( P \leq 0.0001 \)) at 90 days. Thrombolysis was not significantly associated with mortality and clinical outcome at 90 days. However, there was a tendency toward favourable outcome in patients who received thrombolysis (OR = 1.33; 95% CI = [0.94, 1.9]; \( P = 0.111 \)).

Multivariate analysis for the prediction of stroke mortality using stroke severity, age, admission to stroke unit, and thrombolysis as independent variables demonstrated that stroke severity was strongest predictor of mortality (OR = 1.26; 95% CI = [1.12, 1.2]; \( P \leq 0.0001 \)) [Table 2]. Age was no longer significantly associated with mortality (OR = 1.01; 95% CI = [0.99, 1.03]; \( P = 0.303 \)). Admission to stroke unit (OR = 0.21; 95% CI = [0.10, 0.42]; \( P \leq 0.0001 \)) and thrombolysis (OR = 0.58; 95% CI = [0.36, 0.95]; \( P = 0.03 \)) were negatively associated with stroke mortality. In terms of unfavourable outcome, stroke severity was the dominant factor (OR = 1.16; 95% CI = [1.13, 1.2]; \( P \leq 0.0001 \)), followed by age (OR = 1.03; 95% CI = [1.01, 1.05]; \( P = 0.001 \)) while controlling for the effects of admission to stroke unit and thrombolysis.

Receiver–operator characteristic analysis

The ROC analyses revealed that the model with stroke severity and age, while controlling for the effects of stroke unit admission and thrombolysis, demonstrated higher predictive ability for mortality (ROC area = 0.80; specificity = 97%; overall rate of correct classification = 81%; positive predictive value = 61%) versus overall unfavourable outcome (ROC area = 0.78; specificity = 71%; Overall rate of correct
classification = 70%; positive predictive value = 67\%\) at 90 days [Table 3]. Figure 3 shows the ROC (sensitivity vs. 1-specificity) analyses curves for the prediction of mortality and overall clinical outcomes at 90 days.

**Influence on stroke outcome and in-hospital placement**

Moderate positive correlation was observed between NIHSS score at admission and mRS scores at 90 days \(r = 0.47; P < 0.001\) [Figure 4]. However, no correlation was observed between age and mRS at 90 days \(r = 0.15, P = 0.0006\).

Severe (OR = 16, 95% CI = [7.6, 35], \(P \leq 0.0001\)) and moderate (OR = 4.8; 95% CI = [2.13, 10.7]; \(P \leq 0.0001\)) strokes were significantly associated with mortality at 90 days [see Table 4]. Patients aged 75 and above were two times more likely to be associated with mortality at 90 days. However, the association was not statistically significant \(P = 0.153\). In terms of unfavourable outcome, severe stroke (OR = 12; 95% CI = [6.7, 21.6]) had a dominant effect in comparison to that of age \(\geq 75\) (OR = 4; 95% CI = [1.75, 10.55]), as evident from higher odds ratios of 12 vs. 4.

The influence of NIHSS at admission and age on length of hospital stay or in-hospital placement is shown in Table 5. Multivariate regression analysis revealed that increasing NIHSS at admission was significantly associated with longer in-hospital placement \(P < 0.0001\). Severe strokes were more likely to have a longer in-hospital placement \(P < 0.0001\).

**Discussion**

This study aimed to compare the independent influence of both initial stroke severity and age on the likelihood of unfavourable clinical outcome and death within 90 days of an acute ischaemic stroke. We found that the initial stroke severity showed the strongest independent association with the risk of death and unfavourable outcome within 90 days. Previous studies have also indicated that stroke severity is an important determinant of patient outcome in stroke [1129-1131]. Initial stroke severity is also a significant predictor of responses to treatment in ischemic stroke [112, 760, 1132-1134]. Our results indicate that it is important to measure stroke severity and make an adjustment for stroke severity when reporting for both functional outcome
and mortality rates. At present, in most of the administrative datasets, stroke severity measurements are not available. This is pertinent as there is an increasing number of administrative dataset publications reporting stroke mortality rates where adjustment for stroke severity is absent or limited [1052, 1135]. Indeed, studies have shown that if the severity of stroke case-mix is not taken into account; it can lead to skewed or even misleading mortality estimates, which in turn may have implications for funding and health-care policy. A recent study in the United States by Fonarow et al [1052] has generated interest in the practice of performance evaluation for acute stroke services. The authors noted that risk models potentially used to measure hospitals performance, which discounts initial stroke severity, can be misleading and could lead to misalignment of incentives. The authors re-evaluated rankings of hospitals post-stroke severity adjustment and found that a significant proportion of hospitals initially ranked as mortality rate outliers fell within the specified boundaries post-adjustment. Therefore, stroke risk models using either administrative data or clinical data that do not include severity have inferior discrimination, substantial unaccounted for a variance, and can result in misclassification of hospital performance.

Our findings also suggest that stroke severity is an independent predictor of overall functional outcome. We found a positive correlation between increasing admission NIHSS score and the unfavourable modified Rankin at 90 days. This further establishes the need for appropriate case-mix adjustment in comparison of functional outcome performance across hospitals [1136, 1137]. We also found that increasing stroke severity was associated with longer in-hospital placement. At present, most administrative datasets do not contain many of the key covariates necessary to perform appropriate case-mix adjustment. Our study also showed that the length of in-hospital placement was significantly influenced by the stroke severity. This is in agreement with the findings reported elsewhere [1138].

Our study has several limitations and we acknowledge that bias could have been introduced. First, the retrospective nature of our study limits acquisition of some data elements and the tertiary hospital-based acute stroke under assessment for reperfusion therapy sampling frame has led to a collection of hyperacute and severe stroke case-mix. Higher rates of thrombolysis implementation in our cohort are an indicator of this selection bias. Importantly, however, intravenous tissue plasminogen
activator or Alteplase does not significantly alter ischemic stroke mortality rates [732, 1139]. Bias could also be introduced due to the kind of treatment regimen or the treatment pathway the patient was on.

In conclusion, this study shows that baseline stroke severity is a dominant influence on the overall functional outcome, in-hospital placement, and mortality post-stroke. Stroke severity was independently associated with the likelihood of death following an acute stroke. Baseline stroke severity is an essential covariate in any analysis of stroke outcome. Reliable prognostic modelling in acute stroke requires the use of a valid adjustment for baseline stroke severity. Our results are an addition to the evidence base for clinicians and researchers to use stroke severity for early risk stratification. Analyses of stroke outcome not including baseline stroke severity should be considered inadequate and potentially misleading.

ACKNOWLEDGMENTS

The authors would like to thank the patients and carers, and the doctors, administrators, radiographers, stroke clinical audit and programming staff who contributed to the collection of data. We are especially grateful for administrative support from Michelle K. Wyborn and Kristy Morris.

COMPETING INTERESTS

The author(s) declare that they have no competing interests.
FIGURE LEGENDS

**Figure 1.** Inclusion and exclusion algorithm. I64 = stroke, not specified as haemorrhage or infarction, I63 = cerebral infarction, I62 = other nontraumatic intracranial haemorrhage, I61 = intracerebral haemorrhage, I60 = subarachnoid haemorrhage.

**Figure 2.** Chart showing the distribution of age profiles and corresponding stroke severity categories. Age categories: <55, 55 to 64, 65 to 74, and ≥75 years. Stroke severity categories: Mild (NIHSS<8), moderate (NIHSS: 8 to 16) and severe (NIHSS: 17 and above).

**Figure 3.** Receiver operating characteristic (ROC) curves for mortality (a), favourable outcome (b) and unfavourable outcome (c). The area under the curve (AUC) is a measure of the discriminatory power of the risk model.

**Figure 4.** Correlation of presenting NIHSS score and modified Rankin score (at 90 days). Baseline NIHSS score was predictive of overall functional outcome at 90 days. The regression line is obtained by plotting the fitted values of 90-day mRS scores and the NIHSS at admission.
Figure 1

ICD-10 Discharge
(n=756)

164  (n=54)  163  (n=554)  161  (n=109)  162  (n=3)  160  (n=8)

Not with primary discharge diagnosis of stroke
(n=28)

Included in the study
(n=608)

Excluded from the study
(n=148)

Datasets

With 90 days mortality & in-hospital placement data
(n=588)

With the 90 days overall outcome (mRS) data
(n=486)

Excluded due to unavailable mRS at 90 days scores
(n=122)
Figure 2.
Figure 3.

(i) Mortality (mRS=6)  
(ii) Unfavourable outcome (mRS 3-6)  
(iii) Favourable outcome (mRS 0-2)
## Table 1: Demographic and clinical characteristics of the study patients. Values are expressed as number (percentages) unless otherwise indicated.

<table>
<thead>
<tr>
<th>Gender (n=608)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>316</td>
<td>(52)</td>
</tr>
<tr>
<td>Female</td>
<td>292</td>
<td>(48)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age (n=608); mean±SD</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &lt; 55</td>
<td>49</td>
<td>(8.06)</td>
</tr>
<tr>
<td>55≤Age≤64</td>
<td>61</td>
<td>(10.03)</td>
</tr>
<tr>
<td>65≤Age≤74</td>
<td>135</td>
<td>(22.20)</td>
</tr>
<tr>
<td>Age ≥ 75</td>
<td>363</td>
<td>(59.70)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment factors</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombolytic treatment</td>
<td>325</td>
<td>(53.45)</td>
</tr>
<tr>
<td>Stroke unit admission</td>
<td>542</td>
<td>(89.14)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NIHSS at admission (n=588); median (IQR)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild (0≤NIHSS≤7)</td>
<td>185</td>
<td>(31.46)</td>
</tr>
<tr>
<td>Moderate (8≤NIHSS≤16)</td>
<td>204</td>
<td>(34.69)</td>
</tr>
<tr>
<td>Severe (17≤NIHSS≤44)</td>
<td>199</td>
<td>(33.84)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NIHSS at 24 hours (n=426); median (IQR)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of hospital stay; median (IQR)</td>
<td>9</td>
<td>(15)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dead at 90 days (n=608)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 90 mRS (n=504); median (IQR)</td>
<td>3</td>
<td>(4.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Good Clinical Outcome (mRS= 0-2)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>75</td>
<td>(14.88)</td>
</tr>
<tr>
<td>1</td>
<td>106</td>
<td>(21.03)</td>
</tr>
<tr>
<td>2</td>
<td>53</td>
<td>(10.52)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bad Clinical Outcome (mRS= 3-6)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>51</td>
<td>(10.12)</td>
</tr>
<tr>
<td>4</td>
<td>42</td>
<td>(8.33)</td>
</tr>
<tr>
<td>5</td>
<td>51</td>
<td>(10.12)</td>
</tr>
<tr>
<td>6</td>
<td>126</td>
<td>(25)</td>
</tr>
</tbody>
</table>

*P<0.05 as threshold for statistical significance.
Table 2: Odds ratios (95% confidence intervals) for prediction of 90 days mortality (Model I), the favourable outcome at 90 days (Model II), and unfavourable outcome at 90 days (Model III).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mortality (n=588)</th>
<th>Favourable Outcome (mRS 0-2) (n=486)</th>
<th>Unfavourable Outcome (mRS 3-6) (n=486)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Simple OR (95% CI); P &gt;</td>
<td>Mixed OR (95% CI); P &gt;</td>
<td>Simple OR (95% CI); P &gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIHSS at admission</td>
<td>1.15 (1.11-1.18); P &lt;</td>
<td>1.16 (1.12-1.2); P =</td>
<td>1.16 (1.10-1.17); P =</td>
</tr>
<tr>
<td></td>
<td>0.0001*</td>
<td>&lt;0.0001*</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Age</td>
<td>1.02 (1.00-1.02); P =</td>
<td>1.01 (0.99-1.03); P =</td>
<td>1.03 (1.01-1.05); P =</td>
</tr>
<tr>
<td></td>
<td>0.049*</td>
<td>0.303</td>
<td>0.001*</td>
</tr>
<tr>
<td>Admission to Stroke Unit</td>
<td>0.26 (0.15-0.44); P &lt;</td>
<td>0.21 (0.10-0.42); P &lt;</td>
<td>0.35 (0.18-0.57); P =</td>
</tr>
<tr>
<td></td>
<td>0.0001*</td>
<td>0.0001*</td>
<td>0.001*</td>
</tr>
<tr>
<td>Thrombolysis</td>
<td>0.77 (0.52-1.15); P =</td>
<td>0.58 (0.36-0.95); P =</td>
<td>0.75 (0.53-0.50); P =</td>
</tr>
<tr>
<td></td>
<td>0.203</td>
<td>0.03*</td>
<td>0.001*</td>
</tr>
</tbody>
</table>
Table 3. Sensitivity and Specificity Analysis for age and stroke severity controlling for the effects of thrombolysis and admission to a stroke unit, as a test for prediction of 90 days mortality (Model I), the favourable outcome at 90 days (Model II), and unfavourable outcome at 90 days (Model III).

<table>
<thead>
<tr>
<th></th>
<th>Model I</th>
<th>Model II</th>
<th>Model III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(90 days mortality (mRS=6)); n=588</td>
<td>(Favourable Outcome (mRS=0-2) at 90 days); n=486</td>
<td>(Unfavourable Outcome (mRS=3-6) at 90 days); n=486</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>21.01%</td>
<td>69.60%</td>
<td>70.66%</td>
</tr>
<tr>
<td>Specificity</td>
<td>96.59%</td>
<td>70.66%</td>
<td>69.60%</td>
</tr>
<tr>
<td>PPV</td>
<td>60.98%</td>
<td>67.52%</td>
<td>72.62%</td>
</tr>
<tr>
<td>NPV</td>
<td>82.82%</td>
<td>72.62%</td>
<td>67.52%</td>
</tr>
<tr>
<td>Overall rate of correct classification</td>
<td>81.29%</td>
<td>70.16%</td>
<td>70.16%</td>
</tr>
<tr>
<td>Area under the ROC curve</td>
<td>0.80</td>
<td>0.78</td>
<td>0.78</td>
</tr>
</tbody>
</table>

NPV = negative predictive value, PPV = positive predictive value, ROC = receiver-operating characteristic.
Table 4: Multivariate analysis with stratified stroke severity and age groups showing odds ratios (95% confidence intervals) for the prediction of prediction of 90 days mortality (Model I), the favourable outcome at 90 days (Model II), and the unfavourable outcome at 90 days (Model III).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mortality at 90 days (n=588)</th>
<th>Unfavourable Outcome (n=486)</th>
<th>Favourable Outcome (n=486)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dead vs alive at 90 days, n (%)</td>
<td>Mixed OR (95% CI); P&gt;</td>
<td>z</td>
</tr>
<tr>
<td>NIHSS at admission</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild (0≤NIHSS≤7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 (8.4) vs 175 (27.31); P&lt;0.0001*</td>
<td>35 (13.5) vs 94 (41.4); P&lt;0.0001*</td>
<td>1 94 (41.4) vs 35 (13.51); P&lt;0.0001*</td>
</tr>
<tr>
<td>Moderate (8≤NIHSS≤16)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 (25.21) vs 174 (37.10); P=0.017*</td>
<td>85 (32.82) vs 91 (40.1); P=0.108</td>
<td>3.8 (2.18-6.64); P&lt;0.0001*</td>
</tr>
<tr>
<td>Severe (17≤NIHSS≤44)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>79 (66.39) vs 120 (25.59); P=0.0001*</td>
<td>139 (53.67) vs 42 (18.5); P=0.0001*</td>
<td>11.99 (6.66-21.6); P&lt;0.0001*</td>
</tr>
<tr>
<td>Age</td>
<td>P=0.383</td>
<td>P=0.005</td>
<td>P=0.005</td>
</tr>
<tr>
<td>Age &lt; 55</td>
<td>4 (3.17) vs 45 (9.34); P=0.026*</td>
<td>8 (2.96) vs 27 (11.54); P&lt;0.0001*</td>
<td>1 27 (11.54) vs 8 (2.96); P&lt;0.0001*</td>
</tr>
<tr>
<td>55≤Age≤64</td>
<td>14 (11.11) vs 47 (9.75); P=0.621</td>
<td>31 (11.48) vs 21 (8.97); P=0.381</td>
<td>4.69 (1.59-13.79); P=0.005*</td>
</tr>
</tbody>
</table>
| 65≤Age≤74                 | 22 (17.46) vs 113             | 1.63 (0.48- vs 49 (18.15) vs 58 | 2.76 (1.06-7.22); | 58 (24.79) vs 49 | 0.36 (0.14-
<table>
<thead>
<tr>
<th></th>
<th>Age ≥ 75</th>
<th>Stroke unit admission</th>
<th>Thrombolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>(23.44); P=0.185</td>
<td>(24.79); P=0.081</td>
<td>(18.15); P=0.081</td>
<td>0.95); P=0.039*</td>
</tr>
<tr>
<td>5.52); P=0.429</td>
<td>5.52); P=0.429</td>
<td>5.52); P=0.429</td>
<td>5.52); P=0.429</td>
</tr>
<tr>
<td>0.039*</td>
<td>0.001*</td>
<td>0.004*</td>
<td>0.005*</td>
</tr>
<tr>
<td>Age ≥ 75</td>
<td>86 (68.25) vs 277 (57.47); P=0.032*</td>
<td>2.3 (0.73-7.2); P=0.153</td>
<td>61 (48.41) vs 264 (54.77); P=0.229</td>
</tr>
<tr>
<td></td>
<td>182 (67.41) vs 128 (54.7); P=0.004*</td>
<td>4.3 (1.75-10.55); P=0.001*</td>
<td>146 (54.07) vs 143 (61.1); P=0.003*</td>
</tr>
<tr>
<td></td>
<td>128 (54.70) vs 182 (67.41); P=0.004*</td>
<td>0.23 (0.09-0.57); P=0.001*</td>
<td>143 (61.11) vs 146 (54.07); P=0.125</td>
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<td>0.23 (0.09-0.57); P=0.001*</td>
<td>0.23 (0.09-0.57); P=0.001*</td>
<td>0.23 (0.09-0.57); P=0.001*</td>
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<tr>
<td>Stroke unit admission</td>
<td>96 (76.19) vs 446 (92.53); P=0.0001*</td>
<td>0.21 (0.11-0.42); P=&lt;0.001*</td>
<td>61 (48.41) vs 264 (54.77); P=0.229</td>
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<td>234 (86.67) vs 222 (94.87); P=0.002*</td>
<td>0.29 (0.12-0.68); P=0.005*</td>
<td>146 (54.07) vs 143 (61.1); P=0.003*</td>
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<td>222 (94.87) vs 234 (86.67); P=0.002*</td>
<td>3.49 (1.47-8.27); P=0.005*</td>
<td>143 (61.11) vs 146 (54.07); P=0.125</td>
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<td>0.23 (0.09-0.57); P=0.001*</td>
<td>0.23 (0.09-0.57); P=0.001*</td>
<td>0.23 (0.09-0.57); P=0.001*</td>
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Table 5. Multivariate linear regression analysis for prediction of in-hospital placement or length of hospital stay.

| Variable (n=588) | Coefficients (95% confidence interval) | P>|t| |
|------------------|----------------------------------------|-----|
| **Model (A)**    |                                        |     |
| NIHSS at admission | 0.49 (0.24, 0.74)                        | <0.0001* |
| Age              | -0.17 (-0.31, -0.03)                    | 0.018* |
| Stroke unit admission | 7.84 (1.71, 13.96)                      | 0.012* |
| Thrombolysis     | 0.16 (-3.74, 4.05)                      | 0.936 |
| **Model (B)**    |                                        |     |
| NIHSS at admission | <0.00001*                              |     |
| Mild (0≤NIHSS≤7) | 1                                      |     |
| Moderate (8≤NIHSS≤16) | 8.96 (4.34, 13.59)                    | <0.0001* |
| Severe (17≤NIHSS≤44) | 11.08 (6.41, 15.74)                  | <0.0001* |
| Age              |                                        |     |
| Age < 55         | 1                                      | 0.001* |
| 55≤Age≤64        | 8.26 (-0.27, 16.79)                    | 0.058 |
| 65≤Age≤74        | -0.44 (-7.79, 6.91)                    | 0.907 |
| Age ≥ 75         | -4.14 (10.91, 2.63)                    | 0.230 |
| Stroke unit admission | 7.96 (1.85, 14.07)                  | 0.011* |
| Thrombolysis     | -0.44 (-0.43, 3.45)                    | 0.825 |
“In spirituality, the searching is the finding and the pursuit is the achievement.”

- Abraham J. Twerski
CHAPTER 4: LESION TOPOGRAPHY AND PERI-LESIONAL HYPERPERFUSION IN ISCHEMIC STROKE

4.1. INTRODUCTION

Grouping of acute stroke patients into specific imaging profiles can allow greater understanding of prognosis and can assist in the selection of patients for specific acute therapies or interventions [646, 1140]. A similar strategy may also be clinically relevant in guiding stroke rehabilitation interventions; however, understanding of specific imaging profiles that are predictive of longer-term stroke recovery is limited. Perfusion imaging is a technique now widely used in stroke research and clinical practice. Arterial spin labelling (ASL), a quantitative magnetic resonance imaging (MRI) perfusion measurement technique that harnesses blood water as an endogenous contrast agent, is able to demonstrate hypoperfusion and hyperperfusion patterns in stroke patients [1141]. Unlike contrast based perfusion MRI, ASL doesn’t require injectable gadolinium-based contrast agents and shows strong agreement with MRI perfusion measurements [1142].

Focal hyperperfusion is caused by local or regional increases in cerebral blood flow. Hyperperfusion on ASL perfusion MRI has drawn growing interest lately, a few groups including the authors' have shown that ASL hyperperfusion is associated with haemorrhagic transformation (HT) as well as good or poor clinical outcome depending on the spatial localization and temporal dynamics. While there is a general consensus that post-operative hyperperfusion following carotid endarterectomy and carotid artery stenting is detrimental and is associated with poor clinical outcomes [1143, 1144]; post-ischemic hyperperfusion may have positive or negative prognostic impact [8, 1145, 1146]. In 1996, Marchal & colleagues noted the phenomenon of hyperperfusion on acute stage perfusion positron emission tomography images (PET) and co-registered with chronic-stage computed tomography (CT) scans in patients with acute middle cerebral artery (MCA) stroke [9]. The authors noted that the areas with hyperperfusion were both significantly larger than the final infarct, and topographically distinct from the site of final infarction. The hyperperfusion patterns were more commonly found in infarct topographies covering deep MCA regions including striatocapsular territory consistent with lenticulostriate artery mouth occlusion during transient MCA embolism. The authors postulated that spontaneous non-haemorrhagic hyperperfusion may be a marker for favourable tissue outcome [9, 363]. A recent study
using ASL MRI has shown that a select group of patients with ischemic stroke show focal regions of perilesional hyperperfusion (PLH) on ASL at 24 hours and that these patients have better clinical recovery from their initial stroke [8]. It is also recognised that focal hyperperfusion early after brain ischemia can be identified following transient ischemic attacks, and is associated with reversible deficits [1147]. Extensive studies on animal models have also shown that hyperperfusion is the hallmark of early and efficient recanalization [1148-1150]. However, there are limited data on the association of PLH with clinical outcomes and more human studies are needed. Moreover, factors associated with late-stage (12-24 hours) PLH are poorly defined.

Leptomeningeal collaterals are secondary collateral pathways that act as anastomotic channels, towards maintaining blood flow to brain regions distal to an arterial occlusion, in conditions such as acute ischemic stroke where cerebral blood flow is pathologically altered [1151, 1152]. They play an important role in sustaining brain viability by facilitating ‘penumbral survival’ until reperfusion. Good baseline collaterals are strongly associated with better clinical outcomes in acute ischemic stroke patients. Leptomeningeal collaterals are relatively dense in and around the cortical superior middle cerebral vascular territory. Therefore, arterial collaterals supplying the perilesional areas around the infarct topographies involving cortical superior MCA, and lenticulostriate (feeding the striatocapsular region) arterial territories may have a role in focal PLH patterns observed on ASL. To this end, a study on the association of baseline collateral status and the topography of infarcts with late-stage (12-24 hours) PLH may be useful in understanding the underlying pathophysiological mechanism. In this prospective study, we sought to examine clinical and neuroradiological correlates of late-stage (12-24 hours) post-ischaemic focal PLH in a group of AIS patients using ASL mapping of brain perfusion.

The specific aims of the project were:

(4) To study the association of PLH at 24 hours with baseline collateral status.
(5) To identify infarct topographies that associate with PLH at 24 hours (identified using MRI ASL blood flow measurement).
(6) To investigate the factors associated with early PLH in these infarct topographies.
Our underlying hypotheses were: (a) that better collateral flow grades will be associated with PLH, (b) that there will be variation in the degree of PLH seen depending on the topography of the ischaemic lesion, and (c) that the infarction in the cortical superior MCA and deep MCA territories such as in striatocapsular region (as per Marchal et al [9]) would more commonly show evidence of PLH than other infarct topographies.
4.2. Contributions


75% conception and design of research

75% experimental procedures

75% analyses and interpretation of the findings

75% writing of the paper and critical appraisal of the content

Prof Christopher Levi

Signed: Date: 13/02/2017

A/Prof Peter Stanwell

Signed: Date: 17/02/2017

Prof John Attia

Signed: Date: 15/02/2017
4.3. PUBLICATION
Baseline collateral status and infarct topography in post-ischaemic perilesional hyperperfusion: An arterial spin labelling study

Sonu Bhaskar¹,², Andrew Bivard¹, Peter Stanwell², Mark Parsons¹,², John R Attia³, Michael Nilsson²,⁴ and Christopher Levi¹,²

Abstract
Focal hyperperfusion after acute ischaemic stroke could be of prognostic value depending upon its spatial localisation and temporal dynamics. Factors associated with late stage (12–24 h) perilesional hyperperfusion, identified using arterial spin labelling, are poorly defined. A prospective cohort of acute ischaemic stroke patients presenting within 4.5 h of symptom onset were assessed with multi-modal computed tomography acutely and magnetic resonance imaging at 24±8 h. Multivariate logistic regression analysis and receiver operating characteristics curves were used. One hundred and nineteen hemispheric acute ischaemic stroke patients (mean age = 71 ± 12 years) with 24 h arterial spin labelling imaging were included. Forty-two (35.3%) patients showed perilesional hyperperfusion on arterial spin labelling at 24 h. Several factors were independently associated with perilesional hyperperfusion: good collaterals (71% versus 29%, P < 0.0001; OR = 5, 95% CI = [1.6, 15.7], P = 0.005), major reperfusion (81% versus 48%, P < < 0.0001; OR = 7.5, 95% CI = [1.6, 35.1], P = 0.01), penumbral salvage (76.2% versus 47%, P = 0.002; OR = 6.6, 95% CI = [1.8, 24.5], P = 0.004), infarction in striatocapsular (OR = 9.5, 95% CI = [2.6, 34], P = 0.001) and in cortical superior division middle cerebral artery (OR = 4.7, 95% CI = [1.4, 15.7], P = 0.012) territory. The area under the receiver operating characteristic curve was 0.91. Our results demonstrate good arterial collaterals, major reperfusion, penumbral salvage, and infarct topographies involving cortical superior middle cerebral artery and striatocapsular are associated with perilesional hyperperfusion.

Keywords
Stroke, perfusion imaging, arterial spin labelling, hyperperfusion, collaterals, topography

Received 11 February 2016; Revised 4 May 2016; Accepted 9 May 2016

Introduction
Grouping of acute stroke patients into specific imaging profiles can allow greater understanding of prognosis and can assist in the selection of patients for specific acute therapies or interventions.¹,² A similar strategy may also be clinically relevant in guiding stroke rehabilitation interventions; however, understanding of specific imaging profiles that are predictive of longer term stroke recovery is limited. Perfusion imaging is a technique now widely used in stroke research and clinical practice. Arterial spin labelling (ASL), a quantitative magnetic resonance imaging (MRI) perfusion measurement technique that harnesses blood water as an endogenous contrast agent, is able to demonstrate
hypoperfusion and hyperperfusion patterns in stroke patients. Unlike contrast-based perfusion MRI, ASL does not require injectable gadolinium-based contrast agents and shows strong agreement with MRI perfusion measurements.4

Focal hyperperfusion is caused by local or regional increases in cerebral blood flow. Hyperperfusion on ASL perfusion MRI has drawn growing interest lately, a few groups including the authors' have shown that ASL hyperperfusion is associated with haemorrhagic transformation (HT) as well as good or poor clinical outcome depending on the spatial localisation and temporal dynamics. While there is a general consensus that post-operative hyperperfusion following carotid endarterectomy and carotid artery stenting is detrimental and is associated with poor clinical outcomes,5,6 post-ischaemic hyperperfusion may have positive or negative prognostic impact.7–9 In 1996, Marchal et al. noted the phenomenon of hyperperfusion on acute stage perfusion positron emission tomography (PET) images and co-registered with chronic-stage computed tomography (CT) scans in patients with acute middle cerebral artery (MCA) stroke.10 The authors noted that the areas with hyperperfusion were both significantly larger than the final infarct and topographically distinct from the site of final infarction. The hyperperfusion patterns were more commonly found in infarct topographies covering deep MCA regions including striatocapsular territory consistent with lenticulostriate artery mouth occlusion during transient MCA embolism. The authors postulated that spontaneous non-haemorrhagic hyperperfusion may be a marker for favourable tissue outcome.10,11 A recent study using ASL MRI has shown that a select group of patients with ischaemic stroke show focal regions of perilesional hyperperfusion (PLH) on ASL at 24 h and that these patients have better clinical recovery from their initial stroke.8 It is also recognised that focal hyperperfusion early after brain ischaemia can be identified following transient ischaemic attacks and is associated with reversible deficits.12 Extensive studies on animal models have also shown that hyperperfusion is the hallmark of early and efficient recanalisation.13–15 However, there are limited data on the association of PLH with clinical outcomes and more human studies are needed. Moreover, factors associated with late stage (12–24 h) PLH are poorly defined.

Leptomeningeal collaterals are secondary collateral pathways that act as anastomotic channels, towards maintaining blood flow to brain regions distal to an arterial occlusion, in conditions such as acute ischaemic stroke (AIS) where cerebral blood flow is pathologically altered.16,17 They play an important role in sustaining brain viability until reperfusion. Good baseline collaterals are strongly associated with better clinical outcomes in AIS patients. Leptomeningeal collaterals are relatively dense in and around the cortical superior middle cerebral vascular territory. Therefore, arterial collaterals supplying the perilesional areas around the infarct topographies involving cortical superior MCA and lenticulostriate (feeding the striatocapsular region) arterial territories may have a role in focal PLH patterns observed on ASL. To this end, a study on the association of baseline collateral status and the topography of infarcts with late stage (12–24 h) PLH may be useful in understanding the underlying pathophysiological mechanism. In this prospective study, we sought to examine clinical and neuroradiological correlates of late stage (12–24 h) post-ischaemic focal PLH in a group of AIS patients using ASL mapping of brain perfusion. The specific aims of the project were as follows:

1. To study the association of PLH at 24 h with baseline collateral status.
2. To identify infarct topographies that associate with PLH at 24 h (identified using MRI ASL blood flow measurement).
3. To investigate the factors associated with early PLH in these infarct topographies.

Our underlying hypotheses were: (a) that better collateral flow grades will be associated with PLH, (b) that there will be variation in the degree of PLH seen depending on the topography of the ischaemic lesion, and (c) that the infarction in the cortical superior MCA and deep MCA territories such as in striatocapsular region (as per Marchal et al.10) would more commonly show evidence of PLH than other infarct topographies.

Materials and methods

Study design and patient selection

Consecutive acute ischaemic stroke patients admitted to the acute stroke unit at an academic medical centre were prospectively studied applying the following inclusion criteria: (a) presented within 4.5 h of stroke symptom onset, (b) evaluated for the eligibility to receive thrombolysis, (c) age >18 years, (d) hemispheric stroke, and (d) ASL MRI acquired within 24 h of symptom onset. Patients with lacunar infarctions (small artery occlusion) were excluded. Patients with motion artefacts on ASL source images were also excluded. All patients were managed according to the discretion of the treating stroke physician and based on local guidelines. This study was approved by the Hunter New England Human Research Ethics Committee (Newcastle, NSW) in accordance with the National
MRI was also obtained at 24 h diffusion-weighted imaging (DWI) and ASL perfusion (dCTA) at baseline. Follow-up MRI including non-contrast CT (NCCT), CT perfusion (CTP) with dynamic CT angiography (dCTA) at baseline. Stroke severity was assessed using the National Institutes of Health Stroke Scale (NIHSS) at baseline (immediately before acute CT) and at 24 h. Stroke severity at admission was categorised into mild (for NIHSS = 0–7), moderate (NIHSS = 8–16), and severe (NIHSS = 17 and above).19 Onset to treatment time, the time delay between stroke onset and administration of tPA, was also recorded.

Imaging acquisition

In accordance with our routine stroke imaging protocol, all the patients underwent non-contrast CT (NCCT), CT perfusion (CTP) with dynamic CT angiography (dCTA) at baseline. Follow-up MRI including diffusion-weighted imaging (DWI) and ASL perfusion MRI was also obtained at 24 ± 8 h. CTA was also obtained at 24 h for measuring the adequacy of reperfusion. All the CT scans were obtained using a 320-detector row 640-slice cone beam multi-detector CT (Aquilion One, Toshiba Medical Systems). NCCT was followed by dCTA and CTP which were acquired simultaneously in two 60 s series. CTP/CTA was obtained in the axial plane before and after the intravenous bolus injection of contrast agent (50 ml of Ultravist 370; Bayer HealthCare, Berlin, Germany) injected at a rate of 6 ml/s chased by 50 ml of saline (acquisition parameters: 120 kV, 128 mA s, scanning coverage (SC) = 240 mm) and scanning width = 5 mm). After a delay of 7 s post-contrast administration pulsed full rotation scan with 19 time points was acquired over 60 s with a total pulse image acquisition time of 9.5 s. In order to examine the extracranial vessels, CTA of the extracranial segment was also acquired using bolus tracking with 50 ml of contrast injected at 6 ml/s chased by 50 ml of saline. The total radiation exposure was 5.5–6.0 mSv.

Following baseline CT examination, follow-up MR imaging was performed at 24 ± 8 h based on routine stroke imaging protocol, that included an axial isotropic DWI spin-echo echo-planar imaging sequence, time-of-flight MR angiography, and whole brain perfusion-weighted imaging (PWI) with bolus-tracking dynamic susceptibility contrast-PWI and axial T2-weighted echo planar sequence, on a 3T MRI (Magnetom Verio; Siemens Verio, Erlangen, Germany) with a 32-channel receive-only head coil.20 ASL was performed using a pulsed sequence with a 32 channel receive-only head coil, using quantitative perfusion imaging with a single subtraction, with thin-slice TI1 periodic saturation (Q2TIPS) technique,8,21 having following image parameters: TR 2500 ms, TI1 500 ms, TI2 1500 ms, inversion time (TI2) 1700 ms, FOV 240 × 240 mm, matrix 64 × 64. This acquired nine slices at 8 mm thickness with 28 repetitions for a scan time of 4:02 min. Images were inspected for the presence of artefacts.

Imaging analysis

All CTP/CTA and perfusion MR data were de-identified prior to analysis, following which both CTP and MR perfusion maps, including mean transit time and cerebral blood volume were generated with MIStar software (Apollo Medical Imaging Technology, Melbourne, Australia), which uses a deconvolution algorithm (using single value deconvolution with delay and dispersion correction) to process the data.22 An arterial input function and venous outflow function was semi-automatically selected from the non-stroke (contralateral) hemisphere MCA/ACA and sagittal sinus, respectively. Previously validated thresholds were applied in order to measure the volume of the acute perfusion lesion (relative delay time (DT) ≥ 3 s) and acute infarct core (relative CBF ≤ 30%).23 Penumbral volume was calculated from the volume of the perfusion lesion (DT threshold ≥ 3 s) minus the volume of the infarct core (relative CBF threshold < 30% within the DT ≥ 3 s lesion). The choice of DT ≥ 3 s as the threshold was based on recent studies.23,24 In addition to the extent of the penumbra in terms of acute penumbral volume, we also recorded presence/absence of acute penumbra for statistical analyses.

ASL imaging data were processed using MIStar as per the protocol described in our previous work.9 PLH was defined as hyperperfusion pattern observed on ASL-MRI as a local CBF of > 130%, surrounding the lesion compared to contralateral healthy tissue (in areas of penumbral salvage), within the acutely hypoperfused area but topographically separate from the 24 h DWI lesion.7,8

Neuroradiological evaluation

Any infarction on the DWI was assigned to either one or multiple arterial territories depending upon its topographical location, i.e. anterior (ACA), middle (cortical superior and inferior divisions) (MCA), posterior cerebral (PCA), lenticulostrate (covering the
striatocapsular region) (SCI), anterior choroid, and thalamic artery (see Supplementary Information (SI), Figure 1). Presence/absence of any infarct in a given arterial territory was assigned as 1/0. For example, for a large cortical MCA infarct involving multiple arterial territories including cortical superior MCA and striatocapsular areas, both cortical superior MCA and striatocapsular were assigned 1. Infarcts were also classified into distinct (involving one territory) and multi-territorial (involving multiple territories) topographies. The extent of infarction in a given arterial territory (see Supplementary Figure 1) on DWI image was determined based on whether the infarct covers >80% of arterial territory (‘complete’) or it covers <80% of arterial territory (‘partial’). Watershed infarcts occur in the border zones between major cerebral arterial territories. Watershed territory infarcts were classified into: (a) external watershed infarction (EWI): border zone of ACA/MCA and MCA/PCA, and (b) internal watershed infarction: border zone between lenticulostriate perforators and the deep penetrating cortical branches of the MCA or at the border zone of deep white matter branches of the MCA and the ACA.

For static collateral grading, we reviewed CTA axial source images and the multiplanar reformats in the sagittal and coronal planes. Collateral circulation status was assessed from the CTA data based on the degree of reconstitution of the MCA up to the distal end of its occlusion and was divided into ‘good’ or ‘poor/reduced’ as described in the protocol used by Miteff et al. The exact location of the thrombus or clot (M1 proximal (M1P), M1 distal (M1D), M2, M3, or internal carotid artery (ICA)) was determined. Furthermore, the precise location of the thrombus was also recorded using three-dimensional volume rendering of dCTA. We defined proximal clot as any thrombus/occlusion in M1P or ICA. Any thrombus/occlusion in M1D, M2, or M3 was classified as a distal clot. Clinical, CT, and MRI imaging data were

Figure 1. Multimodal imaging (acute CTP and follow-up 24 h MRI) showing perilesional hyperperfusion (PLH) on ASL at 24 h. Patient I (upper row): CTP demonstrated acute left MCA territory infarct with a small core and large penumbra (a), and decreased rCBF (b). Post-thrombolysis, patient showed excellent recanalisation. PLH patterns were observed on ASL-MRI at 24 h (c). Area of restricted diffusion was observed on follow-up DWI-MRI (d). PWI-CBF at 24 h is also depicted (e). Patient II (bottom row): Acute CTP shows large penumbra (f) and decreased rCBF (g) over a large part of the right MCA territory indicating a large area of hypoperfused but likely viable tissue (penumbra). Post-thrombolysis, the patient showed excellent response to therapy. The regions that were previously hypoperfused showed PLH (white arrow) at 24 h on ASL (h). DWI-MRI (i) demonstrated an area of restricted diffusion. Hyperperfusion patterns are not clearly evident on PWI-CBF (j). Both patients showed good collaterals on baseline CTA. ASL: arterial spin labelling; CBF: cerebral blood flow; CT: computed tomography; CTA: computed tomographic angiography; CTP: computed tomographic perfusion imaging; DWI: diffusion-weighted imaging; MCA: middle cerebral artery; MIP: maximum intensity projection; PWI: perfusion-weighted imaging.
assessed for the following metrics: (a) aetiological classification based on CCS, (b) extent of infarction in the territory of cerebral artery (classified into complete (>80% territory) and partial (<80% territory), (c) presence of minor and major petechial infarctions (defined by minor: < 5 mm diameter, and major > 5 mm diameter), (d) watershed territory infarction, (e) presentation of HT, (f) reperfusion status, and (g) collateral grading. These images were read by consensus by two experienced readers (SB & CL). All neuroradiological assessment was done blind to the ASL findings.

Outcome measures

Acute HT was defined by hypointensity on T$_2$-MRI located within the infarction zone as described elsewhere. HT was further classified based on extent and severity as per the protocol used in the European-Australasian Acute Stroke Study (ECASS II) trial in the following categories: diffuse haemorrhagic (petechial (HI1) or confluent (HI2)) infarction and parenchymal haemorrhage (PH1 or PH2, defined as space occupying effect). At 24 h a repeat CTA was performed and assessed by an independent blinded reviewer for the adequacy of angiographic reperfusion using thrombolysis in the cerebral infarction (TICI) score. TICI is a widely used angiographic score in endovascular stroke studies. Major reperfusion was assessed as modified TICI (mTICI) grade 2b or 3 (defined as tissue reperfusion in ≥50% of the occluded artery territory). Partial reperfusion was defined as the mTICI grade of 2a (defined as tissue reperfusion in <50% of the occluded artery territory). The mTICI grade of 0–1 was identified as no reperfusion defined either by the presence of no anterograde flow or the flow past the initial occlusion but with no tissue perfusion.

The modified Rankin Scale (mRS) was used to assess clinical outcome in terms of functional status at three months; a score of 0–2 represented a good outcome and a score of 3 and above indicated poor outcome. The early neurological change was categorised as early neurological improvement (ENI) or early neurological deterioration (END). ENI was defined as a gain of NIHSS ≥8 within the first 24 h or NIHSS < 2 at 24 h. END was defined as ≥4-point deterioration on 24 h NIHSS.

Final infarct volume (DWI lesion) at 24 h was calculated. The difference between the acute CTP (PWI lesion) volume and the 24 h DWI lesion volume was used to assess penumbral salvage. Patients with any degree of penumbral salvage were identified as ones with smaller 24 h DWI lesion volume than the acute perfusion lesion volume.

Case presentation

Two cases demonstrating the use of multi-modal (CT & MRI) imaging in patients with right and left MCA territory infarct, respectively, showing PLH on ASL-MRI at 24 h, are shown in Figure 1.

Statistical analysis

All the statistical analyses were performed using STATA (Version 10, 2001; College Station, TX, USA). Numerical values given are the means (±standard deviation) or medians (interquartile range) for age, mRS scores, NIHSS at admission, and NIHSS at 24 h scores as appropriate. For ordinal or continuous data, Mann–Whitney (Wilcoxon rank-sum) test was used. Nominal data were analysed with the Pearson’s chi-squared ($\chi^2$) and the two-tailed Fisher’s exact test. We compared two groups of patients stratified by hyperperfusion status (hyperperfusion versus no hyperperfusion). Group differences were considered significant at values of P < 0.05. Univariate logistic regression analysis was used to test associations between covariates and 24 h PLH. Multivariate analysis was also performed to study the association with the long-term clinical outcome (at 90 days). Results of logistic regression are reported as odds ratios (ORs). Only those variables with P < 0.1 were tested in the subsequent multivariate regression analysis. Before fitting the multivariate model, we also tested the multicollinearity using the variance inflation factor (VIF): a common rule of thumb is that VIFs > 5 identify strongly collinear pairs. If two or more covariates were found to be collinear, we retained the variable that was more strongly associated with the PLH to ensure a stable model. A multivariate regression model, based on a backwards, the stepwise approach was used; only the most important ‘explanatory’ variables were retained to arrive at the most parsimonious model. Comparison between the various multivariate regression models was made using model selection statistics (including the Akaike information criterion (AIC) and the Bayesian information criterion (BIC)). Multivariate normality (of the regression model) was also checked using the Doornik–Hansen test. The sensitivity, specificity, and overall rate of correct classification for each model were estimated using classification statistics using a cut-off (positive outcome threshold) of 0.5. The goodness of fit using Pearson $\chi^2$ test and a number of covariate patterns were calculated for each multivariate model. Finally, the receiver operating characteristic (ROC) curve for the regression model was plotted, and the area under the curve (AUC) was computed to evaluate the predictive ability.
Results

Baseline characteristics

The demographics, risk factors, and other clinical characteristics of the overall study population, stratified by hyperperfusion status, are detailed in Table 1. In total, 119 patients (mean age = 71 ± 12.5 years; the number of males/females = 54 (45.38%) / 65 (54.6%)) with hemispheric ischaemic stroke and 24 h ASL imaging data were included in the study, out of which 42 (35.3%) patients showed PLH within 24 h. Thirty-five patients with small artery occlusion/lacunar infarcts were excluded from the study. One hundred patients (84%) received intravenous recombinant tissue plasminogen activator (rtPA) as early thrombolytic therapy. The median NIHSS score on admission was 14 points (interquartile range (IQR) = 8). The results of the topographic classification of infarcts are also given in Table 1. Distinct watershed infarcts were observed in 26 (21.8%) patients, with the majority of them having EWIs (n = 24). Thirty-seven (31%) patients had infarcts in border zone areas as an extension of territorial infarcts.

PLH versus non-PLH

Good collateral grades at baseline were more common in PLH patients (71% versus 30%; P < 0.0001). Analysis of infarct topography and PLH status demonstrated an association with cortical superior division MCA (76.2% versus 44.2%; P = 0.001) and/or SCI territories (71.4% versus 32.5%; P < 0.0001). Chronic petechial infarction patterns did not differ in proportion between PLH and non-PLH patients. There was a tendency towards higher rates of PLH in patients with large artery atherosclerosis aetiology (64.3% versus 48%, P = 0.124).

In terms of clinical outcome (see Table 2), PLH patients demonstrated significantly higher rates of major reperfusion (81% versus 48%, P < 0.0001), ENI (58.5% versus 37.7%, P = 0.034), and HT (16.7% versus 1.35%, P = 0.003) at 24 h. PLH patients also showed the significantly higher proportion of occurrence of penumbral salvage (76.2% (32/42) versus 46.8% (36/77), P = 0.002). Moreover, mean penumbral salvage volume was significantly greater in PLH patients (22.2 ml versus 3.8 ml, P = 0.028). PLH group showed a tendency towards lower 24 h median NIHSS scores (6 versus 7.5, P = 0.085) and a higher proportion of good clinical outcome at 90 days (61.9% versus 52%, P = 0.338). Non-PLH patients showed significantly higher rates of nil reperfusion at 24 h (39% versus 12%, P = 0.002).

Association with PLH

The results of univariate logistic regression analysis examining the association with the PLH (OR and levels of significance) are shown in Table 3. Neither demographic factors (age, sex), stroke severity at admission, thrombolytic treatment, or other clinical risk factors differed significantly between patients with and without PLH. Good collateral status strongly associated with 24 h PLH. Patients with good baseline collateral grades constituted 71% of PLH patients. Patients who developed PLH had a higher prevalence of infarctions in cortical superior MCA and striatocapsular territories. In logistic regression analysis, the OR for the occurrence of PLH was 5.5 in infarcts with partial lesions in cortical superior MCA territory and 24.6 in infarcts with partial lesions in the striatocapsular territory. Patients who received thrombolytic treatment were 1.6 times more likely to show PLH at 24 h. Large artery atherosclerotic patients were two times more likely to show PLH.

Logistic regression analysis also showed significant association of HT with PLH. In particular, the presence of any developing haemorrhagic infarctions (HI1 or HI2) predicted PLH perfectly. In terms of reperfusion status at 24 h, PLH was significantly associated with major reperfusion. PLH patients were two times more likely to demonstrate ENI within 24 h with 58.5% of PLH patients showing ENI. Moreover, PLH patients were significantly associated with lower 24 h NIHSS scores per unit increase. The presence of any penumbral salvage was associated with PLH. However, a unit increase in penumbral salvage volume did not show significant association with PLH. PLH patients were 1.5 times more likely to have good clinical outcomes at 90 days. However, the association failed to reach statistical significance.

Multivariate regression analysis

Covariates or baseline characteristics with p ≤ 0.1 (any infarction in cortical superior division MCA or SCI territory, collateral status, HT, NIHSS at 24 h, ENI, reperfusion status, penumbral salvage) and other clinically important (OR > 1.5) variables (thrombolytic treatment and large artery atherosclerosis) were tested for collinearity (Supplementary Information, Table 1). No strong multicollinearity was detected. Among the baseline characteristics, good collaterals, infarction in cortical superior MCA territory, and infarction in SCI territory were strongly associated with PLH (see Model 1, Table 4). In reduced model, large artery atherosclerosis and thrombolysis were eliminated because of them not being significantly
**Table 1.** List of baseline characteristics stratified by perilesional hyperperfusion (PLH).

<table>
<thead>
<tr>
<th></th>
<th>All patients n = 119</th>
<th>PLH n = 42</th>
<th>No PLH n = 77</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>119</td>
<td>42 (35.29)</td>
<td>77 (64.71)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>54 (45.38)</td>
<td>16 (38.1)</td>
<td>38 (49.35)</td>
<td>0.255</td>
</tr>
<tr>
<td>Female</td>
<td>65 (54.62)</td>
<td>26 (61.9)</td>
<td>39 (50.65)</td>
<td>0.255</td>
</tr>
<tr>
<td>Age, in years (mean ± SD)</td>
<td>70.9 ± 12.45</td>
<td>69.7 ± 13.9</td>
<td>71.5 ± 11.6</td>
<td>0.4377</td>
</tr>
<tr>
<td>Time to tPA (in mins); mean ± SD</td>
<td>158.7 ± 81</td>
<td>161.9 ± 74.8</td>
<td>156.9 ± 85</td>
<td>0.5593</td>
</tr>
<tr>
<td>OTT (in min); mean ± SD</td>
<td>163.5 ± 84.29</td>
<td>163.6 ± 80</td>
<td>163.4 ± 86.8</td>
<td>0.8694</td>
</tr>
<tr>
<td>Acute penumbra, ml; median (IQR)</td>
<td>47.9 [63.5]</td>
<td>46.35 [84.7]</td>
<td>53.1 [62]</td>
<td>0.2613</td>
</tr>
<tr>
<td>Presence of acute penumbra</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>96 (80.67)</td>
<td>36 (85.71)</td>
<td>60 (77.92)</td>
<td>0.342</td>
</tr>
<tr>
<td>No</td>
<td>23 (19.33)</td>
<td>6 (14.29)</td>
<td>17 (22.08)</td>
<td>0.342</td>
</tr>
<tr>
<td>Occlusion on admission CTA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>73 (61.34)</td>
<td>28 (66.67)</td>
<td>45 (58.44)</td>
<td>0.434</td>
</tr>
<tr>
<td>No</td>
<td>46 (38.66)</td>
<td>14 (33.33)</td>
<td>32 (41.56)</td>
<td>0.434</td>
</tr>
<tr>
<td>Clot location</td>
<td></td>
<td></td>
<td></td>
<td>0.517</td>
</tr>
<tr>
<td>Proximal thrombus (ICA + M1P)</td>
<td>37 (31.09)</td>
<td>13 (30.95)</td>
<td>24 (31.17)</td>
<td>1.000</td>
</tr>
<tr>
<td>Distal thrombus (M1D + M2 + M3)</td>
<td>40 (33.61)</td>
<td>16 (38.10)</td>
<td>24 (31.17)</td>
<td>0.543</td>
</tr>
<tr>
<td>ICA</td>
<td>22 (18.49)</td>
<td>6 (14.29)</td>
<td>16 (20.78)</td>
<td>0.464</td>
</tr>
<tr>
<td>M1 proximal (M1P)</td>
<td>20 (16.8)</td>
<td>9 (21.43)</td>
<td>11 (14.29)</td>
<td>0.32</td>
</tr>
<tr>
<td>M1 distal (M1D)</td>
<td>16 (13.45)</td>
<td>5 (11.9)</td>
<td>11 (14.29)</td>
<td>0.786</td>
</tr>
<tr>
<td>M2 and M3</td>
<td>24 (20.17)</td>
<td>11 (26.19)</td>
<td>13 (16.88)</td>
<td>0.241</td>
</tr>
<tr>
<td>Treatment factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombolysed</td>
<td>100 (84.03)</td>
<td>37 (88.10)</td>
<td>63 (81.82)</td>
<td>0.441</td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>106 (89.08)</td>
<td>38 (90.48)</td>
<td>68 (88.31)</td>
<td>1.000</td>
</tr>
<tr>
<td>Diabetes</td>
<td>37 (31.09)</td>
<td>13 (30.95)</td>
<td>24 (31.17)</td>
<td>1.000</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
<td>54 (45.38)</td>
<td>16 (38.1)</td>
<td>38 (49.35)</td>
<td>0.2555</td>
</tr>
<tr>
<td>Present smoker (n = 119)</td>
<td>25 (21.01)</td>
<td>10 (23.81)</td>
<td>15 (19.48)</td>
<td>0.640</td>
</tr>
<tr>
<td>Past smoker (n = 119)</td>
<td>50 (42.02)</td>
<td>20 (47.61)</td>
<td>30 (38.96)</td>
<td>0.438</td>
</tr>
<tr>
<td>AF</td>
<td>69 (57.98)</td>
<td>26 (61.9)</td>
<td>43 (55.84)</td>
<td>0.564</td>
</tr>
<tr>
<td>Depression</td>
<td>12 (10.08)</td>
<td>4 (9.52)</td>
<td>8 (10.39)</td>
<td>1.000</td>
</tr>
<tr>
<td>History of stroke/TIA (n = 153)</td>
<td>20 (16.81)</td>
<td>6 (14.29)</td>
<td>14 (18.18)</td>
<td>0.798</td>
</tr>
<tr>
<td>Aetiology (CCS classification)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large artery atherosclerosis</td>
<td>64 (53.78)</td>
<td>27 (64.29)</td>
<td>37 (48.05)</td>
<td>0.124</td>
</tr>
<tr>
<td>Cardioembolic</td>
<td>54 (45.38)</td>
<td>15 (35.71)</td>
<td>39 (50.65)</td>
<td>0.128</td>
</tr>
<tr>
<td>Collateral grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>53 (44.54)</td>
<td>30 (71.43)</td>
<td>23 (29.87)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Poor/reduced</td>
<td>66 (55.46)</td>
<td>12 (28.57)</td>
<td>54 (70.13)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Stroke topography (any infarction in given territory)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACA</td>
<td>9 (7.56)</td>
<td>3 (7.14)</td>
<td>6 (7.79)</td>
<td>1.000</td>
</tr>
<tr>
<td>Complete</td>
<td>2 (1.68)</td>
<td>1 (2.38)</td>
<td>1 (1.30)</td>
<td>1.000</td>
</tr>
<tr>
<td>Partial</td>
<td>7 (5.88)</td>
<td>2 (4.76)</td>
<td>5 (6.49)</td>
<td>1.000</td>
</tr>
<tr>
<td>Absent</td>
<td>110 (92.44)</td>
<td>39 (92.86)</td>
<td>71 (92.21)</td>
<td>1.000</td>
</tr>
</tbody>
</table>

(continued)
Multivariate modelling was done by fitting a model with all potential (non-collinear) covariates (Model I). Among the covariates, independent predictors of 24 h PLH selected by stepwise logistic regression were collateral grade, infarction in cortical superior MCA territory, infarction in the striatocapsular territory, reperfusion status, HT, and penumbral salvage. Results of the stepwise backwards multivariate logistic regression are shown in Table 4, Model II. Multivariate regression analysis revealed that stroke severity at admission, thrombolysis, large artery atherosclerosis, NIHSS at 24 h, and ENI did not appear to influence the PLH.

In the reduced model, several factors were positively associated with PLH (Model II): good collaterals, infarction in SCI territory, infarction in cortical superior MCA territory, major reperfusion, and penumbral salvage. HT showed a trend towards association with increasing PLH rates; however, this did not reach statistical significance. The lower AIC and BIC values indicated that the reduced model fitted the data better than the Model I (AIC = 102 versus 120; BIC = 125 versus 131). The overall rate of correct classification of the reduced model was estimated to be 84%, with 92% specificity and 69.1% sensitivity. The ROC area (AUC) of approximately 0.91 indicated excellent discrimination/accuracy for the model. Finally, the independent predictors of PLH were good collaterals, major reperfusion at 24 h, penumbral salvage, infarction in cortical superior MCA territory, and infarction in the striatocapsular territory.

### Table 1. Continued

<table>
<thead>
<tr>
<th></th>
<th>All patients n = 119</th>
<th>PLH n = 42</th>
<th>No PLH n = 77</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cortical superior MCA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete</td>
<td>66 (55.46)</td>
<td>32 (76.19)</td>
<td>34 (44.16)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Partial</td>
<td>41 (34.54)</td>
<td>18 (42.86)</td>
<td>23 (29.87)</td>
<td>0.164</td>
</tr>
<tr>
<td>Absent</td>
<td>25 (21.01)</td>
<td>14 (33.33)</td>
<td>11 (14.29)</td>
<td>0.019*</td>
</tr>
<tr>
<td><strong>Striatocapsular</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete</td>
<td>55 (46.22)</td>
<td>30 (71.43)</td>
<td>25 (32.47)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Partial</td>
<td>35 (29.41)</td>
<td>13 (30.95)</td>
<td>22 (28.57)</td>
<td>0.835</td>
</tr>
<tr>
<td>Absent</td>
<td>20 (16.81)</td>
<td>20 (48.20)</td>
<td>30 (39.69)</td>
<td>0.001*</td>
</tr>
<tr>
<td><strong>Inferior division MCA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete</td>
<td>92 (77.31)</td>
<td>29 (69.05)</td>
<td>63 (81.82)</td>
<td>0.168</td>
</tr>
<tr>
<td>Partial</td>
<td>30 (25.21)</td>
<td>8 (19.05)</td>
<td>22 (28.57)</td>
<td>0.279</td>
</tr>
<tr>
<td>Absent</td>
<td>62 (52.10)</td>
<td>21 (50)</td>
<td>41 (53.25)</td>
<td>0.848</td>
</tr>
<tr>
<td><strong>Anterior choroidal artery</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete</td>
<td>28 (23.53)</td>
<td>9 (21.43)</td>
<td>19 (24.68)</td>
<td>0.822</td>
</tr>
<tr>
<td>Partial</td>
<td>11 (9.24)</td>
<td>2 (4.76)</td>
<td>9 (11.69)</td>
<td>0.324</td>
</tr>
<tr>
<td>Absent</td>
<td>91 (76.47)</td>
<td>33 (79.57)</td>
<td>58 (75.32)</td>
<td>0.822</td>
</tr>
<tr>
<td><strong>PCA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete</td>
<td>17 (14.29)</td>
<td>4 (9.52)</td>
<td>13 (16.88)</td>
<td>0.412</td>
</tr>
<tr>
<td>Partial</td>
<td>4 (3.36)</td>
<td>0</td>
<td>4 (5.19)</td>
<td>0.296</td>
</tr>
<tr>
<td>Absent</td>
<td>13 (10.92)</td>
<td>4 (9.52)</td>
<td>9 (11.69)</td>
<td>1.000</td>
</tr>
<tr>
<td><strong>Watershed Infarcts (EWI or IWI)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>External watershed infarcts (EWI)</td>
<td>24 (20.17)</td>
<td>7 (16.67)</td>
<td>17 (22.08)</td>
<td>0.634</td>
</tr>
<tr>
<td>Internal watershed infarcts (IWI)</td>
<td>2 (1.68)</td>
<td>1 (2.38)</td>
<td>1 (1.30)</td>
<td>1.000</td>
</tr>
<tr>
<td>Both EWI and IWI</td>
<td>102 (85.71)</td>
<td>38 (90.48)</td>
<td>64 (83.12)</td>
<td>0.412</td>
</tr>
<tr>
<td><strong>Infarcts in border zone (extension of territorial infarcts)</strong></td>
<td>37 (31.09)</td>
<td>15 (35.71)</td>
<td>22 (28.57)</td>
<td>0.535</td>
</tr>
</tbody>
</table>

ACSA: anterior cerebral artery; AChA: anterior choroid artery; AF: atrial fibrillation; IQR: interquartile range; MCA: middle cerebral artery; NIHSS: National Institute of Health Stroke Scale Score; OTT: onset to treatment time; PCA: posterior cerebral artery; SCI: striatocapsular, thalamic; SD: standard deviation; TIA: transient ischaemic attack. Continuous or ordinal variables are shown as mean ± SD or median (IQR), as appropriate. Mann–Whitney test was used to compare continuous or ordinal variables. Nominal data were analysed with the Pearson's chi-squared ($\chi^2$) and the two-tailed Fisher's exact test. CCS classification method was used to assess the causative aetiological mechanism underlying the stroke event. Infarct topographies are defined as complete, partial, or no infarction (absent) in that territory. Collateral grading was classified as either good or reduced. Figures in parentheses are percentages or inter-quartile range (IQR). *P < 0.05 as the threshold for statistical significance. Uncorrected for multiple comparisons.
Association with 90 days clinical outcome

Among PLH patients, 62% showed good clinical outcome at 90 days. There was a tendency towards good clinical recovery in PLH patients. However, in this cohort, the PLH did not demonstrate a significant association with 90 days clinical outcome. In a multivariate analysis, following variables were found to be independently associated with clinical outcome at 90 days: NIHSS at admission, ENI, and core volume.

Discussion

In this study, we sought to examine the clinical and radiological correlates of post-acute (12–24 h) focal PLH in a large sample of AIS patients using non-invasive ASL mapping of brain perfusion. We found that PLH is independently associated with good collaterals, cortical superior division MCA stroke, striato-capsular infarction and occurrence of major reperfusion at 24 h. The previously recognised associations using PET and ASL between PLH and more favourable clinical outcomes may imply that there is longer lived penumbra in some patients, and its survival, presumably via later reperfusion, is supported by the presence of PLH. Alternatively, if PLH is occurring in tissue without residual ischaemia, the hyperperfusion may be resulting in other, yet to be defined, protective mechanisms. Whatever the underlying mechanism, the presence of PLH correlates with favourable clinical trajectory.

Table 2. List of clinical outcomes stratified by perilesional hyperperfusion (PLH).

<table>
<thead>
<tr>
<th></th>
<th>All patients (n = 119)</th>
<th>PLH (n = 42)</th>
<th>No PLH (n = 77)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h core volume (in ml); median (IQR)</td>
<td>22.4 [61.1]</td>
<td>16.2 [33.1]</td>
<td>35.3 [63.7]</td>
<td>0.1413</td>
</tr>
<tr>
<td>Median 24 h NIHSS (IQR) (n = 153)</td>
<td>7 (11)</td>
<td>6 (8)</td>
<td>7.5 (13)</td>
<td>0.0850</td>
</tr>
</tbody>
</table>

Any penumbral salvage

- Yes | 68 (57.14) | 32 (76.19) | 36 (46.75) | 0.002* |
- No  | 51 (42.86) | 10 (23.81) | 41 (53.25) | 0.002* |

Penumbral salvage volume (in ml)

- Median (IQR) | 9.9 [91.9] | 22.75 [68.9] | −6.8 [103.4] | 0.0277* |
- Mean ± SD | 10.33 ± 65.87 | 22.23 ± 71.65 | 3.83 ± 62.01 | 0.0277* |

Reperfusion status

- Major reperfusion | 71 (59.66) | 34 (80.95) | 37 (48.05) | <0.0001* |
- Incomplete reperfusion | 15 (12.61) | 3 (7.14) | 12 (15.58) | 0.252 |
- No reperfusion | 35 (29.41) | 5 (11.90) | 30 (38.46) | 0.002* |
- HT | 8 (6.9) | 7 (16.67) | 1 (1.35) | 0.001* |
- HI1 or HI2 | 7 (6.03) | 7 (16.67) | 0 (0) | 0.001* |
- PH1 or PH2 | 1 (0.86) | 0 | 1 (1.35) | 1.000 |

Major petechial (>5 mm) | 94 (81.03) | 33 (78.57) | 61 (82.43) | 0.629 |

Minor petechial (<5 mm) | 109 (93.99) | 40 (95.24) | 69 (93.24) | 1.000 |

ENI (ΔNIHSS ≥ 8 or ΔNIHSS < 2) | 53 (44.92) | 24 (58.54) | 29 (37.66) | 0.034* |

END (ΔNIHSS ≤ −4) | 5 (4.24) | 0 (0) | 5 (6.49) | 0.162 |

Median 90 days mRS (IQR) | 2 (3) | 2 (2) | 2 (4) | 0.1775 |

Good (mRS = 0–2) | 66 (55.46) | 26 (61.9) | 40 (51.95) | 0.338 |

- 0 | 23 (19.33) | 8 (19.05) | 15 (19.48) | 1.000 |

- 1 | 23 (19.33) | 11 (26.19) | 12 (15.58) | 0.224 |

- 2 | 20 (16.81) | 7 (16.67) | 13 (16.88) | 1.000 |

Bad (mRS = 3–6) | 53 (44.54) | 16 (38.1) | 37 (48.05) | 0.338 |

- 3 | 15 (12.61) | 6 (14.29) | 9 (11.69) | 0.775 |

- 4 | 13 (10.92) | 5 (11.90) | 8 (10.39) | 0.769 |

- 5 | 14 (11.76) | 4 (9.52) | 10 (12.99) | 0.768 |

- 6 | 11 (9.24) | 1 (2.38) | 10 (12.99) | 0.094 |

END: early neurological deterioration; ENI: early neurological improvement; HT: haemorrhagic transformation; mRS: modified Rankin score. The clinical outcome at 90 days was assessed using mRS (score range: 0–6), where an mRS score of 0–2 corresponds to a good outcome. Figures in parentheses are percentages or interquartile range (IQR). *P < 0.05 as the threshold for statistical significance. Uncorrected for multiple comparisons.

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### Table 3. Odds ratios (95% confidence intervals) and P-value (only with $P < 0.1$ shown) for the variables associated with perilesional hyperperfusion (PLH) at 24 h using bivariate logistic regression analysis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>% of patients with hyperperfusion within 24 h</th>
<th>OR (95% confidence interval)</th>
<th>$P &gt; \chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke severity at admission (unit increase)</td>
<td>88.1</td>
<td>0.97 (0.9, 1.03)</td>
<td>0.285</td>
</tr>
<tr>
<td>Thrombolytic treatment</td>
<td>88.1</td>
<td>1.6 (0.55, 4.93)</td>
<td>0.375</td>
</tr>
<tr>
<td>Collateral grade ($n = 151$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>71.4</td>
<td>5.87 (2.56, 13.44)</td>
<td>$&lt; 0.0001^*$</td>
</tr>
<tr>
<td>Poor/reduced</td>
<td>28.6</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Aetiology (CCS classification)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large artery atherosclerosis</td>
<td>64.3</td>
<td>1.94 (0.9, 4.2)</td>
<td>0.092</td>
</tr>
<tr>
<td>Cardioembolic</td>
<td>35.7</td>
<td>0.54 (0.25, 1.17)</td>
<td>0.120</td>
</tr>
<tr>
<td><strong>Stroke topography (any infarction in given territory)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infarction in cortical sup MCA territory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>76.2</td>
<td>4.05 (1.75, 9.38)</td>
<td>$0.001^*$</td>
</tr>
<tr>
<td>Absent</td>
<td>23.8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Extent of cortical sup MCA infarction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete</td>
<td>42.9</td>
<td>3.37 (1.34, 8.48)</td>
<td>$0.010^*$</td>
</tr>
<tr>
<td>Partial</td>
<td>33.3</td>
<td>5.47 (1.92, 15.6)</td>
<td>$0.001^*$</td>
</tr>
<tr>
<td>Absent</td>
<td>23.8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Infarction in striatocapsular territory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>71.4</td>
<td>5.2 (2.29, 11.83)</td>
<td>$&lt; 0.0001^*$</td>
</tr>
<tr>
<td>Absent</td>
<td>28.6</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Extent of striatocapsular infarction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete</td>
<td>31</td>
<td>2.56 (1.01, 6.49)</td>
<td>$0.047^*$</td>
</tr>
<tr>
<td>Partial</td>
<td>40.5</td>
<td>24.56 (6.19, 97.47)</td>
<td>$&lt; 0.0001^*$</td>
</tr>
<tr>
<td>Absent</td>
<td>28.6</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Clinical outcomes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIHSS at 24 h (unit increase)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major reperfusion</td>
<td>80.95</td>
<td>5.33 (1.85, 15.34)</td>
<td>$0.002^*$</td>
</tr>
<tr>
<td>Partial reperfusion</td>
<td>7.14</td>
<td>0.58 (0.32, 7.76)</td>
<td>0.572</td>
</tr>
<tr>
<td>No reperfusion</td>
<td>11.9</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Penumbral salvage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>76.19</td>
<td>3.64 (1.57, 8.44)</td>
<td>$0.003^*$</td>
</tr>
<tr>
<td>No</td>
<td>23.8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Penumbral salvage volume (in ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HT</td>
<td>16.7</td>
<td>14.6 (1.73, 123.3)</td>
<td>$0.014^*$</td>
</tr>
<tr>
<td><strong>HT classification</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HI1 or HI2</td>
<td>16.7</td>
<td>Predicts PLH perfectly.</td>
<td></td>
</tr>
<tr>
<td>PH1 or PH2</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>mRS at 90 days</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good (mRS = 0-2)</td>
<td>61.9</td>
<td>1.5 (0.69, 3.2)</td>
<td>0.297</td>
</tr>
<tr>
<td>Bad (mRS 3–6)</td>
<td>38.1</td>
<td>0.67 (0.31, 1.43)</td>
<td>0.297</td>
</tr>
<tr>
<td>Mortality (mRS = 6)</td>
<td>2.38</td>
<td>0.16 (0.02, 1.32)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

HT: haemorrhagic transformation; mRS: modified Rankin Score; SCI: striatocapsular; sup MCA: cortical superior division middle cerebral artery. Continuous or ordinal variables are shown as mean $\pm$ SD or median (IQR). Figures in parentheses are an interquartile range. *Used as reference category. $^*$P < 0.05 as the threshold for statistical significance.
To the best of our knowledge, there are none or limited prior studies that have investigated the link between infarct topography, collateral status, and PLH at 24 h. Moreover, clinical application of ASL in stroke population is still very limited.

The principal finding of this study was that PLH, defined on ASL-MRI within 12–24 h of the stroke onset, was significantly associated with good baseline collateral vessel status measured using CTA at the time of initial baseline acute stroke imaging. Baseline collateral status is now recognised to be important in the evaluation and treatment of cerebral ischaemia.32,33 The presence and a higher grade of collateral supply through leptomeningeal sources correlate with the presence of smaller final infarct volume,37 improved recanalisation rates, and favourable functional outcomes in patients undergoing endovascular revascularisation therapy.27,38–40 Therapeutically effective recanalization in a setting of poor baseline collaterals may result in increased rates of HT,38,41 thereby leading to worsening of neurological status by restricting effective reperfusion. Our results show that collateral flow accurately predicts 24 h PLH patterns, which have been previously shown to be associated with a reduction in neurological severity at 24 h, penumbral survival until reperfusion, and better clinical outcomes at 90 days in AIS. From a therapeutic standpoint, we postulate that patients with the good collateral flow may have infarcts associated with hyperperfusion in perilesional areas. Moreover, good collaterals may be sustaining penumbral survival until reperfusion. This mechanism may be responsible for driving a favourable clinical trajectory. However, in this study, we acknowledge that we did not show causality between collateral status and PLH.

Our findings also show that two infarct topographies, involving cortical superior MCA and striatocapsular territories, are associated with increased rates of PLH patterns. This is in agreement with the previous study by Marchal et al. where they also found that PLH was more commonly seen in patients with small-sized infarcts, located deep in the MCA territory where there is survival of overlying or adjacent cortex, consistent with lenticulostriate artery mouth occlusion during transient MCA embolism.10,42–45 We postulate that the presence of good collaterals is probably an important determinant (possibly a pre-requisite) of the

Table 4. Stepwise-backwards multivariate logistic regression analysis showing the association of baseline characteristics/covariates with PLH at 24 h.

<table>
<thead>
<tr>
<th>OR (95% CI)</th>
<th>P &gt;</th>
<th>z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model I: Baseline characteristics association with PLH (Model parameters: n = 119; dof = 4; pseudo R² = 0.2767; area under the ROC curve = 0.85; goodness of fit (Pearson χ²) test: P = 0.1458; AIC = 119.8; BIC = 130.9; number of covariate patterns = 8; sensitivity = 66.7%; specificity = 89.61%; PPV = 77.8%; NPV = 83.13%; overall rate of correct classification = 81.5%). Good collaterals 8.4 (3.1, 22.5) &lt;0.0001* Infarction in cortical superior MCA territory 3.7 (1.3, 10.6) 0.013* Infarction in striatocapsular territory 4.2 (1.6, 11.1) 0.004*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model II covariates association with PLH (model parameters: n = 116; dof = 8; pseudo R² = 0.4284; area under the ROC curve = 0.91; goodness of fit (Pearson χ²) test: P = 0.1724; AIC = 102.81; BIC = 124.84; number of covariate patterns = 39; sensitivity = 69.1%; specificity = 91.9%; PPV = 82.86%; NPV = 83.95%; overall rate of correct classification = 83.6%). Good collaterals 5.05 (1.62, 15.7) 0.005* Infarction in cortical superior MCA territory 4.7 (1.4, 15.7) 0.012* Infarction in striatocapsular territory 9.45 (2.63, 33.96) 0.001* HT 7.95 (0.52, 122.3) 0.137 Reperfusion status 0.0357* Major reperfusion 7.5 (1.6, 35.1) 0.01* Incomplete reperfusion 3.7 (0.4, 30.2) 0.227 No reperfusion 1 Penumbral salvage Yes 6.64 (1.8, 24.49) 0.004* No 1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
A/BIC: Akaike/Bayesian information criteria; dof: degrees of freedom; ENI: early neurological improvement; HT: haemorrhagic transformation; NPV: negative predictive value; PPV: positive predictive value; ROC: receiver–operator characteristic curve. *Used as reference category. P < 0.1 for enter and stay criteria. The two multivariate models differed in terms of the particular covariates/factors included. *P < 0.05 as the threshold for statistical significance.
development of the PLH patterns seen on ASL-MRI supported by the knowledge: when there are excellent leptomeningeal collaterals, the cortex is spared and hence infarcts develop only in SCI territory. The PLH patterns being more prevalent in cortical superior division MCA as opposed to inferior division MCA are in keeping with the recognised denser collateralisation via leptomeningeal communications from ACA to MCA compared to PCA to MCA. The cortical superior division MCA is potentially fed by the leptomeningeal arteriolar anastomoses (including end-to-end anastomoses, end-to-side connections, and azygos variants) from the ACA, whereas the inferior division MCA is fed by collaterals arising from the PCA.

In our acute stroke cohort, major reperfusion was significantly associated with PLH. This is corroborated by the previous studies that linked the presence of relative hyperperfusion of the ipsilateral hemisphere, ranging from 20% to 44%, observed in complications of the carotid revascularisation such as carotid endarterectomy, to the reperfusion. Recanalisation post t-PA could have been the driver of major reperfusion. Our results also show a significant association between PLH and HT. This is consistent with previous studies by Marchal et al. Moreover, we found that the PLH patients demonstrated a tendency to favourable long-term clinical recovery (64%). Our findings suggest that late stage reperfusion can be harmless, and the fact that PLH may be a marker of favourable tissue outcome concurs with Marchal et al.

We found a strong association between PLH and HT (as expected, since hyperperfusion is a marker of reperfusion of a severely ischaemic tissue with vascular damage), but not with PH1–PH2 (although only one of those occurred). The non-association of PLH with PH1–PH2 could be explained in terms of the small number of HT in our AIS cohort. Owing to the small sample size, it is very well possible that we did not find occurrences of PH1–PH2 due to low power. This may be a matter for future investigation. Multivariate modelling also demonstrated a trend towards higher PLH rates in patients presenting with HT, a subgroup of our cohort who are eight times more likely to show HT than their non-PLH counterparts. Our results are supported by a recent study also showing a strong link between postischaemic hyperperfusion detected by ASL-MRI and HT. Using multivariate logistic regression Yu et al. demonstrated that hyperperfusion was an independent risk factor for HT and that hyperperfusion patients were approximately three times as likely to experience HT compared with patients without hyperperfusion. However, the study included patients presenting with hyperperfusion both within (lesional) as well as around (peri-lesional) DWI lesions. In this study, we also found that patients receiving intravenous thrombolytic treatment were more likely to develop PLH; however, the association was not significant.

Our study revealed that ASL imaging can be used to capture post-ischaemic PLH patterns in AIS patients. Our incidence of PLH (42/119; 35.3%) seen in topographically distinct areas from the site of infarction corresponds well with the previous studies. This is especially appropriate given the known limitations of ASL for reduced sensitivity to regions of low perfusion (underestimation of tissue perfusion) in the presence of prolonged arterial transit delays. The shortening of, or avoidance of prolonged, arterial transit time enables easy detection of hyperperfusion on ASL. In an interesting study by Zaharchuk et al., arterial transit artefacts on ASL was used to identify collaterals in patients with Moyamoya disease for angiographic validation. Assessment of lesion topography, collateral status, reperfusion, and post-ischaemic hyperperfusion patterns using multimodal imaging can provide additional metrics for patient selection towards intervention strategies and prognosis in acute settings. It may also be useful in guiding appropriate recovery intervention strategies; a therapeutic approach with a focus on infarct topography and collateral status may improve the long-term clinical outcomes, and hence impact the quality of life for treated patients. This may have implications for prognostication in hyper-acute settings and patient selection towards identifying potential recovery targets for long-term therapy and rehabilitation. However, further research is warranted to better evaluate the utility of this imaging-based approach.

Limitations

There may be a selection bias in this study as observed in the high rate of thrombolysis in our cohort’s case mix. Endovascular (intra-arterial) treatment is not available at our facility. Therefore, we may not be able to generalise our results to patients receiving intra-arterial therapy. Additionally, it is unknown when patients reperfused exactly while all patients had a relatively variable time of onset from stroke and the majority received rtPA; we cannot be certain how long after rtPA infusion that reperfusion occurred. This may mean that some patients reperfused significantly later than others, thereby limiting their ability to develop hypoperfusion due to poor reperfusion despite the appearance of successful reperfusion 24 h later. However, we consider this variability as strength to our study considering its similitude to the heterogeneity of case mix encountered clinically. Moreover, the large sample size used in this study may reduce the effect of some of the limitations. Nevertheless, future studies on even larger sample size may provide additional insights.
to our findings. The ASL method used in our study is based on a pulsed sequence with T11 of 500 ms and T12 of 1700 ms, resulting in a post-labelling delay of 1200 ms, which is short especially for stroke patients. We acknowledge that this may be relatively ‘old’ with regard to other recent studies on similar lines. As such, the difficulty in differentiation of delayed arterial transit effect from hyperperfusion could possibly have caused confounding effects. It would also be interesting to assess whether hyperglycaemia is associated with PLH. This could potentially be subject of future investigation.

**Conclusion**

Post-ischaemic hyperperfusion in acute settings can be of prognostic value depending on the spatial localisation and temporal dynamics. PLH in AIS patients predicts a more favourable recovery in some patients. We have identified that several lesion topographies and collateral status at baseline are associated with PLH. In terms of clinical outcomes, PLH was also found to be associated with penumbral salvage and major reperfusion at 24 h. This study is novel considering its pathophysiological and clinical standpoints. Predicting post-stroke recovery remains a challenge to stroke physicians. However, profiling patients using imaging metrics may enable clinicians to better understand prognosis and design individualised stroke recovery protocols. Further studies are required to replicate our results in a broader case mix of acute stroke and to measure effects of these synergistic factors and to further evaluate the long-term recovery trajectories of the various imaging defined subgroups. This may have practical significance for therapeutic clinical trials of acute stroke. Our study also shows the potential application of non-invasive imaging using ASL in the acute stroke population. ASL at 12–24 h can be useful for pathophysiological investigations.

**Funding**

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Hunter Medical Research Institute & the University of Newcastle.

**Declaration of conflicting interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Authors’ contribution**

Contributors SB, MP, AB, and CL conceived and designed the study. SB, AB, and CL collected and analysed the data. SB, CL, and JRA contributed in the data analysis. SB, PS, and CL drafted the article, and all authors (SB, CL, PS, AB, JRA, MN, and MP) contributed towards the patient recruitment, study design, data analysis, drafting, and revision of the article.

**Supplementary material**

Supplementary material for this paper can be found at http://jcbfm.sagepub.com/content/by/supplemental-data

**References**

14. Tamura A, Asano T and Sano K. Correlation between rCBF and histological changes following temporary


4.4. SUPPLEMENTARY INFORMATION (SI)

Supplementary Tables

**SI Table 1. Multicollinearity test using Variance Inflation Factor (VIF), Tolerance and R-Squared.**

<table>
<thead>
<tr>
<th></th>
<th>VIF</th>
<th>Tolerance</th>
<th>R-Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLH</td>
<td>1.74</td>
<td>0.57</td>
<td>0.43</td>
</tr>
<tr>
<td>Good Collaterals</td>
<td>1.3</td>
<td>0.77</td>
<td>0.23</td>
</tr>
<tr>
<td>Cortical Sup MCA</td>
<td>1.3</td>
<td>0.77</td>
<td>0.23</td>
</tr>
<tr>
<td>SCI</td>
<td>1.41</td>
<td>0.71</td>
<td>0.29</td>
</tr>
<tr>
<td>Salvage</td>
<td>1.38</td>
<td>0.73</td>
<td>0.27</td>
</tr>
<tr>
<td>HT</td>
<td>1.13</td>
<td>0.88</td>
<td>0.12</td>
</tr>
<tr>
<td>Major Reperfusion</td>
<td>1.28</td>
<td>0.78</td>
<td>0.22</td>
</tr>
</tbody>
</table>

(Note: PLH: peri-lesional hyperperfusion; Sup MCA: cortical superior division MCA territory; SCI: striatocapsular/lenticulostriate territory; Salvage: Penumbral salvage (yes/no); HT: Hemorrhagic transformation).

**SI Figure 1. Schematic showing various cerebral vascular territories. Infarct was assigned to one or multiple vascular territories depending upon its topographic location.**
“One question is always relevant: How can I use this to move forward?”

- Rebbetzin Tziporah Heller
CHAPTER 5: DELAY OF LATE-VENOUS PHASE CORTICAL VEIN FILLING IN ACUTE ISCHEMIC STROKE PATIENTS: A NOVEL NEURORADIOLOGICAL BIOMARKER

5.1. Introduction

CT angiography (CTA) is a non-invasive vascular imaging technique with high sensitivity for the detection of arterial anatomy in the circle of Willis and other vascular structures. CTA also presents a reliable substitute to magnetic resonance angiography (MRA) and an expedient adjunct to conventional digital subtraction angiography (DSA) [12, 1153]. The development of new generation 320-detector row 640-slice multi-detector CT (MDCT) scanners allows for whole-brain, sub-second, and volumetric acquisition of four-dimensional (4D) dynamic time-resolved CTA (dCTA) studies of the brain, in particular visualization of changes in perfusion [10, 11]. In addition to obtaining three-dimensional (3D) evaluation of intracranial vasculature, time-resolved dCTA allows visualization of contrast flow from its arterial to venous phases and has demonstrated superior diagnostic accuracy in comparison to the single-phase conventional CT-angiography [11, 12]. Currently, clinico-neuroradiological assessments using CTA focus primarily on the arterial cerebral circulation. Information on haemodynamics and the drainage topography of the venous cerebral circulation may be useful for prognostication in stroke. Animal studies have shown that the presence of blood flow in cortical veins after ischemic stroke was associated with decreased severity of hemiparesis and lower infarct volumes [1154]. Based on these findings, the authors postulated that cortical venous outflow is more prevalent in strokes with good baseline collaterals, and animals with poor collaterals would demonstrate reduced or absent venous outflow. Other studies have used venous phase timing as a surrogate marker of the adequacy of collaterals at the circle of Willis and for general cerebral blood flow [13]. A recent clinical study found that cortical venous drainage, not the deep venous drainage patterns, identified by CTA, accurately predicted clinical outcomes [16]. Several studies have demonstrated the presence of hypointense prominent cortical veins, in early or mid-venous phase, ipsilateral to the ischemic tissue in acute ischemic stroke patients using susceptibility-weighted imaging (SWI) [14, 15, 17, 18, 1154] [1155]. However, data on venous drainage patterns, temporal aspects of the filling of leptomeningeal collateral vessels [466], and their role in stroke pathophysiology are limited [16, 707, 1155-1157].
Currently, methods used to assess collateral filling are qualitative in nature. An alternative and direct measure of collateral flow can be very useful in clinical practice. In this study, we prospectively evaluated a quantitative measure, being venous drainage, in a group of hyperacute ischemic stroke patients who underwent dCTA angiography as part of their emergent evaluation. We report a novel angiographic pattern of delay in late venous phase cortical vein filling (LCVF) and its relationship to arterial collateral status.

The specific objectives of the study were:

1. To examine the frequency of delayed-LCVF.

2. To investigate the factors or covariates associated with delayed-LCVF.

3. To study the association of delayed-LCVF with baseline collateral status.

We hypothesize that the appearance of delayed-LCVF will be more commonly seen in a sub-group of ischemic stroke patients with poor arterial collaterals.
5.2. Contributions


75% conception and design of research
75% experimental procedures
75% analyses and interpretation of the findings
75% writing of the paper and critical appraisal of the content

Prof Christopher Levi

13/02/2017
Signed: Date:

A/Prof Peter Stanwell

17/02/2017
Signed: Date:

Prof John Attia

15/02/2017
Signed: Date:
Prof Michael Nilsson

Signed: Date: 16/02/2017

Prof Mark Parsons

Signed: Date: 13/02/2017

Dr Andrew Bivard

Signed: Date: 16/02/2017

Signed: Date:

Faculty Assistant Dean (Research Training)

Prof Robert Callister

Signed: Date: 17/2/17

5.3. PUBLICATION
Delay of late-venous phase cortical vein filling in acute ischemic stroke patients: Associations with collateral status

Sonu Bhaskar1,2, Andrew Bivard1, Mark Parsons1,2, Michael Nilsson2,3, John R Attia4, Peter Stanwell2 and Christopher Levi1,2

Abstract
Evaluation of the venous system may be useful in stroke prognostication and patient selection for acute intervention strategies. We report a novel phenomenon, delayed-late venous phase cortical vein filling, observed on dynamic computed tomography angiography obtained using multidetector computed tomography scanner, in acute ischemic stroke patients. The aim of this study was to examine the frequency of delayed-late venous phase cortical vein filling and assess its association to baseline collateral status. Dynamic computed tomography angiography images of acute ischemic stroke patients, being assessed for reperfusion therapy, were prospectively studied. Delayed-late venous phase cortical vein filling was defined by late venous phase opacification of cortical veins despite contrast clearance from contralateral cortical veins on dynamic computed tomography angiography. Time to peak of maximum arterial enhancement was recorded. A total of 117 patients (mean age = 70.6 ± 13.3 years; males = 48%) with hemispheric ischemic stroke who underwent acute dynamic computed tomography angiography were included in the study. Overall, 56 (48%) demonstrated delayed-late venous phase cortical vein filling. Poor collateralization (OR = 13.50; 95% CI = (4.2, 43); p ≤ 0.0001) and longer time to peak of maximum arterial enhancement (OR = 3.2; 95% CI = (1.96, 5.3); p ≤ 0.0001) were positively associated with delayed-late venous phase cortical vein filling. Delayed-late venous phase cortical vein filling was independently associated with poor baseline collateral status (75% vs. 15%; p ≤ 0.0001; OR = 14.38; 95% CI = (4.33, 47.8); p ≤ 0.0001). Delayed-late venous phase cortical vein filling is frequently seen in patients with acute ischemic stroke and is associated with poor baseline collateralization.

Keywords
Stroke, angiography, CTA, venous flow, collateral flow

Received 8 December 2015; Revised 2 February 2016; Accepted 2 February 2016

Introduction
Computed tomography angiography (CTA) is a non-invasive vascular imaging technique with high sensitivity for the detection of arterial anatomy in the circle of Willis and other vascular structures. CTA also presents a reliable substitute to magnetic resonance angiography (MRA) and an expedient adjunct to conventional digital subtraction angiography (DSA).1,2 The development of new generation 320-detector row 640-slice multidetector CT (MDCT) scanners allows for whole-brain, sub-second, and volumetric acquisition of four-dimensional (4D) dynamic time-resolved CTA (dCTA) studies of the brain, in particular visualization of changes in perfusion.3,4 In addition to
obtaining three-dimensional (3D) evaluation of intracranial vasculature, time-resolved dCTA allows visualization of contrast flow from its arterial to venous phases and has demonstrated superior diagnostic accuracy in comparison to the single-phase conventional CT-angiography. Currently, clinico-neuroradiological assessments using CTA focus primarily on the arterial cerebral circulation. Information on haemodynamics and the drainage topology of the venous cerebral circulation may be useful for prognostication in stroke. Animal studies have shown that the presence of blood flow in cortical veins after ischemic stroke was associated with decreased severity of hemiparesis and lower infarct volumes. Based on these findings, the authors postulated that cortical venous outflow is more prevalent in strokes with good baseline collaterals, and animals with poor collaterals would demonstrate reduced or absent venous outflow. Other studies have used venous phase timing as a surrogate marker of the adequacy of collaterals at the circle of Willis and for general cerebral blood flow. A recent clinical study found that cortical venous drainage, not the deep venous drainage patterns, identified by CTA, accurately predicted clinical outcomes. Several studies have demonstrated the presence of hypointense prominent cortical veins, in early or mid-venous phase, ipsilateral to the ischemic tissue in acute ischemic stroke patients using susceptibility-weighted imaging (SWI). However, data on venous drainage patterns, temporal aspects of the filling of leptomeningeal collateral vessels, and their role in stroke pathophysiology are limited.

Currently, methods used to assess collateral filling are qualitative in nature. An alternative and direct measure of collateral flow can be very useful in clinical practice. In this study, we prospectively evaluated a quantitative measure, being venous drainage, in a group of hyperacute ischemic stroke patients who underwent dCTA angiography as part of their emergent evaluation. We report a novel angiographic pattern of delay in late venous phase cortical vein filling (LCVF) and its relationship to arterial collateral status.

The specific objectives of the study were:

1. To examine the frequency of delayed-LCVF.
2. To investigate the factors or covariates associated with delayed-LCVF.
3. To study the association of delayed-LCVF with baseline collateral status.

We hypothesize that the appearance of delayed-LCVF will be more commonly seen in a sub-group of ischemic stroke patients with poor arterial collaterals.

Materials and methods

Patient selection and study design

We studied consecutive acute ischemic stroke patients admitted to a comprehensive stroke centre using the following inclusion criteria: (a) age > 18 years, (b) presented within 4.5 h of stroke symptom onset, (c) being evaluated for reperfusion therapy, (d) hemispheric stroke, and (e) dCTA acquired at baseline. Patients without baseline CTA images, with hemorrhagic or metabolic stroke, were excluded from the study. Patients who were eligible for thrombolysis received 0.9 mg/kg intravenous recombinant tissue plasminogen activator (rtPA). Demographics and risk factors were assessed in a structured case-record form. Baseline clinical measures (immediately before acute CT) were assessed using the National Institutes of Health Stroke Scale (NIHSS). Stroke etiology was assessed using the Causative Classification System (CCS) criteria. This study was approved by the Hunter New England Human Research Ethics Committee (HNEHREC, Newcastle, NSW) in accordance with the National Statement in Ethical Conduct in Human Research 2007. All patients gave informed consent.

Imaging acquisition and neuroradiological evaluation

All the patients included in the study underwent non-contrast CT (NCCT), CT perfusion (CTP) with dCTA at baseline, and follow-up (24 h) magnetic resonance imaging (MRI) (including diffusion-weighted imaging (DWI)) in accordance with our routine stroke imaging protocol. All CT imaging was acquired on a 320-detector row 640-slice cone beam MDCT scanner (Aquilion One, Toshiba Medical Systems). Whole-brain NCCT was performed in one rotation (detector width 16 cm). Subsequent to NCCT, a 4D-dCTA and CTP were acquired simultaneously in two 60-s series. CTA/CTP imaging data were acquired in the axial plane before and after administration of 50 ml of contrast agent (Ultravist 370; Bayer HealthCare, Berlin, Germany) injected intravenously at a rate of 6 ml/s. An axial slice was acquired at 5 mm. Staging 7 s after contrast injection, pulsed full rotation scan with 19 time points acquired over 60 s with a total pulse image acquisition time of 9.5 s was used. For examination of extra cranial vessels, CTA of extracranial segment was also acquired using bolus tracking with 50 ml of contrast (injected at 6 ml per second chased by 50 ml of saline). Total radiation exposure was 5.5–6.0 mSev. MRI was performed at 24 h based on standard stroke imaging routine protocol that includes an axial isotropic DWI spin-echo echo-planar
imaging (SE-EPI sequence, time-of-flight MR angiography (TOF-MRA), and whole-brain perfusion imaging with bolus-tracking perfusion-weighted imaging (PWI), on a 3T MRI (Siemens Verio, Erlangen, Germany) with a 32-channel receive-only head coil. All CTA images were de-identified and reviewed digitally at a workstation (Vitrea®, VX, Version 1.0, Vital Images, Minnetonka, MN, USA). Baseline axial CTA data were formatted and images were analysed using maximum intensity projection (MIP) and multi-planar reformat reconstructions in coronal and sagittal planes. These images were read by consensus by two experienced readers (SB and CL). In order to obtain optimized spatial orientation and precise localization of the ischemic lesion, three-dimensional volume rendering was applied. Delayed-LCVF was defined by late venous phase opacification of cortical veins despite contrast clearance from contralateral cortical veins on MIP images from dCTA (see Supplementary Video). To assess the association of venous drainage with delay in maximized enhancement of arterial collateralization, we recorded time to peak of maximum arterial enhancement (TPME). The baseline (time = 0) was defined as the image immediately preceding the venous filling in early phase, following which, the times taken to reach maximized arterial filling of all M2, M3, and M4 segments, with respect to the contralateral hemisphere, were recorded (each successive image corresponds to 2 s lag; therefore, TPMEs were recorded as even numbers: 2 s, 4 s, 6 s, 8 s...). For morphological assessment of collateralization status, collateral grading was done using dCTA data based on the degree of reconstitution of the MCA up to the distal end of its occlusion and was divided into ‘good’ or ‘reduced or poor’ using a protocol described previously. The extension of the sphenoidal segment from the bifurcation of ICA on the medial end to its bifurcation or trifurcation in the insular region is identified as M1. M2 is defined in terms of proximal (the segment that starts immediately after M1 bifurcation) and distal (beyond M1 region) segments. CTP data were de-identified prior to analysis, following which CTP perfusion maps, including mean transit time (MTT) and cerebral blood volume (CBV) were generated with MiStar software (Apollo Medical Imaging Technology, Melbourne, Australia), which uses a deconvolution algorithm to process the data. Acute perfusion imaging was processed using single-value deconvolution with delay and dispersion correction. An arterial input function and venous outflow function was semiautomatically selected from the non-stroke (contralateral) hemisphere MCA/ACA and sagittal sinus, respectively. Previously validated thresholds were applied in order to measure the volume of the acute perfusion lesion (relative delay time, DT > 3 s) and acute infarct core (relative CBF < 30%). Penumbral volume was calculated from the volume of the perfusion lesion (DT threshold > 3 s) minus the volume of the infarct core (relative CBF threshold < 30% within the DT > 3 s of the lesion).

Case presentations

Two cases with delayed-LCVF are shown in Figures 1 and 2. Early, mid-venous, and late venous phases are depicted. The patterns of delayed-LCVF as seen on MIP reconstructed images of CTA are detailed. Cortical veins showed persistent opacification in late venous phase on the side of the ischemic lesion despite contrast clearance from the contralateral side. The time course of venous flow on dCTA for the patient in case study 1 is shown in Supplementary Video.

Statistical analysis

All the statistical analyses were performed using STATA (Version 10, 2001; College Station, TX, USA). Numerical values given are the means (±standard deviation) or medians (interquartile range) for age, TPME, and NIHSS at admission. For ordinal or continuous data, Mann-Whitney (Wilcoxon rank-sum) test was used. Nominal data were analysed with the Pearson’s chi-squared ($\chi^2$) or the two-tailed Fisher exact test. We compared two groups of patients stratified by delayed-LCVF status (delayed-LCVF vs. no-delayed-LCVF). Group differences were considered significant at p-values < 0.05. Univariate logistic regression analysis was used to test associations between covariates and delayed-LCVF. Results of logistic regression are reported as odds ratios (ORs). Only those variables with p < 0.1 were tested in the subsequent multivariate regression analysis. Before fitting the multivariate model, we also tested the correlations among all covariates with the pairwise Pearson’s correlation coefficient ($r$) to identify collinear pairs. Significance level of the correlation coefficients for each variable was also tested. Multicollinearity was also tested using tolerance and variance inflation factor (VIF): a common rule of thumb is that VIFs > 5 identify strongly collinear pairs. If two or more covariates were found to be collinear, we retained the variable that was more strongly associated with the delayed-LCVF to ensure a stable model. A multivariate regression model, based on a backwards, step-wise approach was used; to arrive at the most parsimonious model by retaining only the most important ‘explanatory’ variables (p < 0.1). Multivariate normality (of the regression model) was also checked using the Doornik-Hansen test. Comparison between the various
Multivariate regression models was made using model selection statistics (including the Akaike information criterion (AIC) and the Bayesian information criterion (BIC)). The sensitivity, specificity, and overall rate of correct classification for each model were estimated using classification statistics using a cut-off (positive outcome threshold) of 0.5. The goodness-of-fit using Pearson $\chi^2$ test and number of covariate patterns were calculated for each multivariate model. Finally, the receiver operating characteristic (ROC) curve for the regression model was plotted, and the area under the ROC curve was computed to evaluate the predictive ability. To study the association with baseline collateral status, univariate and multivariate logistic models were used. The model used TPME, age, and NIHSS. The effect of delayed-LCVF on this model was also studied, and the classification statistics for both the models were compared.

Figure 1. Case study 1. (a) Non-contrast CT (NCCT) demonstrates effacement of the sulci and blurring of the normal grey/white matter differentiation in the left frontoparietal region. (b) Three-dimensional volume-rendered image from MDCT 4D-CTA. The white arrow points at the presence of a left M2 occlusion. (c) (i–iv) CTP (coronal 5 mm average registered) shows the presence of penumbra and demonstrated region of increased mean transit time (MTT) and time to peak (TTP), as well as reduced cerebral blood flow (CBF) and blood volume (CBV) in the left MCA cortex. (d) Baseline MRI using PWI-DWI mismatch also confirms the presence of penumbra in the left MCA infarct involving insula region. (e) CT Angiogram AP view: coronal reformatted 2D MIP spiral dynamic CTA images (i–vii); The presence of left-sided late-venous phase opacification of cortical veins (red arrow) on left side despite contrast clearance from contralateral (right side) cortical veins can be seen on dynamic CTA image, (e) (vii). Early phase is characterized by early filling of venous sinuses (i–ii), followed by mid-venous phase (iii–vi). Late venous phase is depicted in vii (red-arrow). Background: Left MCA infarct in insular region. A 66-year-old woman with a history of diabetes, hypercholesterolemia, smoking and atrial fibrillation presented with expressive dysphasia and dysarthria. NIHSS score was 23 on admission. The patient was not thrombolysed. NIHSS at 24 h was 10. CT showed acute cerebral infarct in the region of the left frontoparietal region, with minimal mass effect or midline shift. Day 90 mRS score was 5. The old left parietal infarct and chronic small vessel disease was also confirmed on both baseline CT and 24 h MRI.
Results

Baseline characteristics

The demographics, risk factors and other clinical characteristics of the overall study population, stratified by overall, and presence or absence of delayed-LCVF, are detailed in Table 1. In total, 117 patients (mean age = 71 ± 13 years; number of males = 56 (48%)) with hemispheric ischemic stroke and baseline dCTA were included in the study, out of which 56 (47.86%) patients showed delayed phase cortical vein filling; 96 (82%) received intravenous tPA. The median NIHSS score on admission was 14 points (IQR = 8).

Delayed-LCVF vs. no-delayed-LCVF

Neither demographics (age, sex), stroke severity at admission, thrombolytic treatment, or other clinical
risk factors differed significantly between patients with and without LCVF. LCVF was associated with longer dynamic TPME (in s) (median (IQR): 8 (2) vs. 6 (2); \(p < 0.001\)); increased rates of unfavourable collateralization (86% vs. 26%; \(p < 0.001\)) and higher frequencies of stroke due to large artery atherosclerosis (54 % vs. 28%; \(p = 0.005\)) (Table 2). Patients who showed delayed cortical vein filling tended to have infarcts with lower penumbral volumes, although this was not significant (median penumbral volume (IQR), in ml = 48 (78) vs. 63 (74); \(p = 0.417\)).

**Table 1.** List of demographic, clinical, and risk factor variables stratified by late-phase cortical vein filling.

<table>
<thead>
<tr>
<th></th>
<th>All patients (n = 117)</th>
<th>delayed-LCVF (n = 56)</th>
<th>Non-delayed-LCVF (n = 61)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>117</td>
<td>56 (47.86)</td>
<td>61 (52.14)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>56 (47.86)</td>
<td>25 (44.64)</td>
<td>31 (50.82)</td>
<td>0.580</td>
</tr>
<tr>
<td>Age, in years (mean ± SD)</td>
<td>70.61 ± 13.32</td>
<td>70.84 ± 13.43</td>
<td>70.39 ± 13.32</td>
<td>0.9521</td>
</tr>
<tr>
<td>Median baseline NIHSS (IQR)</td>
<td>14 (8)</td>
<td>13.5 (8.5)</td>
<td>14 (8)</td>
<td>0.4108</td>
</tr>
<tr>
<td>Treatment factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombolysis</td>
<td>96 (82.05)</td>
<td>47 (83.93)</td>
<td>49 (80.33)</td>
<td>0.639</td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>102 (87.18)</td>
<td>46 (82.14)</td>
<td>56 (91.80)</td>
<td>0.167</td>
</tr>
<tr>
<td>Diabetes</td>
<td>34 (29.06)</td>
<td>17 (30.36)</td>
<td>17 (27.87)</td>
<td>0.840</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>51 (43.59)</td>
<td>24 (42.86)</td>
<td>27 (44.26)</td>
<td>1.000</td>
</tr>
<tr>
<td>Present smoker</td>
<td>32 (27.35)</td>
<td>14 (25)</td>
<td>18 (29.51)</td>
<td>0.679</td>
</tr>
<tr>
<td>Past smoker</td>
<td>53 (45.30)</td>
<td>27 (48.21)</td>
<td>26 (42.62)</td>
<td>0.581</td>
</tr>
<tr>
<td>AF</td>
<td>66 (56.41)</td>
<td>31 (55.36)</td>
<td>35 (57.38)</td>
<td>0.854</td>
</tr>
<tr>
<td>New AF</td>
<td>40 (34.19)</td>
<td>17 (30.36)</td>
<td>23 (37.70)</td>
<td>0.440</td>
</tr>
<tr>
<td>Depression</td>
<td>10 (8.55)</td>
<td>3 (5.36)</td>
<td>7 (11.48)</td>
<td>0.327</td>
</tr>
<tr>
<td>History of stroke/TIA</td>
<td>24 (20.51)</td>
<td>12 (21.43)</td>
<td>12 (19.67)</td>
<td>0.823</td>
</tr>
</tbody>
</table>

Note: Figures in parentheses are percentages. *p < 0.05 as the threshold for statistical significance.

LCVF: late venous phase cortical vein filling; AF: atrial fibrillation; HT: hemorrhagic transformation; TIA: transient ischemic attack; IQR: inter-quartile range; SD: standard deviation; NIHSS: National Institute of Health Stroke Scale Score.

Associations with LCVF using univariate logistic regression

The results of univariate logistic regression analysis examining the associations with the LCVF (OR and levels of significance) are shown in Table 3. LCVF was strongly associated with longer dynamic TPME (OR = 3.5; 95% CI = (2.22, 5.55); \(p \leq 0.0001\)), and poor collateral grades (OR = 16.88; 95% CI = (6.58-43.25); \(p \leq 0.001\)). With respect to the etiology, large artery atherosclerosis was more likely (OR = 2.44; 95% CI = (0.97, 6.19); \(p = 0.059\)) and cardioembolic less likely (OR = 0.78; 95% CI = (0.29, 2.10); \(p = 0.626\)) to be associated with delayed-LCVF. Overall, the etiological mechanism was associated with delayed-LCVF (\(p = 0.03\)). Other clinical data such as age, sex, neurological deficit at baseline or at 24 h, and risk factors (e.g. hypertension, diabetes, history of smoking, atrial fibrillation and preceding transitory ischemic attacks) did not show significant association with delayed-LCVF (see Supplementary Information 1, SI Table 1). Interestingly, the infarct lesion and penumbral volumes were also not affected by this phenomenon in this study population.

Covariates with \(p < 0.1\) (TPME, poor collaterals, etiology) were tested for collinearity, and VIF values were found to be less than 5. Therefore, no strong multicollinearity was detected.

**Multivariate regression model of associations with delayed-LCVF**

Results of the step-wise backwards multivariate logistic regression are shown in Table 4. Multivariate modelling was done by fitting a model with all potential (non-collinear) covariates (Model I). In the reduced model, two factors were positively associated with delayed-LCVF (Model II): poor collateralization (OR = 13.50, 95% CI = [4.23, 43.04]; \(p < 0.0001\)), and longer TPME (OR = 3.22, 95% CI = [1.96, 5.3]; \(p < 0.0001\)). The lower AIC and BIC values indicated that the reduced model (Model II) fitted the data better than Model I (Model II vs. Model I: AIC = 87.43 vs. 89.2; BIC = 95.7 vs. 102.89). The overall rate of correct classification of the reduced model was estimated to be 87.2%, with 90% of the non-delayed-LCVF group correctly classified (specificity), and 84% of the delayed-LCVF group.
correctly classified (sensitivity). The ROC plot for the reduced model is shown in Supplementary Information I, SI Figure 1. The AUC of approximately 0.91 indicated excellent discrimination/accuracy for the model.

Association with poor collateral status

Univariate analysis showed that TPME (OR = 1.79; 95% CI = (1.35, 2.37); p ≤ 0.0001) and delayed-LCVF (OR = 16.88; 95% CI = (6.58, 43.25); p ≤ 0.0001) were associated with poor baseline collateral status. However, when delayed-LCVF was added to the multivariate regression model, while controlling for the effects of age and baseline stroke severity, association of TPME with poor-collateral status became non-significant (OR = 1.15; 95% CI = [0.79, 1.68]; p = 0.464).

Table 2. List of imaging findings stratified by late-stage cortical vein filling.

<table>
<thead>
<tr>
<th>Variable</th>
<th>All patients (n = 117)</th>
<th>delayed-LCVF (n = 56)</th>
<th>Non-delayed-LCVF (n = 61)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPME, in seconds, Mean ± SD</td>
<td>7.35 ± 1.68</td>
<td>8.43 ± 1.31</td>
<td>6.36 ± 1.34</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>TPME, in seconds, Median (IQR)</td>
<td>8 (2)</td>
<td>8 (2)</td>
<td>6 (2)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>Collateral grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced/poor</td>
<td>64 (54.70)</td>
<td>48 (85.71)</td>
<td>16 (26.23)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>Good</td>
<td>53 (43.30)</td>
<td>8 (14.29)</td>
<td>45 (73.77)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>Etiology (CCS classification)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large artery atherosclerosis</td>
<td>47 (40.17)</td>
<td>30 (53.57)</td>
<td>17 (27.87)</td>
<td>0.005a</td>
</tr>
<tr>
<td>Cardio embolic</td>
<td>36 (30.77)</td>
<td>13 (23.21)</td>
<td>23 (37.70)</td>
<td>0.110</td>
</tr>
<tr>
<td>Small artery occlusion</td>
<td>31 (26.50)</td>
<td>13 (23.21)</td>
<td>18 (29.51)</td>
<td>0.531</td>
</tr>
<tr>
<td>Undetermined</td>
<td>3 (2.56)</td>
<td>0 (0)</td>
<td>3 (4.92)</td>
<td>0.245</td>
</tr>
<tr>
<td>Baseline infarct volume, n = 115</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Core, ml; Median (IQR)</td>
<td>13.3 (28)</td>
<td>14.3 (29.45)</td>
<td>13.3 (27.1)</td>
<td>0.9576</td>
</tr>
<tr>
<td>Penumbra, ml; Median (IQR)</td>
<td>57 (80.8)</td>
<td>47.85 (77.55)</td>
<td>62.6 (73.9)</td>
<td>0.4171</td>
</tr>
</tbody>
</table>

TPME: time to peak of maximum arterial enhancement (in seconds); LCVF: late venous phase cortical vein filling; CCS: Causative Classification of Stroke System; IQR: inter-quartile range.

Note: CCS classification method was used to assess the causative etiological mechanism underlying the stroke event. Figures in parentheses are percentages and those in square brackets are interquartile ranges.

#p < 0.05 as the threshold for statistical significance.

Table 3. Odds ratios (95% confidence intervals) for the association with late venous phase cortical vein filling.

<table>
<thead>
<tr>
<th>Variable</th>
<th>% of patients with delayed-LCVF</th>
<th>OR (95% CI)</th>
<th>p &gt; z</th>
<th>p &gt; z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collateral grade (n = 151)</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001c</td>
</tr>
<tr>
<td>Reduced/poor</td>
<td>85.71</td>
<td>16.88 (6.58–43.25)</td>
<td>&lt;0.001c</td>
<td></td>
</tr>
<tr>
<td>Gooda</td>
<td>14.29</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Etiology based on CCS Classification</td>
<td></td>
<td></td>
<td></td>
<td>0.0307c</td>
</tr>
<tr>
<td>Large artery atherosclerosis</td>
<td>53.57</td>
<td>2.44 (0.97–6.19)</td>
<td>0.059</td>
<td></td>
</tr>
<tr>
<td>Cardio embolic</td>
<td>23.21</td>
<td>0.78 (0.29–2.10)</td>
<td>0.626</td>
<td></td>
</tr>
<tr>
<td>Small artery occlusiona</td>
<td>23.21</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undetermined</td>
<td>0</td>
<td>NAb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPME, in seconds</td>
<td>3.50 (2.22–5.53)</td>
<td>&lt;0.0001c</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TPME: Time to peak of maximum arterial enhancement (in seconds); LCVF: late venous phase cortical vein filling; CCS: Causative Classification of Stroke System.

aUsed as reference category.

bNot applicable.

cp < 0.05 as the threshold for statistical significance.
multivariate logistic regression analyses for covariates associated with poor-collateralization. Addition of delayed-LCVF showed significant improvement in model parameters (TPME and Delayed-LCVF vs. TPME: ROC Area = 0.85 vs. 0.77; specificity = 84.91% vs. 67.92%; overall rate of classification = 80.3% vs. 73%; BIC = 134.7 vs. 153.8) (see Supplementary Information 1, SI Table 2).

**Table 4.** Step-wise-backwards multivariate logistic regression analysis showing the association of covariates with delayed-LCVF.

<table>
<thead>
<tr>
<th>Covariate</th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor collaterals</td>
<td>13.77 (4.22–44.88)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>TPME, in seconds</td>
<td>3.04 (1.83–5.03)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Etiology based on CCS classification</td>
<td></td>
<td>0.522</td>
</tr>
<tr>
<td>Large artery atherosclerosis</td>
<td>2.15 (0.57–8.07)</td>
<td>0.256</td>
</tr>
<tr>
<td>Cardio embolic</td>
<td>1.61 (0.37–6.98)</td>
<td>0.526</td>
</tr>
<tr>
<td>Small artery occlusion&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Undetermined</td>
<td>NA&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Model I (first step; final model) (Model parameters: n = 114; dof = 5; pseudo $R^2$ = 0.278; area under the ROC curve = 0.92; goodness of fit (Pearson $\chi^2$) test: $p = 0.1642$; AIC = 89.2; BIC = 102.89; number of covariate patterns = 22; sensitivity = 83.93%; specificity = 89.66%; PPV = 88.68%; NPV = 85.25%; overall rate of correct classification = 86.84%).

Model II (last step; reduced model) (Model parameters: n = 117; dof = 3; pseudo $R^2$ = 0.4973; area under the ROC curve = 0.91; Goodness of fit (Pearson $\chi^2$) test: $p = 0.0838$; AIC = 87.43; BIC = 95.71; number of covariate patterns = 9; sensitivity = 83.93%; specificity = 90.16%; PPV = 88.68%; NPV = 85.94%; overall rate of correct classification = 87.18%).

<sup>a</sup>Used as reference category
<sup>b</sup>Not applicable

**Table 5.** Simple and mixed ORs showing the association of covariates with poor-collateralization.

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Simple OR (95% CI); p</th>
<th>Mixed OR (95% CI); p</th>
<th>Mixed OR (95% CI); p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delayed-LCVF</td>
<td>16.88 (6.58–43.25); &lt;0.0001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.38 (4.33–47.83); &lt;0.0001&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>TPME, in seconds</td>
<td>1.79 (1.35–2.37); 0.0001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.88 (1.39–2.53); &lt;0.0001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.15 (0.79–1.68); 0.464</td>
</tr>
<tr>
<td>Age</td>
<td>1.02 (0.99–1.05); 0.142</td>
<td>1.03 (0.99–1.06); 0.103</td>
<td>1.03 (0.99–1.07); 0.094</td>
</tr>
<tr>
<td>Baseline NIHSS</td>
<td>1.05 (0.98–1.12); 0.172</td>
<td>1.06 (0.99–1.14); 0.115</td>
<td>1.06 (0.97–1.15); 0.185</td>
</tr>
<tr>
<td>Thrombolysis</td>
<td>0.69 (0.27–1.84); 0.465</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Etiology based on CCS classification</td>
<td>0.1518</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large artery atherosclerosis</td>
<td>1.60 (0.63–4.04); 0.325</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardio embolic</td>
<td>0.59 (0.22–1.55); 0.283</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small artery occlusion&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undetermined</td>
<td>0.41 (0.03–5.03); 0.487</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Simple odds ratios (OR) were obtained using univariate logistic regression. Mixed ORs were obtained using multivariate logistic regression analysis using TPME or both TPME and delayed-LCVF while controlling for the effects of age and baseline stroke severity. The multivariate models differed in terms of the particular covariates/factors included.

TPME: time to peak of maximum arterial enhancement (in seconds); LCVF: late venous phase cortical vein filling; CCS: Causative Classification of Stroke System; NIHSS: National Institute of Health Stroke Scale Score.

<sup>a</sup>Used as reference category.
<sup>b</sup>p < 0.05 as the threshold for statistical significance.
Discussion

In this study, we report a novel cortical vein filling pattern observed in the late venous phase on time-resolved dCTA images in acute ischemic stroke patients. To our knowledge, this is the first such study that describes delayed-LCVF. Delayed-LCVF is independently associated with poor baseline collateral status and the morphological extent of baseline collateralization and delay of maximized collateral enhancement were linked with delayed-LCVF. Studies on cortical veins, their role in stroke pathophysiology, and their association with short- and long-term clinical outcomes are limited. Asymmetrically prominent cortical veins, in early or mid-venous phase, have been previously reported in ischemic stroke using T2* and phase-based methods.\textsuperscript{5,8,9,16,25} A recent study by Xia et al.,\textsuperscript{26} undertaken in a cohort of acute ischemia, reported reduced levels of oxygen saturation in these abnormal veins using a quantitative susceptibility mapping (QSM) technique.\textsuperscript{26} They concluded that the presence of deoxyhemoglobin was the most plausible explanation for the presence of asymmetrically prominent cortical veins.\textsuperscript{13,27,28} So far, the appearance of delayed LCVF has not been studied in stroke patients.

There is wide variability in the presence of the three variants of cortical veins, often existing in reciprocal relationship with angiographic assessment showing a predominance of one or two of these three veins (see Supplementary Information II for anatomical details of the cranial venous system).\textsuperscript{29} Alterations in the venous drainage (increase in venous drainage through secondary pathways), and associated reduction in intracranial compliance have been linked to migraine,\textsuperscript{30} mild traumatic brain injury,\textsuperscript{31} and multiple sclerosis.\textsuperscript{32} Vascular assessment in stroke is predominantly based on examination of the arterial system, and venous correlates of collateral perfusion are usually ignored. Venous circulation accounts for the majority of cerebral blood volume. Increased intracranial pressure may lead to the compression of veins after cerebral ischemia due to brain edema\textsuperscript{33} and subarachnoid hemorrhage due to vasospasm of deep cerebral veins.\textsuperscript{34} Veins and venules may play a role in acute stroke caused by arterial occlusion/stenosis.\textsuperscript{33}

Delayed cortical vein filling in late venous phase

As a principal finding of this study, we found that the delayed appearance of cortical vein filling during the late venous phase on baseline time-resolved dCTA images was relatively common in our hyperacute study cohort. In this exploratory study in acute ischemic stroke population, we demonstrate that dCTA provides useful information on the morphologic extent of the collaterals and the collateral blood flow delay. We found that the morphologic extent of the collaterals and the collateral blood flow delay in maximum enhancement (defined in terms of TPME) were independent predictors of delayed-LCVF.

Previous studies have shown that the good collateralization on dCTA predicts a small lesion volume.\textsuperscript{35} One of the shortcomings of these studies is that the assessment of collateral status was based on the maximal morphologic extent of collateral vessels over the entire scan time and lacked the temporal information associated with time point of collateral reconstitution. Data on time point of collateral reconstitution and its predictive value are limited.\textsuperscript{12,18,36} We demonstrate that the time delay in collateral flow provides an additional measure over and above the morphological based assessment. Our study shows that the model using both morphological extent of collateral vessel and the delay in collateral reconstitution showed excellent predictive ability for delayed-LCVF.

Average TPME was significantly greater in delayed-LCVF population. This measurement can provide quantifiable data on the status of arterial and venous flow. The longer the TPME on dCTA, the higher the probability of delayed-LCVF. Longer scanning times could assist in the further characterization of filling defects.

Poor collateral status linked to delayed-LCVF

This study also shows that the baseline delayed-LCVF was independently associated with baseline collateralization status. In multivariate analysis, delay in maximal collateral enhancement or TPME, was significantly associated with morphological extent of the collateral vessels at the baseline, while controlling for the effects of age and baseline stroke severity. However, on addition of delayed-LCVF to the multivariate model, the association of TPME was no longer significantly associated with poor baseline collateralization. Baseline collateral status is considered an important parameter in the evaluation and treatment of cerebral ischemia,\textsuperscript{57} and is linked to infarct volume,\textsuperscript{38} and functional outcomes.\textsuperscript{14,21,39} Leptomeningeal collaterals play a pivotal role in maintaining blood flow to brain regions distal to an arterial occlusion, thereby contributing to sustaining brain viability (by allowing survival of ischemic penumbra) and limiting the ischemic core size.\textsuperscript{39} In acute stroke settings, good collaterals at baseline are strongly associated with better clinical outcomes as it allows the survival of ischemic penumbra. On the contrary, failure of flow due to the presence of poor collaterals at baseline has been observed in some patients and was found to be linked to infarct growth.\textsuperscript{39} A number of plausible mechanisms behind poor collateral status or ‘collateral failure’ have been proposed including rise in...
intracranial pressure, venous steal, collateral vessel thrombosis, blood pressure fluctuations secondary to autonomic dysfunction, and reversed Robin Hood syndrome. We postulate that a select group of patients with poor collaterals may show presence of delayed-LCVF owing to the slowing of venous outflow in the late venous phase.

Presently, evaluation of collateral status is done qualitatively through visual examination and involves indirect assessment of the extent and rate of backfilling of pial arteries which are fed by collateral vessels to maintain blood flow. As such, a more 'direct' measure such as the absence or presence of delayed-LCVF pattern could potentially be used as a more reproducible method to assess collateral status.

**Evaluation of venous drainage using dCTA and identification of delayed-LCVF with MIP reconstruction of dCTA source images**

Conventional arterial angiography using DSA is considered the gold standard in angiographic evaluation. However, it is expensive, time-consuming, and involves more invasive procedures (cut down to the femoral artery, individualized selection of each vessel, etc.) compared to CTA. Hence, it is not routinely performed in the initial workup of majority of ischemic stroke patients. CTA and MRA are used in non-invasive assessment of collateralization. CTA offers a relatively low cost, wide availability, less time-consuming, and non-invasive (in comparison to DSA) alternative to patients. However, assessment of collaterals on conventional CTA is dependent on the image acquisition timing. This causes impaired assessment in setting of delayed collateral filling distal to the occluded artery. The development of whole-brain 4D-dCTA on MDCT scanners has made it possible to generate time-resolved angiograms of brain vasculature, in particular the collaterals, from skull base to the vertex. The dCTA harnesses the ability of MDCT scanners to acquire imaging at multiple time points. This technique has demonstrated improved assessment of collaterals by capturing optimal enhancement of collateral vessels, identification of the origin of the dominant collaterals, and quantifying the delay of maximum enhancement. Moreover, dCTA provides additional hemodynamic information (in contrast to conventional cerebral angiogram) by allowing time-resolved visualization of pial arterial filling in all vascular territories. See Supplementary Information II for a detailed account on applications of CTA in the emergent evaluation of stroke.

Our study shows that dCTA (obtained using 320-detector row 640-slice MDCT scanners) with appropriate reconstructions using an MIP algorithm can be used to investigate venous dynamics or various stages of downstream venous flow. Evaluation of MIP reconstructions of CTA images as a part of routine stroke imaging can provide additional insights into the venous dynamics, and identification of special drainage patterns such as asymmetric prominent cortical veins during the early and mid-venous phase and delayed-LCVF appearance in late venous phase.

**Limitations**

We acknowledge that this study has few limitations, and these data must be interpreted in the context of the study design. Although our study cohort was comparatively larger or at par to many similar studies focussing on cerebral veins, the number of patients was still small. However, we believe that the use of large cohort of consecutive acute ischemic stroke patients and our standardized treatment regimen for patients admitted to our comprehensive stroke centre would have arguably minimized this problem. The assessment of delay in maximized enhancement may have been influenced by additional occlusions distal to the M1 segment and variations in the prominences of M2, M3 and M4 trunks. The data may be influenced by the differences in the filling time of collaterals in different areas of the MCA territory. However, since we recorded the delay in maximized collateralization enhancement all the way through to M4 (tracing M2 and M3 along the way), this discrepancy would not have much of bearing on our final results. We acknowledge that we did not have data on the site of occlusion. We contemplate that the delayed LCVF may not be appreciated in patients with distal MCA occlusions. Therefore, we envisage to investigate this on a larger dataset of patients that are being collected currently – to find out if there exists significant difference between the prevalence of delayed-LCVF in major vessel proximal versus distal occlusion. Future prospective multicenter studies on even larger sample size are recommended for confirming our findings and for investigating the association of this new imaging finding (delayed-LCVF) with penumbra, short-term and long-term functional clinical outcomes.

**Conclusion**

In summary, we have presented evidence around the appearance of delayed cortical vein filling during the late venous phase, using the reconstruction of baseline dCTA images using MIP and other reformatted formats, in acute ischemic stroke patients. We found that the delayed-LCVF is independently associated with poor baseline collateral status. We also found that the morphological extent of baseline...
collateralization and delay of maximized collateral enhancement were linked with delayed-LCVF. Future prospective studies with a larger number of patients are needed to establish the role of delayed-LCVF in stroke prognostication. Our study also suggests that MIP reconstructions of dCTA source images are a valuable angiographic tool to evaluate arterial and venous anatomy. Evaluation of drainage patterns and flow dynamics associated with the downstream venous system may be useful in the prognostic management of acute stroke patients.

Funding
The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Hunter Medical Research Institute & University of Newcastle.

Declaration of conflicting interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors’ contributions
Contributors SB and CL conceived and designed the study. SB and CL collected and analysed the data. SB, CL and JRA contributed in the data analysis. SB, PS and CL drafted the article, and all authors (SB, CL, PS, AB, JRA, MN, and MP) contributed towards the patient recruitment, study design, data analysis, drafting and revision of the article.

Supplementary material
Supplementary material for this paper can be found at http://jcbfm.sagepub.com/content/by/supplemental-data

References


5.4. Supplementary Information I

**Supplementary Figures & Tables**

**SI Table 1.** Odds ratios (95% confidence intervals) of clinical variables examined for association with late-stage cortical vein filling.

**SI Table 2.** Sensitivity and specificity analyses of multivariate models of association with poor collateralization.

**SI Figure 1.** The Receiver–operator characteristic (ROC) curve for the reduced model (obtained using stepwise backward multivariate regression) of delayed-LCVF using both TPME and poor collateral status.

**Supplementary Video.** Maximum intensity projection (MIP) reconstructed temporal profile of cortical vein drainage on dynamic CTA (dCTA). Video file is available online: http://jcb.sagepub.com/content/early/2016/03/10/0271678X16637611/suppl/DC1
SI Table 1. Odds ratios (95% confidence intervals) of clinical variables examined for association with late-stage cortical vein filling. *p<0.05 as threshold for statistical significance.

| Variable, n=117 | OR (95% confidence interval) | P>|z|
|----------------|-------------------------------|------|
| Number of patients |                             |      |
| Male            | 0.78 (0.38-1.62)              | 0.504|
| Age, in years   | 1.00 (0.98-1.03)              | 0.856|
| Baseline NIHSS  | 1.02 (0.95-1.08)              | 0.595|
| NIHSS at 24 hours (n=116) | 1.01 (0.96-1.06) | 0.714|
| **Treatment factors** |                             |      |
| Thrombolysis    | 1.28 (0.49-3.31)              | 0.613|
| **Risk Factors** |                             |      |
| Hypertension    | 0.41 (0.13-1.29)              | 0.127|
| Diabetes        | 1.13 (0.51-2.51)              | 0.767|
| Dyslipidemia    | 0.94 (0.45-1.96)              | 0.878|
| Present Smoker  | 0.80 (0.35-1.80)              | 0.585|
| Past Smoker     | 1.25 (0.60-2.60)              | 0.544|
| AF              | 0.92 (0.44-1.91)              | 0.826|
| New AF          | 0.72 (0.33-1.56)              | 0.403|
| Depression      | 0.44 (0.11-1.78)              | 0.248|
| History of Stroke/TIA | 1.11 (0.45-2.73) | 0.814|
| Core (n=115)    | 1.00 (0.99-1.01)              | 0.450|
| Penumbra (n=115)| 1.00 (0.99-1.01)              | 0.897|

Abbreviations: AF= Atrial Fibrillation; TIA= Transient Ischemic Attack; LCVF=Late venous phase cortical vein filling
SI Table 2. Sensitivity and specificity analyses of multivariate models of association with poor collateralization.

<table>
<thead>
<tr>
<th></th>
<th>TPME</th>
<th>TPME and delayed-LCVF</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROC Area</td>
<td>0.77</td>
<td>0.85</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>76.56%</td>
<td>76.56%</td>
</tr>
<tr>
<td>Specificity</td>
<td>67.92%</td>
<td>84.91%</td>
</tr>
<tr>
<td>Positive predictive value (PDV)</td>
<td>74.24%</td>
<td>85.96%</td>
</tr>
<tr>
<td>Negative predictive value (NPV)</td>
<td>70.59%</td>
<td>75%</td>
</tr>
<tr>
<td>Overall rate of correct classification</td>
<td>72.65%</td>
<td>80.34%</td>
</tr>
<tr>
<td>BIC</td>
<td>153.78</td>
<td>134.69</td>
</tr>
<tr>
<td>AIC</td>
<td>142.73</td>
<td>120.88</td>
</tr>
<tr>
<td>Degrees of freedom (dof)</td>
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<td>5</td>
</tr>
<tr>
<td>Goodness of fit (Pearson χ²) test</td>
<td>0.1856</td>
<td>0.1684</td>
</tr>
<tr>
<td>Number of covariate patterns</td>
<td>116</td>
<td>116</td>
</tr>
</tbody>
</table>

Abbreviations: dof=degrees of freedom; AIC and BIC= Akaike and Bayesian information criteria; PPV= Positive predictive value; NPV= Negative predictive value; ROC: Receiver-operator characteristic curve; TPME=Time to peak of maximum arterial enhancement (in seconds); LCVF=Late venous phase cortical vein filling
Supplementary Figures

SI Figure 1. The Receiver–operator characteristic (ROC) curve for the reduced model (obtained using stepwise backward multivariate regression) of delayed-LCVF using both TPME and poor collateral status.

The area under the curve of approximately 0.91 indicated excellent discrimination for the model. ROC areas were computed using the area under the curve. The sensitivity and specificity of the reduced model were 83.93% and 90.16%, respectively. The model demonstrated the high overall rate of correct classification (87.18%).
5.5. SUPPLEMENTARY INFORMATION II

DISCUSSION

In his seminal work on the development of the human cranial venous system, Paget meticulously documented various venous stages and their relation to arteries [1158]. The cerebral venous circulation consists of superficial and deep systems, both of which drain into the internal jugular veins. The superficial system is comprised of sagittal sinuses, and three distinguished variants of cortical veins including Sylvian middle cerebral vein (SMCV), a superior anastomotic vein of Trolard and the inferior anastomotic vein of Labbé.[1159] SMCV drains the area around the Sylvian fissure. The temporal lobe is drained by the vein of Labbé that connects the veins along the Sylvian fissure, including the SMCV, with the transverse sinus; the vein of Trolard is the largest cortical vein that connects the veins along the Sylvian fissure with the superior sagittal sinus [1160].

Computed tomographic angiography (CTA) in Stroke

CTA has been used in the diagnosis and emergent evaluation (e.g., occlusion of the internal carotid artery bifurcation, extent of carotid stenosis, irregularities in arterial walls including presence/evidence of intimal thickening, calcification, ulceration and plaque formation [540, 1161-1163]) of a number of cardiovascular diseases including occlusive diseases, cerebral aneurysms [1164], planning for surgical interventions (including carotid endarterectomy (CEA) and percutaneous transluminal angioplasty (PTA)), and postoperative evaluation [539, 540]. CTA has also shown potential for
leptomeningeal intracranial collateral assessment [468-471], and for patient selection towards thrombolytic treatment eligibility [525, 527, 1165].
ASSOCIATION OF CORTICAL VEIN FILLING WITH CLOT LOCATION AND CLINICAL OUTCOMES IN ACUTE ISCHEMIC STROKE PATIENTS

CHAPTER 6

“We cannot solve our problems with the same thinking we used when we created them.”

- Albert Einstein
CHAPTER 6: ASSOCIATION OF CORTICAL VEIN FILLING WITH CLOT LOCATION AND CLINICAL OUTCOMES IN ACUTE ISCHEMIC STROKE PATIENTS

6.1. Introduction

Identification of patients who are most likely to benefit from reperfusion therapy using clinical and imaging markers is important in the quest for a more tailored approach to treatment in acute ischemic stroke (AIS). We recently reported a novel CT angiographic (CTA) finding on the presence of delayed cortical vein filling in late-venous phase in AIS patients [3]. Delayed late-phase cortical vein filling (delayed-LCVF) is characterised by late-venous phase opacification of cortical veins despite contrast clearance from contralateral cortical veins on four-dimensional (4D) dynamic time-resolved CTA (dCTA). We found that delayed-LCVF is independently associated with poor baseline collateral status. Currently, assessment of arterial leptomeningeal collateral status is indirect and performed qualitatively through visual examination of the extent and rate of backfilling of pial arteries that are fed by collateral vessels. Therefore, delayed-LCVF, a more ‘direct’ and reproducible measure, may prove to be very helpful to assess collateral status. There is growing interest in the role of leptomeningeal collaterals in AIS [2, 3, 466, 469-471, 700, 1152]. The presence of baseline arterial collaterals is emerging as an important parameter in the evaluation and treatment of cerebral ischemia,[1166] and is linked to infarct core volume [1167], and functional outcomes [470, 707, 1168]. Good collaterals are associated with good clinical outcomes; conversely, poor collaterals are linked to infarct growth [1152]. Studies on the role of cortical veins in stroke pathophysiology and prognosis are limited [3, 13, 16, 466, 707, 1169-1171].

Location and the volume of the thrombus are also important factors in prognostication of AIS [1172-1181]. Proximal, high volume clots have poor clinical outcomes, while, low-volume, distal thrombus is associated with good clinical outcomes. Moreover, the size of thrombus and the anatomical differentiation between a proximal and a distal occlusion also influences the effectiveness of intra-venous thrombolysis (IVT) with recombinant tissue plasminogen activator (rtPA) [1172-1180]. IVT is more efficient in the dissolution of distal clots in comparison to proximal ones. Therefore, further studies
on the association of thrombus location with delayed-LCVF, in AIS patients who received IVT, are important.

In this study, we prospectively studied the association of late stage cortical vein drainage in a group of AIS patients treated with IVT, with tissue at risk, clot location, and clinical outcome. The specific objectives of the study were:

(7) To study the association of delayed-LCVF with ischemic infarct core and tissue at risk.
(8) To study the association of delayed-LCVF with clot location.
(9) To investigate if delayed-LCVF is associated with clinical outcomes including reperfusion status at 24 hours, and 90-day functional outcome.

We hypothesise that patients with delayed-LCVF will have smaller penumbra, higher infarct core volumes, and worse outcomes at 90 days. We also hypothesise that the AIS patients with proximal occlusion (M1 proximal (M1P) or ICA) will demonstrate a higher proportion of delayed-LCVF patterns in comparison to the patients with distal occlusion (M1 distal (M1D) or M2 or M3). We discuss the implications of the location of the clot in evaluating the clinical outcomes post IVT in the AIS cohort.
6.2. Contributions

“As co-authors of the paper, Bhaskar S, Bivard A, Parsons M, Nilsson M, Attia JR, Stanwell P, Levi C. (2016) Association of Cortical Vein Filling with Clot Location and Clinical Outcomes in Acute Ischaemic Stroke Patients. *Nature Scientific Reports*, 6, 38525; doi: 10.1038/srep38525, we confirm that Sonu Bhaskar has made the following contributions:

75% conception and design of research

75% experimental procedures

75% analyses and interpretation of the findings

75% writing of the paper and critical appraisal of the content

Prof Christopher Levi

13/02/2017

Signed: Date:

A/Prof Peter Stanwell

17/02/2017

Signed: Date:

Prof John Attia

15/02/2017

Signed: Date:
6.3. PUBLICATION
Association of Cortical Vein Filling with Clot Location and Clinical Outcomes in Acute Ischaemic Stroke Patients

Sonu Bhaskar1,2, Andrew Bivard1, Peter Stanwell2, John R. Attia3, Mark Parsons1,2, Michael Nilsson2,4 & Christopher Levi1,2

Delay in cortical vein filling during the late-venous phase (delayed-LCVF) is characterized by opacification of cerebral veins despite contrast clearance from contralateral veins on dynamic computed tomography angiography (dCTA) in acute ischemic stroke (AIS) patients. The aim of the study was to investigate the associations of delayed-LCVF with clot location, reperfusion status at 24 hours, and 90-days functional outcome in AIS patients who received reperfusion therapy. A prospective cohort of AIS patients treated with intravenous thrombolysis was studied. Groupwise comparison, univariate, and multivariate regression analyses were used to study the association of delayed-LCVF with clot location and clinical outcomes. Of 93 patients (mean age = 72 ± 12 years) with hemispheric AIS included in the study, 46 (49%) demonstrated delayed-LCVF. Patients with delayed-LCVF demonstrated a significantly higher proportion of proximal occlusion (72% vs 13%, P < 0.0001), and poor reperfusion at 24 hours (41% vs 11%, P = 0.001). The proportion of poor functional outcome at 90 days was not significantly different (22/56 (48%) vs 17/61 (36%), P = 0.297). The appearance of delayed-LCVF on baseline dCTA may be a surrogate for large vessel occlusion, and an early marker for poor 24-hour angiographic reperfusion.

Identification of patients who are most likely to benefit from reperfusion therapy using clinical and imaging markers is important in the quest for a more tailored approach to treatment in acute ischemic stroke (AIS). We recently reported a novel CT angiographic (CTA) finding on the presence of delayed cortical vein filling in late-venous phase in AIS patients. Delayed late-phase cortical vein filling (delayed-LCVF) is characterised by late-venous phase opacification of cortical veins despite contrast clearance from contralateral cortical veins on four-dimensional (4D) dynamic time-resolved CTA (dCTA). We found that delayed-LCVF is independently associated with poor baseline collateral status. Currently, assessment of arterial leptomeningeal collateral status is indirect and performed qualitatively through visual examination of the extent and rate of backfilling of pial arteries that are fed by collateral vessels. Therefore, delayed-LCVF, a more direct and reproducible measure, may prove to be very helpful to assess collateral status. There is growing interest in the role of leptomeningeal collaterals in AIS. The presence of baseline arterial collaterals is emerging as an important parameter in the evaluation and treatment of cerebral ischemia, and is linked to infarct core volume, and functional outcomes. Good collaterals are associated with good clinical outcomes; conversely, poor collaterals are linked to infarct growth. Studies on the role of cortical veins in stroke pathophysiology and prognosis are limited.

Location and the volume of the thrombus are also important factors in prognostication of AIS. Proximal, high volume clots have poor clinical outcomes, while, low-volume, distal thrombus is associated with good clinical outcomes. Moreover, the size of thrombus and the anatomical differentiation between a proximal and a distal...
occlusion also influences the effectiveness of intra-venous thrombolysis (IVT) with recombinant tissue plasminogen activator (rtPA)\(^{18-26}\). IVT is more efficient in the dissolution of distal clots in comparison to proximal ones. Therefore, further studies on the association of thrombus location with delayed-LCVF, in AIS patients who received IVT, are important.

In this study, we prospectively studied the association of late stage cortical vein drainage in a group of AIS patients treated with IVT, with tissue at risk, clot location, and clinical outcome. The specific objectives of the study were:

(1) To study the association of delayed-LCVF with ischemic infarct core and tissue at risk.
(2) To study the association of delayed-LCVF with clot location.
(3) To investigate if delayed-LCVF is associated with clinical outcomes including reperfusion status at 24 hours, and 90-day functional outcome.

We hypothesise that patients with delayed-LCVF will have smaller penumbra, higher infarct core volumes, and worse outcomes at 90 days. We also hypothesise that the AIS patients with proximal occlusion (M1 proximal (M1P) or ICA) will demonstrate a higher proportion of delayed-LCVF patterns in comparison to the patients with distal occlusion (M1 distal (M1D) or M2 or M3). We discuss the implications of the location of the clot in evaluating the clinical outcomes post IVT in the AIS cohort.

Results

Case Presentation. A case study showing the acute CTP and dCTA at baseline is shown in Fig. 1. Figure 2 depicts the follow-up CT & MR imaging findings at 24 hours.

Baseline characteristics. Of the 154 patients, 93 (60.4%) patients (mean age = 71.6 ± 12.4 years; the number of females = 49 (52.7%)) with acute anterior circulation vessel occlusion who received IVT met the inclusion criteria (Table 1). Figure 3 shows the distribution of patients for different clot locations, reperfusion status at 24 hours and functional outcome at 90 days. Out of 93 patients with hemispheric ischaemic stroke included in the study, 46 (49.5%) patients showed delayed-LCVF. The median NIHSS score at admission and 24 hours were 14 (IQR = 8) and 7 (IQR = 9), respectively. Fifty-four (58%) patients demonstrated good functional outcome (mRS 0–2) at 90 days and 52.7% of patients demonstrated major reperfusion at 24 hours. The average OTT for this AIS cohort was 162.6 ± 83.9 minutes. The summary of clinical outcomes for all patients is shown in Table 2.

Delayed-LCVF vs No delayed-LCVF. There were no significant differences between the age, sex, NIHSS at admission and at 24 hours, and other clinical risk factors among patients with, and without, delayed LCVF. Patients with delayed-LCVF demonstrated a significant association with poor baseline collaterals (85% vs 21%, P = <0.001; OR = 20.6; 95% CI = [7, 60], P = <0.001), and longer time to peak of maximum arterial enhancement (TPME) (median TPME (in seconds) = 8 vs 6, P = <0.001; OR = 3.3; 95% CI = [2, 5.4]; P = <0.001) (Table 1). There were no significant differences in onset to treatment time (OTT) between the delayed vs non-delayed LCVF groups (P = 0.215). We also observed no significant association of OTT with 90 days’ functional outcome (P = 0.2090) which is in agreement with previous study.

Occlusion of the internal carotid artery (ICA) (32.6% vs 6.4%, P = 0.002), and M1 proximal (M1P) (29% vs 6.4%, P = <0.001) was significantly higher in the delayed-LCVF group in comparison to non-delayed-LCVF. Conversely, distal M1 (M1D) (15% vs 36%, P = 0.03), and M2 and/or M3 (13% vs 51%, P = <0.001) occlusions were significantly lower in delayed-LCVF group. When pooled, proximal occlusion (ICA and/or M1P) was commonly seen in delayed-LCVF (72% vs 13%, P = <0.001) group, and conversely, occurrence of distal occlusion (M1D and/or M2 and/or M3) was significantly lower in delayed-LCVF group (28% vs 87%, P = <0.001), versus the non-delayed-LCVF group.

Association of delayed-LCVF with infarct core and tissue at risk. Acute core volumes were not significantly different between delayed-LCVF vs non-delayed-LCVF groups (18.85 mL vs 13.15 mL, P = 0.731) (Table 1). Univariate analysis revealed no significant association of the acute core volume and presence of delayed-LCVF (OR = 1; 95% CI = [0.99, 1.02]; P = 0.494). In terms of penumbra volume, no significant difference was found between the two groups, delayed-LCVF vs non-delayed-LCVF (61.05 mL vs 64.45 mL, P = 0.574). Moreover, delayed-LCVF was not associated with penumbra volume (OR = 1.01; 95% CI = [0.99, 1.01]; P = 0.732). No significant association was found between the delayed-LCVF and 24-hour core volume (18 mL vs 12.5 mL, P = 0.37; OR = 1, 95% CI = [1, 1.01], P = 0.247) (Table 2). Moreover, delayed-LCVF was also not associated with penumbra salvage (24 mL vs 43 mL, P = 0.31; OR = 1, 95% CI = [0.99, 1.1], P = 0.272).

Associations with clot location. Results of univariate logistic regression analysis for association with proximal clot is shown in Supplementary Table 2. Independent variables (P ≤ 0.1) and other important covariates (NIHSS at admission) were used for stepwise backward multivariate logistic regression analysis to study the association with incidence of the proximal clot (see Table 3; Model 3A). Finally, TPME, dyslipidemia, and delayed-LCVF were retained in the final multivariate logistic regression model and treated as potential confounders (Table 3; Model 3C). Higher rates of delayed-LCVF (OR = 106.62; 95% CI = [15, 756]; P = < 0.0001) and dyslipidemia (OR = 5.8; 95% OR = [1.6, 21]; P = 0.007) were positively associated with incidence of proximal clot. Interestingly, each unit increase in TPME was negatively associated with presence of proximal clot (OR = 0.56; 95% CI = [0.35, 0.9]; P = 0.02). The model showed good discrimination ability with an area under the receiver operating characteristic (ROC) curve of 0.89 (sensitivity = 82%, specificity = 81.5%). We also compared the reduced models with and without the inclusion of delayed-LCVF (Model 3C (reduced
model with delayed-LCVF) vs Model 3B (reduced model without delayed-LCVF)). We found that the addition of delayed-LCVF significantly increased the discrimination accuracy of the model (BICModel3C vs BICModel3B = 95 vs 131; ROCModel3C vs ROCModel3B = 0.89 vs 0.66; SensitivityModel3C vs SensitivityModel3B = 82% vs 36%; SpecificityModel3C vs SpecificityModel3B = 81.5% vs 81.5%; PPVModel3C vs PPVModel3B = 76.19% vs 58.3%).

Delayed-LCVF association with reperfusion status at 24 hours, and functional outcome at 90 days. Patients with delayed-LCVF demonstrated a significantly higher proportion of poor angiographic reperfusion at 24 hours (68% vs 31.8%, P = 0.001) (Table 2). Moreover, bivariate logistic regression analysis also revealed a significant association of delayed-LCVF with overall angiographic reperfusion at 24 hours (P = 0.001). Delayed-LCVF was positively associated with poor reperfusion at 24 hours (OR = 4.4; 95% CI = [1.8, 10.6]; P = 0.001). Independent variables (with P ≤ 0.1; age, NIHSS at admission, acute core volume, collateral status, delayed-LCVF, clot location and hypertension) (see Supplementary Table 3) were used for stepwise backward multivariate logistic regression analysis to study the association with poor angiographic reperfusion (see Table 4; Model 4A). In the reduced model (Table 4; Model 4C), delayed-LCVF, clot location, and baseline core volume were retained. Higher rates of delayed-LCVF (OR = 3.7; 95% CI = [1.2, 11.28]; P = 0.021), and increasing acute core volume (OR = 1.02; 95% OR = [1, 1.04]; P = 0.021) were significantly associated with poor angiographic reperfusion at 24 hours. Comparison between the reduced models with and without inclusion of delayed-LCVF (Model 4C (reduced model with delayed-LCVF) vs Model 4B (reduced model without delayed-LCVF)) revealed that the addition of delayed-LCVF to the model revealed no added advantage on discriminative accuracy: BIC (BICModel4C vs BICModel4B = 127 vs 128), sensitivity (SensitivityModel4C vs SensitivityModel4B = 70.45% vs 70.45%), or discrimination accuracy (ROCModel4C vs ROCModel4B = 0.74 vs 0.70; SpecificityModel4C vs SpecificityModel4B = 68.75% vs 64.58%);

Figure 1. Case Study 1. Acute stroke imaging. Top Panel: Axial CTP demonstrated the presence of an occlusion in the left MCA. The red arrow points to the presence of a left MCA occlusion. CTP (coronal 5 mm average registered) shows the presence of penumbra and demonstrated region of reduced cerebral blood flow (CBF) and blood volume (CBV), as well as increased mean transit time (MTT) and time to peak (TTP) in the left MCA cortex. 2D MIP spiral dynamic CT angiography (CTA) images formatted in coronal (middle panel) (a–e), and sagittal (bottom panel) planes (f–j); CTA Right-Left view and CTA posteroanterior view. The Early phase is characterized by early filling of venous sinuses (a), followed by mid-venous phase (b–d). Late venous phase is depicted in (e) (blue-arrow). The presence of left-sided late-venous phase opacification of cortical veins (blue arrow) on left side despite contrast clearance from contralateral (right side) cortical veins can be seen on dynamic CTA image, (e,j). Background: A 72-year-old female with a history of hypertension, diabetes, hyperlipidaemia, and atrial fibrillation presented with right-sided facial droop and right-sided hemiparesis with NIHSS score of 21 on admission. Acute CTP demonstrated acute right middle cerebral artery (MCA) ischemia with evidence of a large penumbra. The patient demonstrated delayed-LCVF on baseline dCTA. The patient received intravenous rt-PA therapy at 90 minutes post stroke onset.
NIHSS a thrombus location and clinical outcome.13,17,28–31. vs 116), sensitvROCModel5C vs ROCModel5B changes in the left MCA territory. Long-term prognosis of the patient was poor (mRS the bifurcation despite recanalization. There is evidence of infarction involving the basal ganglia and some patchy recanalization/reperfusion. On 24 hours CTA, poor flow into the branches was observed suggestive of stenosis at the bifurcation despite recanalization. There is evidence of infarction involving the basal ganglia and some patchy changes in the left MCA territory. Long-term prognosis of the patient was poor (mRS = 4 at 90 days).

Figure 2. Case Study 1. Follow-up imaging at 24 hours. Non-contrast CT (NCCT) at 24 hours shows low attenuation within the head of the left caudate nucleus, anterior limb of the left internal capsule and lentiform nucleus, as well as patchy low attenuation in the white matter in the corona radiata and temporal lobe, consistent with the known left middle cerebral artery (MCA) infarct. No evidence of haemorrhagic transformation of areas of parenchymal abnormality was seen. DWI-MRI showed patchy infarcts in left MCA territory. T2-FLAIR also confirmed the presence of patchy infarcts in the left hemisphere. Magnetic resonance angiography (MRA) shows that the left MCA has reanalysed however, there remains some poor flow into the branches with the suggestion of some stenosis at the bifurcation. 24 hour CTA showed evidence of partial recanalization. Poor flow at the bifurcation possibly due to stenosis. Background: Despite thrombolysis, the patient showed only partial recanalization/reperfusion. On 24 hour CTA, poor flow into the branches was observed suggestive of stenosis at the bifurcation despite recanalization. There is evidence of infarction involving the basal ganglia and some patchy changes in the left MCA territory. Long-term prognosis of the patient was poor (mRS = 4 at 90 days).

PPV Model4C vs PPV Model4B = 67.4% vs 64.58%) (Table 4). In the model without delayed-LCVF (Model 4B), proximal clot (OR = 2.9; 95% CI = [1.2, 7.17]; P = 0.017) and acute core volume (OR = 1.02; 95% CI = [1, 1.04]; P = 0.015) were significantly associated with poor angiographic reperfusion status at 24 hours.

Delayed-LCVF was not significantly associated with penumbral salvage (Median Penumbral Salvage, in mL = 24.15 vs 43.45, P = 0.31; median penumbral salvage, in percentage = 52.9% vs 84.2%, P = 0.1) or functional outcome at 90 days (47.8% vs 36%, P = 0.297) (Table 2). To study the association with functional outcome at 90 days using backward stepwise multivariate regression analyses, independent variables (with P ≤ 0.1; NIHSS at admission, baseline core volume, penumbra, dyslipidaemia, and reperfusion status at 24 hours (see Supplementary Table 1) and other important covariates (clot location, delayed-LCVF) were included in the final multivariate regression model (Table 5; Model 5A). In the reduced multivariate regression model (Model 5C), delayed-LCVF was not significantly associated with functional outcome at 90 days, when adjusted for NIHSS at admission, acute core volume, clot location, and 24-hour reperfusion status (Table 3). Increasing acute core volume and reperfusion status at 24 hours were significantly associated with poor functional outcome at 90 days. Comparison of model characteristics between the two models, with and without delayed-LCVF, revealed addition of delayed-LCVF significantly improved the discriminative accuracy; BIC (BIC Model4C vs BIC Model4B = 92.5 vs 116), sensitivity (Sensitivity Model4C vs Sensitivity Model4B = 82% vs 69%), or discrimination accuracy (ROC Model4C vs ROC Model4B = 0.93 vs 0.80; Specificity Model4C vs Specificity Model4B = 88.7% vs 79.25%; PPV Model4C vs PPV Model4B = 84.21% vs 71%) (Table 5).

Discussion
In this study, we sought to investigate associations of novel cortical vein filling pattern, observed during the late venous phase on time-resolution dCTA, with the acute core, tissue at risk, clot location, reperfusion status at 24 hours, and long-term functional outcomes in a cohort of AIS patients treated with IVT. In the current study, we found that the patients with proximal (thrombus in ICA and/or M1P) occlusion are at significantly higher risk of showing delayed-LCVF. We also noted a strong association of delayed-LCVF with poor reperfusion status at 24 hours. We could not demonstrate that the impact of delayed-LCVF on these acute outcomes translated into poor functional outcomes at 90 days, but this may be due to our small sample size and the fact that functional outcome is quite distal in the causal chain we are investigating. The inclusion of delayed-LCVF to the multivariate model significantly improved the predictive accuracy of poor functional outcome. We found no association of delayed-LCVF with ischemic infarct core or tissue at risk. Previously, we reported the presence of delayed cortical vein filling pattern in late-venous phase on dCTA in a cohort of AIS patients, where delayed-LCVF was found to be independently associated with poor baseline arterial collaterals and delay in maximised collateral enhancement.1 There are limited studies on the role of cortical veins in stroke pathophysiology, and their associations with thrombus location and clinical outcome.13,17,28–31

Our findings indicate that delayed-LCVF was significantly more common in patients with M1P and/or ICA occlusion. Delayed-LCVF showed significant improvement in discriminative accuracy when it was added to the multivariate regression model. As such, the appearance of delayed-LCVF on dCTA is a surrogate for proximal large vessel occlusion which is known to be associated with poor outcome. Previous studies have reported that AIS patients with thrombus in M1D, M2, and M3 segments are more likely to undergo recanalization than those with M1P and ICA occlusions.18,27. Large vessel occlusions are less likely to be recanalised after IVT and are more likely to have poor clinical outcomes. The fact that delayed-LCVF is strongly associated with proximal thrombus may be used as an important parameter towards stroke prognostication and selection of patients for IVT. We postulate that proximal large vessel occlusions may lead to delayed-late cortical vein filling.
In this study, we found that patients who showed delayed-LCVF on baseline dCTA demonstrated the significantly higher rate of poor angiographic reperfusion at 24 hours. This is clinically relevant suggesting that patients with the delayed-LCVF pattern on baseline dCTA will be poorly reperfused despite IVT. However, the addition of delayed-LCVF to multivariate regression model didn’t improve the predictive accuracy of the model over and above clot location. Our results show that delayed-LCVF is a statistically significant prognostic indicator of early angiographic reperfusion (at 24 hours). From the current literature, we know that reperfusion status is a significant predictor of long-term (90 days) outcome\(^{22,23}\). In a multivariate logistic regression analysis, delayed-LCVF was not a significant covariate for predicting 90 days’ functional outcome. Although, our point estimate supports an effect on 90 days mRS (OR = 1.6), we do not have sufficient power to demonstrate this at a statistically significant level. We hypothesize that patients with the delayed-LCVF pattern on baseline dCTA may show an unfavourable trajectory. Interestingly, the addition of delayed-LCVF significantly improved the predictive accuracy of functional outcome at 90 days. The presence of delayed-LCVF may aid in identifying patients at risk of 24-hour poor angiographic reperfusion. Other studies have also shown significant association of cerebral-venous flow with prognosis in stroke in both animals and humans\(^{13,17,34}\). Interestingly, animal studies focussing primarily on early and mid-venous phase have shown that the presence of cortical vein filling after ischaemic stroke was associated with decreased severity of hemiparesis and lower infarct volumes. This led to the hypothesis that the cortical venous flow may produce favourable outcomes as it would be more commonly prevalent in strokes with good collaterals\(^{34}\). Another study on humans also found that the cortical venous drainage was associated with good clinical outcomes\(^{17}\).

Imaging biomarkers towards the identification of patients who might benefit from early reperfusion therapy and guiding early intervention options to limit or even freeze infarct progression is crucial for strategies in acute stroke treatment\(^{35,36}\). The advent of cutting-edge next generation 320-detector row 640-slice multi-detector
CT (MDCT) scanners have facilitated the acquisition of whole-brain, sub-second, and volumetric acquisition of 4D-dCTA data. CTP/CTA is not an invasive procedure compared to digital subtraction angiography (DSA), and is routinely obtained during clinical care of stroke patients, has proven to be of added clinical utility in the early evaluation of stroke, facilitating precise localization of site of occlusion, and identification of hypoperfused territory at risk of infarction. The CTP was acquired simultaneously with the CTA with the use of same contrast bolus. Dynamic CTA allows evaluation of intracranial vasculature and visualisation of contrast flow from its arterial to venous phases. Using appropriate reconstructions of dCTA using MIP algorithm, we investigated various stages of venous drainage and downstream venous dynamics; including the assessment of delayed-LCVF appearance in late venous phase. Assessment of impaired cortical venous drainage may provide valuable information over and above arterial collateral assessment, and the presence of delayed-LCVF could have a role in making informed decisions on patient management and prognosis.

We understand that our study has several limitations, including small sample size and the variability in the cortical venous structures. Since the publication of MR CLEAN, REVASCAT, EXTEND-IA, ESCAPE, and SWIFT PRIME, the standard practice now includes intravenous thrombolysis when possible, complementing thrombectomy. However, endovascular treatment was not available at our centre at the time of the study. We tried to account for small sample size by using the Wilcoxon-Rank test, which would be conservative in this case. We acknowledge that additional occlusions distal to the M1 segment and variations in the prominences of M2, M3, and M4 trunks may have an impact on the assessment of delay in maximised enhancement. Moreover, it may also be influenced by the differences in the filling time of collaterals in different areas of the MCA territory.

Table 2. List of short term and long term clinical outcomes stratified by late-stage cortical vein filling. Figures in parentheses are percentages and those in square brackets are interquartile ranges. *P < 0.05 as the threshold for statistical significance. Comparison between two groups for categorical and continuous/ordinal variables was made using 2-sided Fisher’s exact test and Wilcoxon rank-sum (Mann-Whitney) test, respectively. Abbreviations: mRS = Modified Rankin Score. P values shown are uncorrected for multiple comparisons.

| & All patients & Delayed-LCVF & Non-delayed-LCVF & P & OR [95% CI] & P > [z] |
|---|---|---|---|---|---|---|
| NIHSS at 24 hours; Median [IQR] & (n = 93) & (n = 46) & (n = 47) & & & |
| 24-Hour Core Volume (in mL); median [IQR] & 15.8 [32.4] & 18.05 [37.7] & 12.5 [32.9] & 0.3727 & 1 [1, 1.01] & 0.247 |
| Penumbral Salvage (in mL); median [IQR] & 39.55 [79.25] & 24.15 [84.4] & 43.45 [77.4] & 0.3100 & 1 [0.99, 1] & 0.272 |
| % Penumbral Salvage; Median [IQR] & 65.55 [91.8] & 52.92 [141.18] & 84.24 [73.8] & 0.1068 & 1 [0.997, 1] & 0.189 |
| Reperfusion Status at 24 hours & & & & & & |
| Major Reperfusion & 49 (52.69) & 16 (34.78) & 33 (70.21) & 0.001* & 0.48 [0.17, 1.35] & 0.167 |
| Partial Reperfusion & 22 (23.66) & 19 (41.3) & 3 (6.38) & <0.001* & 6.33 [1.14, 27.73] & 0.014* |
| No Reperfusion & 22 (23.66) & 11 (23.91) & 11 (23.40) & 1.0000 & 1 [ref] & |
| Reperfusion Status at 24 hours & & & & & & |
| Major Reperfusion & 49 (52.7) & 16 (32.6) & 33 (67.4) & <0.001* & 0.001* & |
| Partial Reperfusion & 22 (23.6) & 19 (41.3) & 3 (6.38) & <0.001* & 6.33 [1.44, 27.73] & 0.014* |
| No Reperfusion & 22 (23.7) & 11 (23.91) & 11 (23.40) & 1.0000 & 1 [ref] & |
| mRS at 90 days; Median [IQR] & 44 (47.3) & 30 (68.2) & 14 (31.8) & 0.001* & 4.42 [1.85, 10.56] & 0.001* |
| Good (mRS = 0–2) & 54 (58.06) & 24 (52.17) & 30 (63.83) & 0.297 & 0.62 [0.27, 1.42] & 0.256 |
| Bad (mRS = 3–6) & 39 (41.94) & 22 (47.83) & 17 (36.17) & 0.297 & 1.61 [0.71, 3.71] & 0.256 |

Figure 3. Clinical outcome (reperfusion status at 24 hours, and 3-month modified Rankin score (mRS)) and clot location for presence and absence of delayed-LCVF. Good clinical outcome was defined as mRS 0–2 at 3 months.
To conclude, in this study, we sought to study the associations of delayed-LCVF with core volume, tissue at risk, clot location, and clinical outcome (vis a vis reperfusion at 24 hours, functional outcome (in terms of modified Rankin score (mRS)) at 90 days) in a prospective cohort of AIS patients who received intravenous thrombolytic therapy. Endovascular procedures or mechanical thrombectomy was not available at our centre at the time of the study. Delayed-LCVF patterns were more commonly seen in proximal thrombus or large vessel occlusion. It may also be useful in identifying patients at risk of poor angiographic reperfusion at 24 hours. Moreover, given the propensity of proximal thrombus towards poor clinical outcome after IVT, and significant association of delayed-LCVF with both proximal thrombus and poor reperfusion at 24 hours, we postulate that the AIS patients with delayed-LCVF may progress unfavourably, and therefore alternate revascularisation strategies may be considered. We also found that addition of delayed-LCVF significantly improves predictive accuracy of 90 days' functional outcome. However, in the present cohort, we acknowledge that delayed-LCVF was not found to be a determinant factor in predicting functional outcome at 90 days. In light of the paucity of literature on the cerebral venous system and their role in stroke prognostication, we believe this study may be of clinical relevance towards understanding the role of cerebro-venous system, in particular, cortical veins, in the prognosis of AIS patients. We propose that delayed-LCVF is a marker that will allow clinicians to study may be of clinical relevance towards understanding the role of cerebro-venous system, in particular, cortical veins, in the prognosis of AIS patients. We propose that delayed-LCVF is a marker that will allow clinicians to

Materials and Methods

Study design and patient selection. Consecutive acute ischaemic stroke patients admitted to the comprehensive stroke unit, Department of Neurology at our academic medical centre were prospectively studied provided they satisfied the following inclusion criteria: (a) aged 18 and above years, (b) acute anterior circulation vessel occlusion followed by IVT, (c) hemispheric stroke, and (d) dCTA data available at baseline and 24 hours. Patients without identifiable thrombus on the baseline dCTA were excluded. Patients received 0.9 mg/kg intravenous recombinant tissue plasminogen activator (rtPA). Baseline clinical characteristics included age, sex, and clinical risk factors (hypertension, diabetes, dyslipidaemia, history of smoking (past/present), atrial fibrillation
(AF), depression and history of stroke and/or transient ischemic attack (TIA)). Clinical data were procured from the patient records. National Institutes of Health Stroke Scale (NIHSS) scores at the time of initiation of the rtPA (time to tPA) or onset to treatment (OTT) was also recorded. Management of patients was in accordance with local guidelines and as per the discretion of the treating stroke physician. This study was approved by the Hunter New England Human Research Ethics Committee (HNEHREC, Newcastle, NSW) in accordance with the National Statement on Ethical Conduct in Human Research 2007. All methods were carried out in accordance with the approved guidelines. Informed consent was obtained from the patient in accordance with the Declaration of Helsinki.

All the patients underwent non-contrast CT (NCCT), CT Perfusion (CTP) and CT angiography (CTA) at baseline and follow-up (24 h) NCCT, CTA, and magnetic resonance imaging (MRI), following our routine stroke imaging protocol. Volumes of the acute perfusion lesion (relative delay time (DT) ≥ 3 seconds) and acute infarct core (relative CBF ≤ 30%) were calculated using previously validated thresholds. Penumbra volume was defined as the volume of the perfusion lesion (DT threshold ≥ 3 seconds) minus the volume of the infarct core (relative CBF threshold < 30% within the DT ≥ 3 sec lesion). The threshold of DT ≥ 3 seconds was based on previous studies.

Maximum intensity projection (MIP) and multiplanar reformat (MPR) reconstructions in coronal and sagittal planes of baseline axial CTA were obtained on the imaging workstation (Vitrea® fX, Version 1.0, Vital Images, Minnetonka, MN, USA). These images were reviewed by consensus by two experienced readers (SB & CL). Three-dimensional volume rendering was applied to obtain the optimized spatial orientation and precise localization of ischemic lesion. The determination of the location of the clot was based on the most proximal position of the occlusion. Clot location was divided into two groups: (a) proximal clot: any thrombus/occlusion in the M1 proximal (M1P) or ICA, and (b) distal clot: any thrombus/occlusion in M1 distal (M1D), M2 or M3. The exact location of the thrombus or clot M1 proximal (M1P), M1 distal (M1D), M2, M3, or internal carotid artery (ICA)) was determined. ICA occlusion was determined based on the presence of a clot in ICA terminus. The M1 segment of the MCA was divided into two parts of equal length, namely the proximal (M1P) and the distal half

Table 4. Stepwise backwards multivariate regression modelling for associations with 24 hours’ poor angiographic reperfusion. P < 0.1 for enter and stay criteria. The two multivariate models differed in terms of the particular covariates/factors included. *P < 0.05 as the threshold for statistical significance. Abbreviations: dof = degrees of freedom; OR = Odds Ratio; AIC and BIC = Akaike and Bayesian information criteria; PPV = Positive predictive value; NPV = Negative predictive value; ROC = Receiver-operator characteristic curve; CI = Confidence interval; LCVF = Late venous phase cortical vein filling; NIHSS = National Institute of Health Stroke Scale Score.
Model 5A

Final model: First Step

<table>
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<tr>
<th>Model characteristics: n = 92; dof = 8; AIC = 109.27; BIC = 129.44; Sensitivity = 71.79%; Specificity = 79.25%; PPV = 71.79%; NPV = 79.25%; Overall rate of classification = 76.09%; ROC area = 0.8036; Pseudo R2 = 0.2562</th>
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<tr>
<td>Delayed-LCVF</td>
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<tr>
<td>NIHSS at admission</td>
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<td>Acute Core volume (in mL)</td>
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<td>Penumbra volume (in mL)</td>
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Table 5. Stepwise backwards multivariate regression modelling for associations with 90 days’ bad functional outcome. *Used as reference category. P < 0.1 for enter and stay criteria. The two multivariate models differed in terms of the particular covariates/factors included. *P < 0.05 as the threshold for statistical significance. Abbreviations: dof = degrees of freedom; OR = Odds Ratio; AIC and BIC = Akaike and Bayesian information criteria; PPV = Positive predictive value; NPV = Negative predictive value; ROC: Receiver-operator characteristic curve; CI = Confidence interval; TPME = Time to peak of maximum arterial enhancement (in seconds); LCVF = Late venous phase cortical vein filling; NIHSS = National Institute of Health Stroke Scale Score.

M1 MCA was defined as a vessel extending from the ICA bifurcation to the origin of the first major branch in the Sylvian sulcus. Delayed-LCVF was identified by late venous phase opacification of cortical veins despite contrast clearance from contralateral cortical veins on maximum intensity projection (MIP) images from dCTA. The time to peak of maximum arterial enhancement (TPME) was also recorded. Collateral grading was done to assess the morphological status using dCTA data based on the degree of reconstitution of the MCA up to the distal end of its occlusion. Collateral grading was classified as ‘good’, ‘reduced’ or ‘poor’ using the Miteff scale. Good collateral grading was assigned if the entire MCA distal to the occluded segment was reconstituted, i.e., if collaterals reconstituted vessels in the: (a) distal portion of the occluded vessel, or (b) proximal portion of the segment adjacent to the occluded vessel (e.g., if there was proximal M1 occlusion, the distal M1 or proximal M2 segments reconstituted). Poor collateral grading was assigned if the reconstitution of the distal MCA was only partial, i.e., if collaterals reconstituted vessels in the: (a) distal portion of the segment adjacent to the occluded vessel, or (b) two segments distal to the occluded vessel, or (c) little or no significant reconstitution of the territory of the occluded vessel.
**Outcome measures.** The modified Rankin Scale (mRS) was used to assess clinical outcome in terms of functional status at 3 months. Patient outcomes were dichotomized into good (mRS 0–2) versus poor/bad (mRS 3–6). Angiographic assessment of the degree of reperfusion was done by an independent blinded reviewer on a repeat CTA acquired at 24 hours using modified thrombolysis in cerebral infarction (mTICI) score. An mTICI grade of 2a, defined as tissue reperfusion in <50% of the occluded artery territory, was identified as partial reperfusion. Major reperfusion corresponded to tissue reperfusion in ≥50% of the occluded artery territory with grades of 2b or 3 on the mTICI scale. All patients with partial or nil angiographic reperfusion at 24 hours were lumped together into “poor reperfusion” category. Penumbral salvage was defined as the difference between the acute CTP lesion volume (PWI lesion) and the 24-hour DWI lesion volume. We identified penumbral salvage lumped together into “poor reperfusion” category. Penumbral salvage was defined as the difference between the acute CTP lesion volume (PWI lesion) and the 24-hour DWI lesion volume. We identified penumbral salvage in patients where 24-hour DWI lesion volume was smaller than the acute perfusion lesion volume. Percentage of penumbral salvage was defined as (penumbral salvage volume/penumbral volume) × 100.

**Statistical analysis.** All the statistical analyses were performed using STATA (Version 10, 2001; College Station, TX, USA). Numerical values given are the means ± standard deviation or medians (interquartile range) for age, core and penumbral volumes, mRS scores, NIHSS at admission, NIHSS at 24 hours, and change in NIHSS scores as appropriate. For ordinal or continuous data, Mann-Whitney (Wilcoxon rank-sum) test was used. Nominal data were analysed with the Pearson's chi-squared (χ²) and the two-tailed Fisher exact test. Groupwise comparison was made between the patients with and without delayed-LCFV. Group differences were considered significant at values of P ≤ 0.05. To test the independent association of significant variables with delayed-LCFV, logistic regression models were fitted. Baseline infarct lesion volume and penumbral volume was dichotomized into small (≤ 25 mL) or large (>25 mL) pertinent to the findings on 25 mL threshold of core volume that accurately predicted the presence of penumbra (tissue at risk), response to thrombolysis, and excellent outcome. A stepwise backwards multivariate logistic regression analyses was used to study the association of delayed-LCFV with clot location, 24-hour angiographic reperfusion status, and 90 days functional outcome. Independent variables with P ≤ 0.1 (on univariate regression) and other important clinical covariates were included in the multivariate logistic regression model. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and the overall rate of correct classification for the multivariate models were estimated. Finally, the receiver operating characteristic (ROC) curve for the regression model was plotted, and the area under the curve was computed to evaluate the discriminative ability.

**References**


**Acknowledgements**

The authors would like to acknowledge the financial support from Hunter Medical Research Institute (HMRI), Hunter New England Health (HNE Health, NSW) and University of Newcastle. We would also like to thank the patients and carers, and the physicians, administrators, radiographers, stroke clinical audit and programming staff who contributed to the collection of data. We are especially grateful for administrative support from Louise-Anne Jordan, Malcolm Evans, Michelle K. Wyborn and Kristy Morris.

**Author Contributions**

Contributors S.B. and C.L. conceived and designed the study. S.B., M.P., A.B., and C.L. collected the data. S.B., J.A., P.S., and C.L. analysed the data. S.B. and C.L. drafted the article and all authors contributed to its revision.

**Additional Information**

**Supplementary information** accompanies this paper at http://www.nature.com/srep

**Competing financial interests:** The authors declare no competing financial interests.

**How to cite this article:** Bhaskar, S. et al. Association of Cortical Vein Filling with Clot Location and Clinical Outcomes in Acute Ischaemic Stroke Patients. *Sci. Rep.* **6**, 38525; doi: 10.1038/srep38525 (2016).

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### 6.4. SUPPLEMENTARY INFORMATION (SI)

**Supplementary Table 1.** Demographic and baseline characteristics by the outcome (mRS) at 3 months after intravenous thrombolysis. Odds ratio (OR) were obtained by bivariate logistic regression analysis for association with good functional outcome (mRS=0-2).

<p>| Characteristics                        | Good Outcome; N (%)=54 (58.06) | Bad outcome; N (%)=39 (41.94) | P       | OR (95% CI)          | P&gt;|z|   |
|----------------------------------------|---------------------------------|--------------------------------|---------|----------------------|-------|
| Age; Mean±SD                           | 70±18                           | 76±14                          | 0.118   | 0.97 [0.94, 1.01]    | 0.117 |
| NIHSS at admission; Median [IQR]       | 12 [8]                          | 15 [8]                         | 0.057   | 0.92 [0.86, 1.00]    | 0.039*|
| NIHSS at 24 hours; Median [IQR]        | 4 [4]                           | 13 [9]                         | &lt;0.001  | 0.73 [0.64, 0.83]    | &lt;0.001*|
| OTT (in mins); Mean±SD                 | 152.28±74.71                    | 176.77±94.29                   | 0.2090  | 1 [0.99, 1]          | 0.172 |
| Acute Core Volume; Median [IQR]        | 10 [20.2]                       | 20.4 [55.5]                    | 0.003*  | 0.97 [0.95, 0.99]    | 0.004*|
| Penumbra; Median [IQR]                 | 51.9 [86.2]                     | 71.8 [60.3]                    | 0.081   | 0.99 [0.98, 1]       | 0.086 |
| TPME; Median [IQR]                     | 8 [2]                           | 8 [2]                          | 0.847   | 1.08 [0.84, 1.38]    | 0.567 |
| Delayed-LCVF                           | 24 (44.44)                      | 22 (56.41)                     | 0.297   | 0.62 [0.27, 1.42]    | 0.256 |
| Female; n (%)                          | 27 (50)                         | 22 (56.41)                     | 0.674   | 0.77 [0.34, 1.77]    | 0.542 |
| Good Collaterals; n (%)                | 28 (51.85)                      | 16 (41.03)                     | 0.400   | 1.55 [0.67, 3.56]    | 0.303 |
| Poor Collaterals; n (%)                | 26 (48.15)                      | 23 (58.97)                     | 0.400   | 0.65 [0.28, 1.48]    | 0.303 |
| Clot location                          |                                 |                                | 0.191   |                     | 0.2016|
| Proximal thrombus (ICA + M1P)          | 22 (40.74)                      | 17 (43.59)                     | 0.833   | 0.89 [0.39, 2.04]    | 0.784 |
| Distal thrombus (M1D + M2 + M3)        | 32 (59.26)                      | 22 (56.41)                     | 0.833   | 1 [Ref]             | 1 [Ref]|</p>
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td><strong>ICA</strong></td>
<td>12 (22.22)</td>
<td>6 (15.38)</td>
<td>0.441</td>
<td>0.86 [0.24, 3]</td>
<td>0.809</td>
</tr>
<tr>
<td><strong>M1 Proximal (M1P)</strong></td>
<td>10 (18.52)</td>
<td>11 (28.21)</td>
<td>0.319</td>
<td>0.39 [0.12, 1.24]</td>
<td>0.111</td>
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<tr>
<td><strong>M1 Distal (M1D)</strong></td>
<td>11 (20.37)</td>
<td>13 (33.33)</td>
<td>0.230</td>
<td>0.36 [0.12, 1.11]</td>
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<tr>
<td><strong>M2 and M3</strong></td>
<td>21 (38.89)</td>
<td>9 (23.08)</td>
<td>0.122</td>
<td>1 [ref]</td>
<td></td>
</tr>
<tr>
<td><strong>24-Hour Core Volume (in mL); median [IQR]</strong></td>
<td>16.1 [32.4]</td>
<td>15.8 [35]</td>
<td>0.5911</td>
<td>1 [1, 1.01]</td>
<td>0.323</td>
</tr>
<tr>
<td><strong>Reperfusion Status</strong></td>
<td></td>
<td></td>
<td>&lt;0.0001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Major Reperfusion</strong></td>
<td>40 (74.07)</td>
<td>9 (23.08)</td>
<td>&lt;0.0001*</td>
<td>1 [ref]</td>
<td></td>
</tr>
<tr>
<td><strong>Poor Reperfusion</strong></td>
<td>14 (25.93)</td>
<td>30 (76.92)</td>
<td>&lt;0.0001*</td>
<td>0.11 [0.04, 0.27]</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td><strong>Risk factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hypertension</strong></td>
<td>50 (92.59)</td>
<td>32 (82.05)</td>
<td>0.192</td>
<td>2.73 [0.74, 10.1]</td>
<td>0.131</td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td>18 (33.33)</td>
<td>9 (23.08)</td>
<td>0.357</td>
<td>1.67 [0.65, 4.25]</td>
<td>0.284</td>
</tr>
<tr>
<td><strong>Dyslipidemia</strong></td>
<td>23 (42.59)</td>
<td>17 (43.59)</td>
<td>1.000</td>
<td>2.59 [1.11, 6.05]</td>
<td>0.028*</td>
</tr>
<tr>
<td><strong>Present Smoker</strong></td>
<td>15 (27.78)</td>
<td>10 (25.64)</td>
<td>1.000</td>
<td>1.12 [0.44, 2.83]</td>
<td>0.807</td>
</tr>
<tr>
<td><strong>Past Smoker</strong></td>
<td>23 (42.59)</td>
<td>15 (38.46)</td>
<td>0.831</td>
<td>1.46 [0.63, 3.37]</td>
<td>0.378</td>
</tr>
<tr>
<td><strong>AF</strong></td>
<td>31 (57.41)</td>
<td>20 (51.28)</td>
<td>0.673</td>
<td>0.93 [0.41, 2.13]</td>
<td>0.870</td>
</tr>
<tr>
<td><strong>New AF</strong></td>
<td>20 (37.04)</td>
<td>10 (25.64)</td>
<td>0.270</td>
<td>0.72 [0.3, 1.77]</td>
<td>0.478</td>
</tr>
<tr>
<td><strong>Depression</strong></td>
<td>6 (11.11)</td>
<td>1 (2.56)</td>
<td>0.232</td>
<td>1.04 [0.22, 4.94]</td>
<td>0.959</td>
</tr>
<tr>
<td><strong>History of Stroke/TIA</strong></td>
<td>10 (18.52)</td>
<td>9 (23.08)</td>
<td>0.612</td>
<td>0.77 [0.27, 2.16]</td>
<td>0.615</td>
</tr>
</tbody>
</table>
**Supplementary Table 2.** Demographic and baseline characteristics of all patients and by the locus of the thrombus. Odds ratio (OR) were obtained by bivariate logistic regression analysis for association with proximal clot.

<p>| Characteristics                              | Proximal thrombus (ICA + M1P) (n=39) | Distal thrombus (M1D + M2 + M3) (n=54) | P       | OR (95% CI)         | P&gt;|z| |
|----------------------------------------------|--------------------------------------|---------------------------------------|---------|---------------------|---------|
| Age (in years); Mean±SD                      | 72±14                                | 72.5±18                               | 0.711   | 1.01 [0.98, 1.05]   | 0.420   |
| Female; n (%)                                | 17 (43.59)                           | 32 (59.26)                            | 0.147   | 0.53 [0.23, 1.11]   | 0.137   |
| NIHSS at admission; Median [IQR]             | 14 [8]                               | 12.5 [8]                              | 0.184   | 1.04 [0.97, 1.11]   | 0.300   |
| NIHSS at 24 hours; Median [IQR]              | 7 [12]                               | 6.5 [10]                              | 0.343   | 1.01 [0.96, 1.07]   | 0.682   |
| OTT (in mins); Mean±SD                       | 159.49±67.65                         | 164.76±94.44                         | 0.636   | 1 [0.99, 1]         | 0.764   |
| Acute Core Volume; Median [IQR]              | 16.3 [25]                            | 12.8 [27.1]                           | 0.884   | 1.0 [0.99, 1.01]    | 0.847   |
| Penumbra; Median [IQR]                       | 60 [70.8]                            | 66.8 [77.9]                           | 0.391   | 1.0 [0.99, 1.01]    | 0.384   |
| TPME; Median [IQR]                           | 8 [4]                                | 8 [2]                                 | 0.019   | 1.3 [1, 1.71]       | 0.054   |
| Delayed-LCVF                                 | 33 (84.62)                           | 13 (24.07)                            | &lt;0.001  | 17.35 [5.95, 50.59] | &lt;0.001  |
| Baseline Collateral Status                   |                                     |                                       | &lt;0.001  |                    |         |
| Good Collaterals; n (%)                      | 9 (23.08)                            | 35 (64.81)                            | &lt;0.001  | 0.16 [0.06, 0.41]   | &lt;0.001  |
| Poor Collaterals; n (%)                      | 30 (76.92)                           | 19 (35.19)                            | &lt;0.001  | 6.14 [2.42, 15.58]  | &lt;0.001  |
| Reperfusion Status                           |                                     |                                       | 0.0209  |                    |         |
| Major Reperfusion                            | 15 (38.46)                           | 34 (62.96)                            | 0.022   | 1 [Ref]            |         |
| Poor Reperfusion                             | 24 (61.54)                           | 20 (37.04)                            | 0.022   | 2.72 [1.16, 6.36]   | 0.021   |
| 24-Hour Core Volume (in mL); median [IQR]    | 11.2 [23.8]                          | 18 [38]                               | 0.3874  | 1 [0.99, 1]         | 0.339   |
| mRS at 90 days; Median [IQR]                 | 2 [3]                                | 2 [4]                                 | 0.803   |                    |         |</p>
<table>
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<th>Good (mRS= 0-2)</th>
<th>Bad (mRS= 3-6)</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>p-Value</th>
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<tr>
<td></td>
<td>22 (56.41)</td>
<td>17 (43.59)</td>
<td>0.833</td>
<td>0.89 [0.39, 2.05]</td>
<td>0.784</td>
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<tr>
<td>Risk factors</td>
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<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>32 (82.05)</td>
<td>50 (92.59)</td>
<td>0.192</td>
<td>0.37 [0.1, 1.4]</td>
<td>0.131</td>
</tr>
<tr>
<td>Diabete</td>
<td>12 (30.77)</td>
<td>15 (27.78)</td>
<td>0.819</td>
<td>1.16 [0.47, 2.85]</td>
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<tr>
<td>Dyslipidemia</td>
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<td>18 (33.33)</td>
<td><strong>0.034</strong></td>
<td>2.59 [1.11, 6.05]</td>
<td><strong>0.028</strong></td>
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<tr>
<td>Present Smoker</td>
<td>11 (28.21)</td>
<td>14 (25.93)</td>
<td>0.817</td>
<td>1.12 [0.44, 2.83]</td>
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</tr>
<tr>
<td>Past Smoker</td>
<td>18 (46.15)</td>
<td>20 (37.04)</td>
<td>0.400</td>
<td>1.46 [0.63, 3.37]</td>
<td>0.378</td>
</tr>
<tr>
<td>AF</td>
<td>21 (53.85)</td>
<td>30 (55.56)</td>
<td>1.000</td>
<td>0.93 [0.41, 2.13]</td>
<td>0.870</td>
</tr>
<tr>
<td>New AF</td>
<td>11 (28.21)</td>
<td>19 (35.19)</td>
<td>0.509</td>
<td>0.72 [0.3, 1.77]</td>
<td>0.478</td>
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<tr>
<td>Depression</td>
<td>3 (7.69)</td>
<td>4 (7.41)</td>
<td>1.000</td>
<td>1.04 [0.22, 4.94]</td>
<td>0.959</td>
</tr>
<tr>
<td>History of Stroke/TIA</td>
<td>7 (17.95)</td>
<td>12 (22.22)</td>
<td>0.795</td>
<td>0.77 [0.27, 2.16]</td>
<td>0.615</td>
</tr>
</tbody>
</table>
Supplementary Table 3. Univariate logistic regression analysis for 24 hours’ poor angiographic reperfusion. *p<0.05 as threshold for statistical significance

| Characteristics                          | Poor angiographic reperfusion | Major angiographic reperfusion | P      | OR (95% CI)                | P>|z| |
|-----------------------------------------|------------------------------|-------------------------------|--------|---------------------------|-----|
| Age (in years); Mean±SD                 | 74.16±10.34                  | 69.31±13.68                   | 0.067  | 1.03 [0.998, 1.07]         | 0.064 |
| Female; n (%)                           | 23 (52.27)                   | 26 (53.06)                    | 1.000  | 0.97 [0.43, 2.19]          | 0.939 |
| NIHSS at admission; Median [IQR]        | 14.5 [8]                     | 13 [9]                        | 0.096  | 1.08 [1, 1.16]             | 0.05* |
| NIHSS at 24 hours; Median [IQR]         | 11.5 [11.5]                  | 4 [5]                         | <0.0001* | 1.21 [1.11, 1.33]        | <0.0001* |
| OTT (in mins); Mean±SD                  | 166.93±88.01                 | 158.61±80.69                  | 0.7695 | 1.001 [0.996, 1.006]       | 0.632 |
| Acute Core Volume; Median [IQR]         | 20.35 [54.15]                | 9.9 [19.25]                   | 0.0639 | 1.02 [1.003, 1.04]         | 0.023* |
| Penumbra; Median [IQR]                  | 73.5 [61.95]                 | 54.65 [78.8]                  | 0.1152 | 1.007 [0.998, 1.02]        | 0.11  |

Baseline Collateral Status

| Good Collaterals; n (%) | 14 (31.82) | 30 (61.22) | 0.007* | 0.296 [0.13, 0.696] | 0.005* |
| Poor Collaterals; n (%) | 30 (68.18) | 19 (38.78) | 0.007* | 3.38 [1.44, 7.96]   | 0.005* |
| TPME; Median [IQR]      | 8 [2]      | 8 [2]      | 0.1584 | 1.15 [0.89, 1.47]   | 0.289  |
| Delayed-LCVF            | 30 (68.18) | 16 (32.65) | 0.001* | 4.42 [1.85, 10.56]  | 0.001* |

Clot location 0.0209*
<table>
<thead>
<tr>
<th></th>
<th>Proximal thrombus (ICA + M1P)</th>
<th>Distal thrombus (M1D + M2 + M3)</th>
<th>24-Hour Core Volume (in mL); median [IQR]</th>
<th>mRS at 90 days; Median [IQR]</th>
<th>Risk factors</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td>0.4813</td>
<td>&lt;0.00001*</td>
<td>Dyslipidemia</td>
</tr>
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<td>Present Smoker</td>
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<td>Past Smoker</td>
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<td></td>
<td>AF</td>
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<td>New AF</td>
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<td>Depression</td>
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<td>History of Stroke/TIA</td>
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</tbody>
</table>

* indicates statistical significance.
“Like the entomologist in search of colourful butterflies, my attention has chased in the gardens of the grey matter cells with delicate and elegant shapes, the mysterious butterflies of the soul, whose beating of wings may one day reveal to us the secrets of the mind.

In summary, all great work is the fruit of patience and perseverance, combined with tenacious concentration on a subject over a period of months or years.”

- Santiago Ramon y Cajal
CHAPTER 7: CONCLUSION & FUTURE DISCUSSIONS
7.1. Conclusion

This thesis examined the role of clinical and neuroradiological markers in acute ischemic stroke (AIS). We used advanced computed tomography (CT) and magnetic resonance imaging (MRI) techniques along with clinical parameters in the pathophysiological investigation, and selection of patients towards predicting the therapeutic outcome of reperfusion therapy in AIS. We identified sub-group of AIS patients who are more likely to show early neurological improvement or good long-term clinical outcome. We found that individual patient selection based on these factors may augment the tailored benefit of thrombolytic treatment to a patient. Results of our investigations concerning the thesis are discussed at length in corresponding individual chapters. Here, we will briefly revisit relevant results, and give insights into future on the implications of patient profiling based approach to acute stroke therapy.

The main conclusions of this thesis are:

7.1.1. Profiling based on stroke severity: Is baseline stroke severity an essential covariate in any analysis of stroke outcome?

In the first study, chapter 3 [1], using multivariate regression and receiver operating curve (ROC) analyses, we demonstrated that initial stroke severity has a strongest independent association with the risk of death within 90 days. Age was of less significant independent influence. Baseline stroke severity independently predicted 90 days’ mortality and unfavourable outcome. Moreover, a higher national institute of health stroke scale (NIHSS) score was significantly correlated with longer in-hospital placement. Based on these findings, we concluded that initial stroke severity has a major impact on the risk of mortality and should be factored in for prediction of the length of the in-hospital placement and overall functional outcome following an AIS. Predicting the clinical course of patients with acute stroke continues to be a prognostic challenge for stroke physicians [1109]. Early risk stratification of acute stroke patients has contributed significant clinical estimates of mortality risk using reliable and simple prognostic models [1052, 1110]. At present, prognostic models of mortality are used in the economic and performance evaluation of stroke care centres. These models often lack appropriate case-mix adjustment of initial stroke severity. Initial severity of stroke and age are both recognised to influence the likelihood of an unfavourable functional outcome and/or mortality following an acute stroke. However, the extent of
the interaction is uncertain. Therefore, epidemiological studies on the extent of the interaction and/or influence of stroke severity and age on mortality and overall functional outcomes are important. Understanding of factors contributing to the progression of stroke and/or death may have an important impact on future stroke trials and patient management.

7.1.2. Perilesional hyperperfusion (PLH) on arterial spin labelling (ASL) perfusion MRI: Is PLH marker of good tissue outcome? Are good collaterals associated with PLH at 24 hours?

In chapter 4 [2], we showed that the peri-lesional hyperperfusion (PLH) seen on ASL-MRI at 24 hours is associated with good baseline collaterals and good angiographic reperfusion outcomes. PLH is a marker of tissue outcome. To the best of our knowledge, there are none or limited prior studies that have investigated the link between infarct topography, collateral status and PLH at 24 hours. In this prospective study, using multi-modal computed tomography (CT) acutely and magnetic resonance imaging (MRI) at 24±8 hours, we found PLH is independently associated with good collaterals, superior cortical division MCA stroke, striatocapsular infarction and occurrence of major reperfusion at 24 hours. With regards to clinical outcomes, PLH was also found to be associated with penumbral salvage and major reperfusion at 24 hours. The previously recognised associations using PET and ASL between PLH and more favourable clinical outcomes may imply that there is longer lived penumbra in some patients, and its survival, presumably via later reperfusion, is supported by the presence of PLH. Alternatively, if PLH is occurring in tissue without residual ischemia, the hyperperfusion may be resulting in other, yet to be defined, protective mechanisms. Whatever the underlying mechanism, the presence of PLH correlates with favourable clinical trajectory. From a therapeutic standpoint, we postulate that patients with the good collateral flow may have infarcts associated with hyperperfusion in perilesional areas. Moreover, good collaterals may be sustaining penumbral survival until reperfusion. This mechanism may be responsible for driving a favourable clinical trajectory. However, in this study, we acknowledge that we did not show causality between collateral status and PLH.

Assessment of lesion topography, collateral status, reperfusion, and post-ischaemic hyperperfusion patterns using multimodal imaging can provide additional metrics for
patient selection towards intervention strategies and prognosis in acute settings. It may have implications for prognostication in hyper-acute settings and patient selection towards identifying potential recovery targets for long-term therapy and rehabilitation. However, further research is warranted to evaluate the utility of this imaging-based approach better. Post-ischaemic hyperperfusion in acute settings can be of prognostic value depending on the spatial localisation and temporal dynamics. PLH in AIS patients predicts a more favourable recovery in some patients. We have identified that several lesion topographies and collateral status at baseline are associated with PLH.

This study is novel considering its pathophysiological and clinical standpoints. Predicting post-stroke recovery remains a challenge to stroke physicians. However, profiling patients using imaging metrics may enable clinicians to predict an accurate prognosis, and design individualised stroke intervention strategies. Further studies are required to replicate our results in a broader case mix of acute stroke and to measure effects of these synergistic factors and to evaluate the long-term recovery trajectories of the various imaging defined subgroups. Our study also shows the potential application of non-invasive imaging using ASL in the acute stroke population. This may have practical significance for therapeutic clinical trials of acute stroke. ASL at 12-24 hours can be useful for pathophysiological investigations.

7.1.3. Delayed-cortical venous filling on dCTA: A novel prognostic biomarker for AIS?

In chapter 5 [3], we reported the prevalence of a novel neuroradiological marker, delayed cortical vein filling in late venous phase (delayed-LCVF), in AIS. Delayed-LCVF was defined by late venous phase opacification of cortical veins despite contrast clearance from contralateral cortical veins on dynamic CTA (dCTA). As a principal finding of this study, we found that the delayed appearance of cortical vein filling during the late venous phase on baseline time-resolved dCTA images was relatively common in our hyperacute study cohort.

To our knowledge, this is the first such study that describes delayed-LCVF. We found that delayed-LCVF is frequently seen in patients with AIS. Our study also suggests that MIP reconstructions of dCTA source images are a valuable angiographic tool to evaluate arterial and venous anatomy. Evaluation of drainage patterns and flow dynamics associated with the downstream venous system may be useful in the
prognostic management of acute stroke patients and patient selection for acute and long-term intervention strategies. Currently, clinico-neuroradiological assessments using CTA focus primarily on the cerebral arterial circulation. Additional imaging surrogates (or early markers) of potentially salvageable brain tissue may be useful in prognosis and theranostics in acute stroke. Future prospective studies with a larger number of patients are needed to establish the role of delayed-LCVF in stroke prognostication.

7.1.4. Delayed-LCVF: a surrogate measure of collateral flow?

In this exploratory study (chapter 5), in AIS cohort, we demonstrated that dCTA provides useful information on the morphologic extent of the collaterals and the collateral blood flow delay. We found that the morphologic extent of the collaterals and the collateral blood flow delay in maximum enhancement (defined in terms of time to peak of maximum arterial enhancement (TPME)) were independent predictors of delayed-LCVF. We demonstrated that delayed-LCVF pattern could potentially be used as a surrogate measure to assess collateral status. Patients with delayed-LCVF are more likely to have poor collateral status and unfavourable clinical outcome.

Presently, evaluation of collateral status is done qualitatively through visual examination and involves indirect assessment of the extent and rate of backfilling of pial arteries which are fed by collateral vessels to maintain blood flow [466, 467, 469]. As such, a more ‘direct’ measure such as the absence or presence of delayed-LCVF pattern could potentially be used as a more reproducible method to assess collateral status. Previous studies have shown that the good collateralization on dCTA predicts a small lesion volume. One of the shortcomings of these studies is that the assessment of collateral status was based on the maximal morphologic extent of collateral vessels over the entire scan time and lacked the temporal information associated with the time point of collateral reconstitution. Data on time point of collateral reconstitution and its predictive value are limited. We demonstrate that the time delay in collateral flow provides an additional measure over and above the morphologically based assessment. Our study shows that the model using both morphological extent of the collateral vessel and the delay in collateral reconstitution showed the excellent predictive ability for delayed-LCVF. Average TPME was significantly greater in delayed-LCVF population. This measurement can provide quantifiable data on the
status of arterial and venous flow. The longer the TPME on dCTA, the higher the probability of delayed-LCVF. Longer scanning times could assist in the further characterization of filling defects.

7.1.5. Does the location of clot influence delayed-LCVF?

Our findings in chapter 6 indicate that delayed-LCVF was significantly more common in patients with M1P and/or ICA occlusion [4]. Delayed-LCVF showed significant improvement in discriminative accuracy when it was added to the multivariate regression model. As such, the appearance of delayed-LCVF on dCTA is a surrogate for proximal large vessel occlusion which is known to be associated with poor outcome. Previous studies have reported that AIS patients with thrombus in M1D, M2, and M3 segments are more likely to undergo recanalization than those with M1P and ICA occlusions. Large vessel occlusions are less liable to be recanalized after IVT and are more likely to have poor clinical outcomes. The fact that delayed-LCVF is strongly associated with proximal thrombus may be used as an important parameter towards stroke prognostication and selection of patients for IVT. We postulate that proximal large vessel occlusions may lead to delayed-LCVF. Based on these findings, the appearance of delayed-LCVF on dCTA can be used as a surrogate for proximal thrombus or large vessel occlusion. Moreover, given the propensity of proximal thrombus towards poor clinical outcome after IVT, and significant association of delayed-LCVF with both proximal thrombus and poor reperfusion at 24 hours, we postulate that the AIS patients with delayed-LCVF may progress unfavourably, and therefore alternative revascularization strategies may be considered.

7.1.6. Does delayed-LCVF associate with clinical outcome in AIS?

In chapter 6, we show that delayed-LCVF may have a role in worsening of clinical outcomes in a select group of patients who progress unfavourably [4]. Delayed-LCVF was significantly associated with poor 24 hours early angiographic reperfusion status. There was a tendency towards worse clinical outcomes at 90 days, though, the association did not reach statistical significance. We hypothesise that patients with the delayed-LCVF pattern on baseline dCTA may show an unfavourable trajectory. Interestingly, the addition of delayed-LCVF significantly improved the predictive accuracy of functional outcome at 90 days. The presence of delayed-LCVF may aid in identifying patients at risk of 24-hour poor angiographic reperfusion. Other studies
have also shown significant association of cerebral venous flow with prognosis in stroke in both animals and humans. Interestingly, animal studies focusing primarily on early and mid-venous phase have shown that the presence of cortical vein filling after ischaemic stroke was associated with decreased severity of hemiparesis and lower infarct volumes. This led to the hypothesis that the cortical venous flow may produce favourable outcomes as it would be more commonly prevalent in strokes with good collaterals. Another study on humans also found that the cortical venous drainage was associated with good clinical outcomes.

There are limited studies on the role of cortical veins in stroke pathophysiology, and their associations with thrombus location and clinical outcome [13-18]. Future studies on the role of AIS and its impact on clinical outcomes will give further insights on the role of cortical veins in AIS prognostication. We propose that delayed-LCVF is a marker that will allow clinicians to extract more prognostic information from imaging that is already routinely acquired. We caution that these results must be understood as preliminary and within the context of the study design. Further prospective studies are recommended to investigate the role of cortical veins in stroke prognostication.

7.1.7. ASL and dCTA can be used to identify surrogate imaging markers which may be of prognostic value.

Chapter 5 & 6 used dCTA, obtained using advanced CT scanners, in neurovascular imaging of AIS. Dynamic CTA obtained using new generation 320-detector row 640-slice multidetector CT (MDCT) scanners with appropriate reconstructions can be used to investigate venous dynamics or various stages of downstream venous flow [3, 4]. It is a promising technique for the dynamic assessment of the cerebral vasculature. MDCT scanners can be used for angiographic assessment of baseline collateral status. Also, in combination with NCCT, CTA/CTP can be rapidly obtained with minimal delay in treatment, and is widely available in emergency departments, and is well tolerated. CTA has been used in the diagnosis and emergent evaluation (e.g., occlusion of the internal carotid artery bifurcation, extent of carotid stenosis, irregularities in arterial walls including presence/evidence of intimal thickening, calcification, ulceration and plaque formation) [540, 1161-1163] of a number of cardiovascular diseases including occlusive diseases, cerebral aneurysms [1164], planning for surgical interventions (including carotid endarterectomy (CEA) and
percutaneous transluminal angioplasty (PTA)), and postoperative evaluation [539, 540]. CTA has also shown potential for leptomeningeal intracranial collateral assessment [468-471], and for patient selection towards thrombolytic treatment eligibility [525, 527, 1165]. Imaging biomarkers towards the identification of patients who might benefit from early reperfusion therapy and guiding early intervention options to limit or even freeze infarct progression are crucial for strategies in acute stroke treatment. The advent of cutting-edge next generation 320-detector row 640-slice multi-detector (MDCT) scanners has facilitated the acquisition of whole-brain, sub-second, and volumetric acquisition of 4D-dCTA data [3]. CTP/CTA is not an invasive procedure compared to digital subtraction angiography (DSA), and is routinely obtained during clinical care of stroke patients, has proven to be of added clinical utility in the early evaluation of stroke, facilitating precise localization of site of occlusion, and identification of hypoperfused territory at risk of infarction. The CTP was acquired simultaneously with the CTA with the use of same contrast bolus. Dynamic CTA allows evaluation of intracranial vasculature and visualisation of contrast flow from its arterial to venous phases. Using appropriate reconstructions of dCTA using MIP algorithm, we investigated various stages of venous drainage and downstream venous dynamics; including the assessment of delayed-LCVF appearance in late venous phase. Assessment of impaired cortical venous drainage may provide valuable information over and above arterial collateral assessment, and the presence of delayed-LCVF could have a role in making informed decisions on patient management and prognosis. Assessment of collaterals on conventional CTA is dependent on the image acquisition timing. This causes impaired evaluation in the setting of delayed collateral filling distal to the occluded artery. The development of whole-brain 4D-dCTA on MDCT scanners have made it possible to generate time-resolved angiograms of brain vasculature, in particular, the collaterals, from skull base to the vertex. The dCTA harnesses the ability of MDCT scanners to acquire imaging at multiple time points. This technique has demonstrated improved assessment of collaterals by capturing optimal enhancement of collateral vessels, identification of the origin of the dominant collaterals, and quantifying the delay of maximum enhancement. Moreover, dCTA provides additional hemodynamic information (in contrast to conventional cerebral angiogram) by allowing time-resolved visualisation of pial arterial filling in all vascular territories. Chapter 4 demonstrated usefulness of ASL as a quantitative cerebral perfusion measurement technique which can detect early PLH patterns in AIS. Clinical
application of ASL in stroke population is still very limited. AIS harnesses blood water as an endogenous contrast agent, and our group and others have shown that it is capable of demonstrating hypoperfusion and hyperperfusion patterns in clinical application [1141]. In comparison to conventional bolus-tracking methods, ASL does not need gadolinium-based contrast and show good repeatability and quantification of absolute cerebral blood flow. However, ASL does have a major limitation of intrinsically low signal to noise ratio (SNR), which may compromise its ability to reliably estimate the cerebral perfusion given the intricacy of cerebral vasculature and etiological mechanisms in AIS. Previous studies have shown substantial agreement in perfusion measurements of ASL and conventional bolus based approaches [1142, 1182].

7.2. Future Works and Discussions
Patient profiling, using advanced neuroimaging and clinical parameters, based approach overcome the restricted time window criteria for patient selection in AIS [242, 480, 525, 532, 585, 646, 657, 810, 847, 902, 1165]. It is used to identify common pathophysiological subtypes of ischemic stroke that have prognostic relevance. The identification of precise stroke-subtype can guide clinicians to choose and prescribe optimal individualised acute and rehabilitation intervention to the stroke patients [480, 517, 902, 1165]. Further studies to investigate the long-term outcome of cortical vein based marker (on dCTA) and peri-lesional hyperperfusion (on ASL) on a larger sample size is underway [3, 4]. We envisage to investigate whether the venous imaging-based biomarker is independent of the reperfusion therapy as the data were acquired using thrombolytic reperfusion therapies. The clinical utility of this radiologic sign is limited at present, however, in the future with the widespread adoption of time-resolved CTA may become important. At present, the endovascular facility is limited to a very select group of patients [646]. We hope that use of advanced neuroimaging and clinical biomarkers will help guide optimal treatment pathway for our patients. We envision that we will be able to translate our results in future clinical trials, thereby laying a roadmap to establish the role of patient profiling in clinical decision making, the efficacy of novel thrombolytic treatment, and planning for rehabilitation. Clinical trials to determine the role of patient profiling in clinical decision making and planning for rehabilitation are being planned. It will enable us to better plan or design clinical trials so as to achieve an optimal therapeutic outcome for our patients.
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APPENDICES

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Title: Baseline collateral status and infarct topography in post-ischaemic perilesional hyperperfusion: An arterial spin labelling study

Author: Saurabh Bhaskar, Andrew Bivard, Peter Stanwell, et al

Publication: Journal of Cerebral Blood Flow & Metabolism

Publisher: SAGE Publications

Date: 06/02/2016

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Title: Delay of late-venous phase cortical vein filling in acute ischemic stroke Patients: Associations with collateral status

Author: Sonu Bhaskar, Andrew Bivard, Mark Parsons, et al

Publication: Journal of Cerebral Blood Flow & Metabolism

Publisher: SAGE Publications

Date: 02/01/2017

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The Graduates Walk, at the corner of the Auchmuty Library, University of Newcastle (UoN), celebrates the graduates who become the life-long member of the ‘Global UoN’ family. The Latin inscription, attolens umero famamque et fata nepotum, translates to ‘lifting onto their shoulders the fame and fate of their children’s children’. L’Chaim!

Dated 3rd of February, 2017