Radiological, Clinical and Genetic Markers of Ischaemic Stroke Outcome

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Abstract

Acute ischaemic stroke is caused by a blocked blood vessel in the cerebral circulation. It is the most common form of stroke worldwide and a major cause of disability and death. Over the past 20 years, major advances in acute stroke treatment and management have led to a reduction in stroke-related mortality, and thus an unavoidable side effect has been the concomitant rise in survivors living with life-changing disability, requiring ongoing clinical management and care. However, stroke outcome is not entirely represented as mortality rates and level of disability – there are a range of neurological sequelae that contribute an important additional burden to patients.

Up to 13% of patients who have suffered an ischaemic stroke will develop seizures within 2 years. For clinicians the development of seizures represents a clinical challenge to manage, is difficult to predict and treat, and associated with poorer patient quality of life. However, at present no indications for antiepileptic drugs in preventing post stroke seizures and epilepsy exist and to date, no blood biomarkers and only few genetic biomarkers have been identified as being associated with an increased risk of post stroke seizure development. This multi-centre (China, Brazil and Australia), multidisciplinary thesis examines novel imaging, genetic and clinical markers as methods for identifying patients at higher risk of developing seizures.

Reperfusion therapies with thrombolysis and, more recently, endovascular thrombectomy have transformed outcomes for patients. This body of research targeted patient groups treated with modern cerebrovascular stent devices and revascularization techniques, in order to assess the implications of these novel stroke interventions, particularly in terms of the development of post stroke seizures. Multiple advanced neuroimaging techniques were used to determine capacity to identify patients at the highest risk of developing post stroke seizures. Results from these investigations showed that the Alberta Stroke Program Early CT Score on non-contrast CT, cortical involvement on CT perfusion parameters and extent of haemorrhagic transformation on non-contrast CT, can be used as radiological markers for stroke outcome, including the identification of higher risk patients for post stroke seizure development. Additionally, unlike previous work, international sites were included along with Australian sites, allowing the interrogation of whether ethnicity and environment influences the development of post stroke seizures. Results from this investigation revealed that, not only does occurrence of seizures differ across populations from different countries, but certain clinical markers, such as presence and treatment of atrial fibrillation, may influence seizure occurrence across populations. Our exploratory study assessing the genetic influence on the development of post stroke seizures has also laid important groundwork in developing genetic biomarkers for future studies and results from this thesis have identified potential genetic variants warranting further investigation.

The results presented in this thesis have the potential to guide identification of individuals at higher risk of developing post stroke seizures and represent a step towards personalised medicine. In the future, if antiepileptogenic drugs become available, these results may inform the selection of an enriched population for trials and guide recruitment for biomarker studies of epileptogenesis.

Declaration

This is to certify that:

(i) The thesis comprises only my original work towards the PhD except where indicated in the Preface;

(ii) Due acknowledgement has been made in the text to all other material used;

(iii) The thesis is fewer than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

Jillian J Naylor **17/07/2018**

Preface

The work presented in this thesis was undertaken in the Department of Neurology, Royal Melbourne Hospital in association with the Departments of Medicine and Radiology, Faculty of Medicine, Dentistry and Health Sciences, University of Melbourne. However, a number of collaborations were formed to complete this work. This includes with the Department of Neurology at the Jinling Hospital Nanjing, the University of Campinas in Brazil, the John Hunter Hospital in Newcastle Australia and the Prince of Wales Hospital in Hong Kong. A substantial component of the work presented in this thesis has been published or is currently in submission for publication. Components of this work have also been presented or accepted for presentation at various scientific conferences.

Publications relevant to this thesis

Naylor J, Churilov L, Rane N, Campbell B.C.V, Yan B. Reliability and Utility of the Alberta Stroke Program Early Computed Tomography Score in Hyperacute Stroke. *J Stroke Cerebrovasc Dis* 2017;26:2547-2552

Naylor J, Churilov L, Chen Z, Koome M, Rane N, Campbell B.C.V. Reliability, Reproducibility and Prognostic Accuracy of the Alberta Stroke Program Early CT Score on CT Perfusion and Non-Contrast CT in Hyperacute Stroke. *Cerebrovasc Dis* 2017;44:195-202

Naylor J, Thevathasan A, Churilov L, Guo R, Xiong Y, Koome M, Chen Z, Chen Z, Liu X, Kwan P, Campbell B.C.V. Association between different acute stroke therapies and development of post stroke seizures. *BMC Neurol* 2018;18:61

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Manuscripts in submission

Naylor J, Churilov L, Thevathasan A, Johnstone B, Koome M, Chen Z, Chen Z, Mitchell P, Kwan P, Campbell B.C.V. A comparison of the association between haemorrhagic transformation and post ischemic stroke seizure development in patients receiving reperfusion therapies. *Submitted to BMC Neurology*

Naylor J, Levi C, Parsons M, Garcia-Esperon C, Longting L, Gawariker Y, Patel R, Yan B, Lee A. A registry and clinical and MRI outcomes following early versus late initiation of anticoagulation after ischaemic stroke or transient ischaemic attack in patients with atrial fibrillation. *Submitted to BMC Cardiovascular*

Other collaborative papers

Thevathasan A, **Naylor J**, Churilov L, Mitchell P.J, Dowling R.J, Yan B, Kwan P. Association between hemorrhagic transformation after endovascular therapy and poststroke seizures. *Epilepsia* 2018;59:403-409

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Conferences and Academic Seminars

E-poster discussion – European Stroke Organisation Conference Gothenburg, May 2018 - *A comparison of the association between haemorrhagic transformation and post ischemic stroke seizure development in patients receiving reperfusion therapies.*

Poster Presentation – American Epilepsy Conference Washington DC, December 2017 - *Association between different acute stroke therapies and development of post stroke seizures*

Oral Presentation – Melbourne Brain Centre Academic Seminar, Melbourne, April 2017 – *Investigating the genetic influence on epilepsy development post ischaemic stroke: a multi-centre, international, case-controlled, candidate-gene approach study*

Oral Presentation – Asia Pacific Stroke Conference, Brisbane, July 2016 – *An MRI registry of clinical and radiological outcomes following initiation of anticoagulation after ischaemic stroke or transient ischaemic attack*

Poster Presentation – European Stroke Organisation Conference, Barcelona, May, 2016 – *Alberta Stroke Program Early CT score (ASPECTS) has high inter-rater variability in stroke onset under 100 minutes*

Oral Presentation – Asia Pacific Stroke Conference, Melbourne, July, 2015 – *An MRI registry of clinical and radiological outcomes following initiation of anticoagulation after ischaemic stroke or transient ischaemic attack*

Other

Australian Awards- Endeavour Fellowship 2016 – a merit-based scholarship providing the opportunity to strengthen collaborative research internationally.

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Abbreviations

ACA	Anterior cerebral artery
AEDs	Antiepileptic drugs
AF	Atrial fibrillation
AIS	Acute ischaemic stroke
ASPECTS	Alberta Stroke Program Early CT Score
ATP	Adenosine triphosphate
AUC	Area under the curve
BBB	Blood brain barrier
BIC	Bayesian information criterion
CI	Confidence Interval
CT	Computed Tomography
CTA	Computed Tomography Angiogram
CTP	Computed Tomography Perfusion
CBV	Cerebral Blood Volume
CBF	Cerebral Blood Flow
DALYs	Disability adjusted life years
DOAC	Direct oral anticoagulant
DSA	Digital subtracted angiogram
DWI	Diffusion Weighted Imaging
DM	Diabetes Mellitus
ECASS	European Cooperative Acute Stroke Study
ECR	Endovascular clot retrieval
EIC	Early ischaemic change
EOS	Early onset seizures

FLAIR	Fluid Attenuated Inversion Recovery
GWAS	Genome-wide association studies
HAS	Hyperdense Artery Sign
HT	Haemorrhagic transformation
HU	Hounsfield unit
IAT	Intra-arterial therapy
ICA	Intracerebral artery
ICH	Intracerebral haemorrhage
IV-tPA	Intravenous tissue-plasminogen activator
ILAE	International League Against Epilepsy
κ	Kappa
LAA	Large artery atherosclerosis
LOS	Late onset seizures
MCA	Middle cerebral artery
MRI	Magnetic resonance imaging
mRS	Modified Rankin Score
MMPs	Metalloproteinases
NCCT	Non-contrast computed tomography
NIHSS	National Institute of Health Stroke Scale
OR	Odds ratio
PCA	Posterior cerebral artery

PSE	Post stroke epilepsy
PSS	Post stroke seizures
RCT	Randomized controlled trial
ROC	Receiver operating characteristic
sICH	Symptomatic intracerebral haemorrhage
SNP	Single nucleotide polymorphisms
TNK	Tenecteplase
Tmax	Time to Maximum of the tissue residue function after deconvolution

Introduction

Stroke is a leading cause of mortality and morbidity worldwide, with ischaemic stroke accounting for 80%-85% of all strokes (1, 4). Over the course of the last 20 years, there have been major advances in ischaemic stroke treatments and managements, resulting in changes in the type of stroke outcomes seen. Despite large geographic variation, between the years 1990-2006, many countries have reported continued decreases in the overall stroke mortality rates. Reasons for decreasing mortality rate have been related to higher income, greater management of risk factors for stroke (e.g., hypertension, cardiovascular risk factor), control interventions, and better diagnosis and health care systems (5, 6). However, the increasing prevalence of stroke survivors forecasts substantial growth in health care costs and socioeconomic burdens and thus there is a greater need for longer term stroke follow up and comprehensive post stroke rehabilitation services.

In 1995, a randomised controlled trial comparing the efficacy of novel thrombolysis treatment with placebo was published. This study found that patients treated with thrombolysis within 3 hours were at least 30% more likely to have minimal or no disability at three months post stroke (7). In 2015, five randomized controlled trials reported the therapeutic benefits of a combination of intra-arterial therapy and thrombolysis in acute stroke patients. In individual patient data meta-analysis, independent functional outcome was increased from 26.5 to 46% with a number needed to treat to achieve an extra independent patient of 5 and to

improved functional outcome by at least 1 point on the modified Rankin Scale of 2.6 (8, 9). Collectively, these trials demonstrate the high efficacy of combined intra-arterial therapy and thrombolysis in eligible patients, leading to a significant increase in likelihood of a better functional outcome compared with best medical therapy alone (10).

Another factor shown to be critical to the efficacy of these treatments is time, with studies showing that patients treated earlier after onset have the greatest chance of benefitting from reperfusion therapies (11). These findings have had, and continue to have global implications for the structure of health care systems, with a focus on the development of systems, tools and technologies to optimise the number of patients receiving treatment earlier (12). To do this, there has been a focus on education in recognising stroke symptoms, implementing efficient code stroke methods and the introduction of telemedicine, all of which has led to an increase in the proportion of stroke patients arriving at hospital earlier (13). In addition, the preliminary implementation of mobile stroke units has allowed the use of pre-hospital treatment (onsite CT imaging, mobile laboratory, telemedicine equipment and access to thrombolysis treatment) to reduce the median time from stroke onset to therapy decision to as little as 35 minutes (14).

Although all these factors have led to a reduction in stroke-related mortality, an unavoidable side effect has been the concomitant rise in survivors living with life-changing disability. Specifically, a Lancet paper published in 2014 suggested that

if these trends in stroke incidence, mortality and disability-adjusted life years lost continue, by 2030 there will be almost 12 million stroke deaths, 70 million stroke survivors and more than 200 million disability adjusted life years lost globally (1). Given the improved immediate outlook of acute stroke, there is a need to understand whether the novel stroke interventions and management will also affect longer-term outcomes of stroke. Due to the marked advances made in ischaemic stroke treatments and management, this thesis will focus solely on patients with acute ischaemic strokes.

Literature Review

Introducing acute ischaemic stroke

Ischaemic stroke comprises approximately 80% of stroke in Australia and is similar in most Western countries (15, 16). Haemorrhagic stroke largely accounts for the remaining 20% and is due to the rupturing of small blood vessels. Stroke in general is the second leading cause of mortality worldwide and the leading cause of adult disability. In terms of absolute numbers worldwide, in 2010 an estimated 11,569,538 ischaemic stroke events occurred; of which 2,835,419 individuals died and 39,038,763 disability-adjusted life years were lost (DALYs) (17). The absolute number of people with incident ischaemic stroke, the number of deaths due to ischaemic stroke and the number of DALYs for people with ischaemic stroke is increasing, particularly in low and middle-income areas where the prevalence and significance of stroke risk factors are greater and the accessibility to health care and services is lower (1).

Defining ischaemic core and penumbra

The most common cause of acute ischaemic stroke is the sudden occlusion of intracranial arteries by a thrombus or embolism, resulting in an immediate reduction in oxygen and glucose delivery to a region of brain tissue (18). Within seconds to minutes, an ischaemic centre (or core) develops representing the region

of brain that is irreversibly damaged (or infarcted). Determining the extent of irreversibly damaged infarct core is important as it has been demonstrated to directly influence the likelihood of good stroke outcome with reperfusion therapies (19, 20) and has been associated with risk of haemorrhagic transformation (21) and malignant oedema (22).

Surrounding the ischaemic core is a volume of tissue that may either survive or die. This is known as the penumbra (18). By definition, the penumbra is a region of hypoperfused, electrically silent and functionally impaired but viable tissue, or a region of tissue that is at risk of being recruited into the ischaemic core (23). Thus, it is the target for reperfusion therapies (24), which will be discussed in detail further on. In contrast, the ischaemic core will remain irreversibly injured regardless of subsequent reperfusion. As stroke is time-dependent, establishing the penumbra versus the core on acute stroke imaging is crucial for treating patients near or outside time thresholds or in situations where stroke onset is unknown. Poor sensitivity and specificity in penumbral selection can lead to inappropriate treatment or patients excluded from therapies that would otherwise be beneficial (24). A pertinent question surrounds optimizing imaging techniques and tools to ensure this. This will be discussed in detail further on.

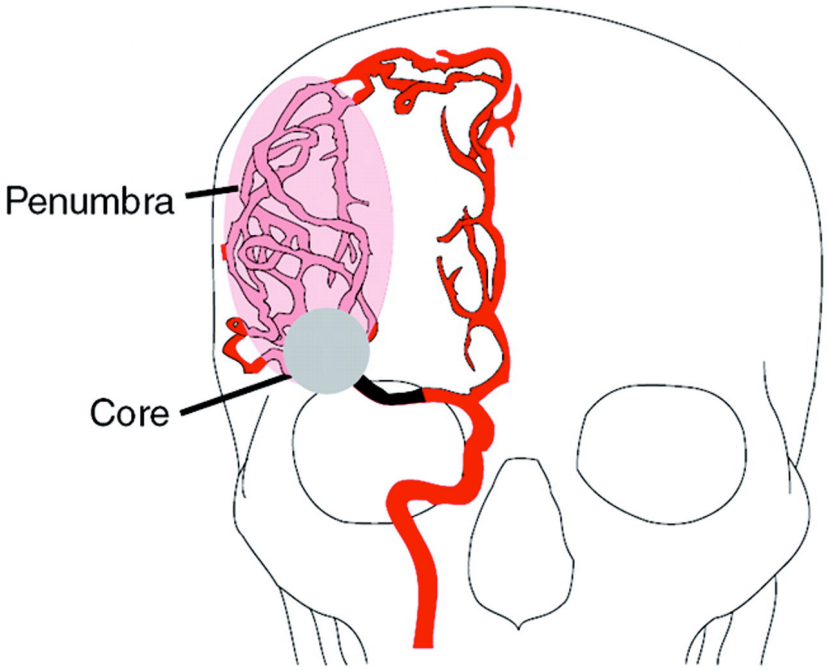

Figure 1: A diagrammatic representation of the ischaemic core and penumbra following an occlusion of the right middle cerebral artery. The perfusion of blood flow to the vascular territory of the occluded artery is depicted. This results in two regions: 1. The ischaemic core, irreversibly injured brain tissue (regardless of reperfusion therapies) and 2. The ischaemic penumbra, the region of tissue that is at risk of being recruited into the ischaemic core (thus is the target for reperfusion therapies) (25).

Epidemiology

This thesis will primarily focus on stroke in Australia and in China, as these will be the target populations for the proceeding studies.

In Australia, stroke is the leading cause of morbidity and the second leading cause of mortality. It is estimated to cost a total $5 billion in health care costs, lost productivity, carer costs and total burden of disease cost. In 2017, there are more than 475,000 people living with the effects of stroke, estimated to rise to 1 million people by 2050. Stroke is indiscriminate: affecting men and women equally from diverse backgrounds and with 30% of survivors under the age of 65 (26).

In China, stroke is now the leading cause of adult death and disability, exceeding heart disease, with 2.5 million incident strokes each year and 7.5 million stroke survivors (27). It has been suggested that with a rapidly developing economy in China, the risk factors for stroke have increased substantially. For example, there was a 97% increase in diabetes prevalence and a 13% increase in obesity in urban china and 85% increase in rural China between 1994 and 2002 (27). A high proportion of the Chinese population smoke (62.4% of men and 3.4% of women) (28). Data from the Chinese National Stroke Registry has shown that 21.5% of patients with acute strokes presented to emergency within 3 hours of stroke onset, with 12.6% of those eligible for thrombolytic treatment and 1.6% overall receiving IV-tPA. (27) According to the Nanjing Registry for first-time strokes, of a total of 752 patients, 142 (18.9%) were classified as ICH, 120 (16.0%) as large

artery atherosclerosis, 123 (16.4%) as small vessel stroke, 160 (21.3%) as cardioembolic stroke and 216 (28.7%) as undetermined causes (29). Stroke risk factors and their significance will be discussed in the following section.

Aetiology

There are many risk factors for stroke including increased age, male sex, previous stroke history, atrial fibrillation, hypertension, diabetes mellitus, cigarette smoking, excessive alcohol, cardiac disease, social status and heredity. These known risk factors had been thought to account to approximately 60% of the stroke risk (30). However, the INTERSTROKE case-control study of first acute stroke identified 10 risk factors (hypertension, smoking, diabetes, a diet-risk score, lack of regular physical exercise, depression, cardiac causes and the ratio of apolipoproteins B to A1 and waist-to-hip score) to contribute 90% of attributable stroke risk (31). In addition, these factors have also been shown to contribute to increased likelihood of worse outcome post stroke and increased chances of recurrent stroke, discussed below.

Age

Age is the single most important risk factor for stroke, with the risk of stroke doubling each successive decade after the age of 55 years (32). The reason for this is multifaceted. Older individuals are more likely to have hypertension,

hypercholesterolemia, diabetes and coronary heart disease, all known factors contributing to stroke. Furthermore, post stroke disability is higher in elderly populations as patients are often unable to undertake intensive rehabilitation and physiotherapy. Men have a 1.25 greater incidence of stroke than women but women have an increased disability after stroke relative to men (33). This is thought to be related to women having a higher life expectancy (more likely living alone at time of stroke) and increased disability with age in comparison to men (33). It is important to note that in the last decade the incidence of ischaemic stroke in younger adults is also rising. This is thought to be because of a rising prevalence of some important traditional vascular risk factors including hypertension, hypercholesterolaemia, diabetes mellitus and obesity in this age group (34). This is particularly important for long-term prognosis after stroke from the perspective of young patients usually having a life expectancy of several decades and should not just include risk factor management but also psychosocial support for other consequences (e.g. returning to work or planning a family) (34). This patient group is also more subject to secondary stroke sequelae such as post stroke epilepsy (reported to affect 2.4-14.4% of young patients with ischaemic stroke (35, 36)) and pain management where to date, little has been examined on post stroke pain in a younger stroke group. It is also suggestive that additional preventative measures are needed in this age group.

Diabetes mellitus

Diabetes mellitus is a strong risk factor for the development of stroke. It is associated with increased mortality; independent of stroke severity (37). The relative risk of stroke is approximately doubled in patients with diabetes compared to non-diabetics (37). The high risk of stroke in diabetics is due to the interplay between hemodynamic and metabolic components of the diabetic syndrome, possibly through increasing peripheral resistance and accelerating the atherosclerotic process (38). It has been suggested that 15% of the cost of managing cerebrovascular disease is attributable to diabetes (38).

Blood pressure

Population-based studies have shown continuous linear relationships between systolic and diastolic blood pressure and the risk of stroke (39). Higher blood pressure is associated with stroke independent of other risk factors (40). Elevated blood pressure accelerates the atherosclerotic process, increasing the likelihood of cerebral lesions from stenosis and emboli originating from large extracranial vessels (41). Reducing blood pressure in patients post stroke is one of the most potent interventions to reduce the risk of recurrent stroke (40).

Alcohol

Alcohol consumption is a major risk factor for hypertension, which, as mentioned, is a leading risk factor for stroke. Its association is dose dependent (42). That is, in both male and female populations heavy drinkers are at an increased risk of all stroke types than non-drinkers (43). However, moderate consumption (<3 drinks per day) seems to have a protective effect for ischaemic stroke, which is not seen in non-drinkers (43). The most likely explanation for this trend is that heavy drinkers have increased systolic and diastolic blood pressures as compared to persons who drink alcohol only occasionally or not at all. Alcohol also increases the risk of ischaemic strokes by increasing the likelihood of heart arrhythmias (particularly atrial fibrillation), cardiac wall mobility disorders, arterial hypertension, activation of the coagulation cascade and impaired blood flow to the brain (44).

Tobacco

Cigarette smoking has shown to be an independent risk factor for stroke in men and women (45). Smoking doubles the risk of a stroke compared with non-smokers (45). It is estimated that approximately 19% of the burden of stroke is attributable to smoking (45). Former smokers of both sexes have a lower risk of stroke than current smokers, with no indication of this being sex specific (45). Cigarette smoking increases atherosclerosis in all vascular beds, but is particularly important in predisposing the patient to stroke.

Clinical predictors of acute stroke outcome

Overview

There are certain clinical predictors of stroke outcome that treating physicians use to guide treatment decision-making in the acute setting. These include the National Institutes of Health Stroke Scale (NIHSS) score, age of patient at stroke onset, the presence of atrial fibrillation (AF), time from stroke onset to arrival to treating centre and brain imaging appearances. Description of these clinical predictors is pertinent as these factors are all considerations for design and execution of stroke research.

The National Institutes of Health Stroke Scale (NIHSS) score

The National Institutes of Health Stroke Scale (NIHSS) score is a standardized neurological assessment that measures neurological impairment and is used as a clinical predictor for treatment and potential outcome (46). It is a 42 point ordinal scale containing 15 items; including level of consciousness, visual field defects and motor and sensory involvement, which demonstrate the level of severity of stroke symptoms, see below (47).

Table 1: Summary of the National Institute of Health Stroke Scale (NIHSS) score

1a) Level of consciousness	0=Alert 1=Not Alert 2=Not alert 3=Responds only with reflex motor or autonomic effects or totally unresponsive, flaccid.
1b) Loss of consciousness questions	0=Answers both questions correctly 1=Answers one question correctly 2=Answers neither question correctly
1c) Loss of consciousness commands	0=Performs both tasks correctly 1=Performs one task correctly 2=Performs neither task correctly
2) Best Gaze	0=Normal 1=Partial gaze palsy 2=Forced deviation
3) Visual	0=No visual loss 1= Partial hemianopia 2= Complete hemianopia 3= Bilateral hemianopia
4) Facial Palsy	0=Normal 1= Minor paralysis 2= Partial Paralysis 3= Complete Paralysis
5) Motor Arm	0=No drift 1=Drift

	2=Some effort against gravity 3=No effort against gravity 4=No movement 5a) Left Arm 5b) Right Arm
6) Motor Leg	0=No drift 1=Drift 2=Some effort against gravity 3=No effort against gravity 4=No movement 6a) Left Leg 6b) Right Leg
7) Limb Ataxia	0=Absent 1=Present in one limb 2=Present in two limbs
8) Sensory	0=Normal 1=Mild-to-moderate sensory loss 2=Severe-to-total sensory loss
9) Best language	0=No aphasia 1= Mild-to-moderate aphasia 2= Severe aphasia 3=Mute, global aphasia
10) Dysarthria	0=Normal 1= Mild-to-moderate dysarthria 2= Severe dysarthria

11) Extinction and Inattention (Neglect)	0= No Abnormality 1= Visual, tactile, auditory or personal inattention 2= Profound hemi-inattention or extinction to more than one modality

The items were selected based on expert opinion and literature review, hence satisfying the requirements for content validity (47). Inter-rater reliability of the NIHSS has been demonstrated by neurologists, physicians and non-physicians (47). The scale correlates with measures of neurological outcome and activities of daily living (ADL) scales (47), and is a strong predictor of recovery after stroke; a score of $\geq$16 indicating a higher probability of death or severe disability and a score of $\leq$6 indicating a high probability of good recovery (48). Severe strokes (NIHSS 21-42) account for 20% of the total population, of which the majority will be fatal, and more than half of the survivors will have severe disabilities without intra-arterial therapies (49). It is these patients who represent major therapeutic challenges. This is because in deciding to treat, for example, hypertensive patients with more severe strokes and a higher risk for symptomatic intracranial haemorrhage (sICH) it can be difficult to balance risk and benefit. The NIHSS can also be used to recruit patients for trials and a measurement of clinical outcome in trials. See appendix for the full NIHSS.

Age

Stroke outcome can be greatly improved by the administration of timely and effective treatment, such as thrombolysis or endovascular clot retrieval, which will be discussed in detail further on. In particular, the use of thrombolysis for acute stroke is associated with better functional and neurological outcomes in adult patients, irrespective of their age (50). In age groups >80 years old, less favorable outcomes are more likely than in age groups <80 years old (50). Less favorable outcomes are expected to occur in the elderly mainly due to greater comorbidities, additional disabilities, medical histories and brain atrophy (51). In the rehabilitation setting, advanced aged is associated with worse functional outcome due to limited physical tolerance in intense rehabilitation programs and slower functional recovery (51), contributing to less favourable outcomes.

Atrial Fibrillation

Atrial fibrillation (AF) accounts for 18-28% of all strokes and is both a risk factor for stroke and clinical prognostic factor for stroke outcome (52). AF is the most common cardiac arrhythmia affecting the elderly. It is present in 1% of the general population and, in those older than 65 years, its prevalence increases to 5.9% (53). It also increases stroke severity, mortality and is associated with worse outcomes at 3-months (54). In addition, patients with atrial fibrillation have longer hospital stays and are less likely to be discharged home (55). It is thought that the adverse effect of AF is due to greater volumes of more severely hypo-

perfused tissue, leading to larger infarct size and an increased risk of sICH (56). The left atrial appendage (LAA) is the remnant of the embryonic left atrium and is largely considered to be non-functional (57). However, the LAA site is considered to be the primary site of thrombus formation in a majority (>90%) of patients with AF (58). AF also adversely affects cardiac haemodynamics due to loss of atrial contraction and irregularity of ventricular rate (59). It is initiated by rapid electrical activity, which eventually causes structural remodelling. These changes are initially reversible, however, if sinus rhythm is not restored, permanent structural changes may result such as enlarged cardiac chambers (59).

Stroke prevention in patients with AF is one of the most common indications for the use of anticoagulant therapies (60). However, the largest consideration for physicians is the balance in the individual patient of the efficacy of these therapies in preventing recurrent stroke versus the risk of intracerebral haemorrhage (ICH). ICH is the most common and least treatable neurological complication of these therapies (61).

Imaging Predictors of acute stroke outcome

Overview

Traditionally, the main role of neuroimaging in acute stroke has been to identify intracerebral haemorrhage and, in the absence of haemorrhage, infer a diagnosis of ischaemic stroke based on clinical features. The role of imaging has expanded and it has become a critical tool in the evaluation and management of acute ischaemic stroke (62). Imaging can now make a positive diagnosis of ischemic stroke, identify patients with large vessel occlusion who require endovascular thrombectomy and inform determination of stroke mechanism. Imaging is also a powerful prognostic tool (62). The ultimate goal of imaging is to maximize the number of appropriately treated patients and minimize delays to treatment.

Stroke reperfusion treatments are time critical with improved outcomes associated with even a few minutes reduction in onset to treatment time (63, 64). However, individual variation in stroke pathophysiology means that the appearance of brain imaging can provide a more accurate prognosis than time alone (65).

MRI with diffusion weighted imaging (DWI) measures the net movement of water in tissue due to random molecular motion of water (66). In early ischaemia, energy depletion leads to failure of Na+/K+ ATPase leading to net influx of water from the extracellular to intracellular compartment (cytotoxic oedema).

Intracellular water has restricted diffusion and this increase is evident as hyperintensity on DWI (and hypointensity on apparent diffusion co-efficient maps). DWI is more sensitive than CT, particularly for small ischaemic lesions and brainstem strokes, and also for patients presenting early after stroke as the ionic and vasogenic oedema required to create early ischaemic change on CT (through increased total tissue water content) take longer to evolve (66). DWI shows hyperintense ischaemic areas within a few minutes to a few hours post stroke onset. Other MRI sequences are highly sensitive for intracerebral haemorrhage (67). However, major disadvantages of MRI for acute stroke evaluation render CT as still the most widely used imaging tool. Patient contraindications, such as claustrophobia, agitation and metallic implants (and the ability to screen patients for these implants) are a major disadvantage to the use of this technique (68). Furthermore, despite improvements in medical infrastructure, many stroke centers do not have the resources, funding, time or staff to perform a DWI/MRI immediately after admission (69). Consequently, in a situation where time is critical, it is not always efficient or effective to evaluate whether the patient is safe for a MRI/DWI and may lead to potentially longer acquisition times, Due to these logistical obstacles, safety, cost and the feasibility of emergency setting MRI scans, computed tomography scans (NCCT, CTP, CTA) are still the most widespread modality, and CT-based imaging is used at the Royal Melbourne Hospital.

The Alberta Stroke Program Early CT Score (ASPECTS) is a commonly used approach to assessing early ischaemic change on non-contrast computed tomography (NCCT). More extensive ischaemic changes (lower ASPECTS) have been associated with less favourable functional outcome and sICH after reperfusion therapies (70-72). CT Perfusion (CTP) can be rapidly acquired immediately after NCCT and improves diagnostic confidence through detection of the delayed blood flow characteristic of vessel occlusion (73-75). CTP has the added advantage of distinguishing potentially salvageable tissue from irreversibly injured ischaemic core (76). Knowledge of cerebral blood volume (CBV), cerebral blood flow (CBF) and Tmax may also influence decision-making for reperfusion therapies, (76) with recent work showing CTP can be used to predict favorable outcome after thrombolysis (77-79). Collateral circulation, which can be assessed using CTP, CT Angiogram (CTA) or catheter Digital Subtracted Angiogram (DSA), refers to a subsidiary network of vascular channels that compensate during arterial insufficiency due to thromboembolism, compromised hemodynamic factors or a combination of both (80). Collaterals have been recognized to influence recanalization, reperfusion, haemorrhagic transformation and subsequent neurological outcomes after stroke (81). These imaging tools will be discussed in turn.

Computed Tomography (CT)

For the evaluation of any stroke, it is the CT scan that is the most widely used imaging tool (82). Most emergency departments have 24 hour access to CT, there are no contraindications related to metallic implants, patients can be more easily visualized and monitored compared to MRI and acquisition times are rapid., allowing fast treatment and the possibility of predicting response to thrombolytic treatment (83). CT is highly sensitivity for the detection of ICH. Modern intravenous iodinated contrast has a low risk of allergic reactions and contrast nephropathy (84). Ionizing radiation does carry some long-term risk but this needs to be balanced against the urgent clinical need for information to guide treatment. Scatter doses in current generation CT scanners are low which is relevant when considering imaging women of child-bearing age (85, 86).

CT scans measure x-ray beam attenuation through the brain, with attenuation directly proportional to tissue density. The object is examined by rapidly rotating the X-ray source around the object. The multiple x-ray beam projections can then be reconstructed into a 3D representation of that object. For each x-ray beam that is passed through the body, the exiting intensity is measured. The exiting beam is known as the linear attenuation coefficient. Attenuation values are represented as Hounsfield Units (HU) - a linear density scale in which water has an arbitrary unit of zero. Thus, denser tissues such as bone, muscle and blood have a positive HU value and a grey-scale brightness that is lighter than water. Conversely, tissues

that are less dense than water, such as fat and air, have a negative HU value and appear darker than water on the CT brain scan (87).

The pathophysiology of ischaemic stroke is the lack of sufficient blood flow to perfuse cerebral tissue. In the acute stroke setting, it is mandatory to perform brain imaging to exclude intracerebral haemorrhage and mass lesions, evaluate the extent of ischaemic changes and assess a patient's eligibility for reperfusion therapies (69). Cerebral ischemia occurs as a consequence of focal disruption of the cerebral circulation, resulting in neuronal dysfunction occurring within minutes. The immediate biochemical response is a cessation of oxidative phosphorylation and thus the depletion of ATP stores. Under these ischaemic conditions, brain tissue responds by switching to the less efficient glycolytic pathway to generate ATP, converting glucose to lactic acid (87). In human adults, the brain is responsible for 20% of total body oxygen consumption, with approximately 70% of the metabolic demand attributable to Na+/K+-ATPase pumps that maintain ion gradients (88). Under ischaemic conditions, mitochondrial production of ATP cease and intracellular ATP stores deplete within 2 minutes. The cell membrane subsequently depolarizes leading to an influx of calcium and sodium, and an efflux of potassium. Cytotoxic oedema is a sign of cell death, characterized by the influx of Na+ down its concentration gradient, and resultant influx of Cl- and water until eventually the membrane ruptures leading to necrotic cell death (89). This results in the redistribution of water from extracellular spaces to intracellular spaces (88).

After several hours, the tight junctions linking endothelial cells become disrupted, affecting the blood brain barrier. The earliest phase of endothelial dysfunction in ischaemia is characterized by the formation of ionic oedema, which is what plain CT reflects. Formation of ionic oedema involves transport of Na+ across the blood-brain barrier, generating an electrical gradient for Cl- and an osmotic gradient for water in the extracellular space, replenishing Na+, Cl- and water in the extracellular space that was depleted from the formation of cytotoxic oedema (89). Cells within this ischaemic core become irreversibly destroyed through degradative processes and can lead to haemorrhagic conversion.

As summarised by Simard et al. there are thought to be 3 phases to the blood brain barrier injury that leads to haemorrhagic conversion. As described, phase 1 is the formation of ionic oedema, the earliest phase of endothelial dysfunction in ischaemia. Phase 2 is the formation of vasogenic oedema and is characterized by the breakdown of the blood-brain barrier, with leakage of plasma proteins into extracellular space. And the third phase is the haemorrhagic conversion, marked by catastrophic failure of capillary integrity leading to the extrusion of all constituents of blood into the brain parenchyma. Some degree of haemorrhagic transformation is common and part of the natural history of large infarcts. However, symptomatic intracerebral haemorrhage due to a large parenchymal haematoma can be a cause of early mortality in patients with acute ischaemic stroke. The phases are thought to occur sequentially, but this most likely depends

on factors such as duration and depth of hypoxia during perfusion or prior to reperfusion.(89)

Plain CT reflects the phase 1 process of ionic oedema, but it is unable to detect the earliest signs of ischemia (cytotoxic oedema) as there is only redistribution rather than increase in tissue water (89). On CT, ionic oedema is reflected by areas of low attenuation change due to influx of water in the extracellular spaces and loss of grey-white matter differentiation. For every 1% increase in the tissue water content, there is a reduction in the X-ray attenuation by 3-5% (an equivalent of a reduction of 2.5 HU on CT) (87). CT shows existing irreversible infarction, but it does not show tissue at risk. The hyperdense artery sign (HAS) has long been known as an indicator of occluding clots in cases of acute ischemia, first reported in 1983 by Gács et al (90). It is the earliest sign and is visible long before parenchymal changes, which are known as early ischemic signs (91). Hyperdense arteries represent acute thrombus and can aid diagnosis when identified on the initial CT scan. They have always been associated with a poor clinical outcome, large volume strokes and severe neurological deficits (92). In patients with a HAS, some studies have reported a benefit from reperfusion therapies (intra-venous thrombolysis and intra-arterial therapy) (93, 94), however other studies do not (92, 95). The unreliability of the HAS is probably due to the larger slice thickness on NCCT compared to the size of the vessel resulting in partial volume effects. However, thin slices (<2.5mm) reduce partial volume effects and improve

sensitivity (96). Arterial calcification and high haematocrit can confound interpretation.

With the exception of the HAS, during the first few hours after an acute ischaemic stroke the initial CT scan may not show any parenchymal abnormalities (97, 98). Subtle early ischaemic signs are loss of gray-white differentiation in the cortex and particularly the poorly collateralized (and therefore severely hypoperfused) basal ganglia and insula. Early swelling due to increased cerebral blood volume in maximally dilated vessels can be seen as a loss of sulci and can be potentially reversible with rapid reperfusion (99). More severe mass effect causing compression of the ventricular system can occur in subsequent days (87).

In the case of an acute stroke, the goals of imaging are to confirm the infarct location, determine the extent of ischemia, exclude haemorrhages and other non-stroke diagnoses (e.g. tumour), and to determine treatment (87). As time progresses, the intensity of brain swelling and mass effect increases, thus representing the subacute phase (100). In addition to defining the anatomical location and extent of lesion, in the subacute phase the goal of imaging is to determine the difference between ischaemic lesions and infarcted lesions. Therapy and prognosis differ depending on whether the infarction is acute, subacute or chronic (100).

The findings on CT scan vary among individuals depending on the size, duration and severity of the infarct, the metabolic state of brain tissue and the presence of collateral blood flow (87). The Alberta Stroke Program Early CT Score (ASPECTS) encourages a systematic approach to non-contrast CT interpretation and is intended to improve the reliability of detection of early ischaemic change, correlating to poorer stroke outcome with thrombolytic therapies (70).

Alberta Stroke Program Early CT Score (ASPECTS)

The Alberta Stroke Program Early CT Score (ASPECTS) tool was developed to provide a standard CT scan with a reproducible grading system (101). It is a semi-quantitative method of defining infarct extent in the MCA territory (102). It was designed as a predictor of outcome in terms of dependence, independence and sICH (102). It is more reliable than the hyperdense middle cerebral artery sign and has been shown to be a predictive marker for outcome after thrombolytic treatment (102). ASPECTS was designed for conventional non-contrast CT but has been established as a reliable predictor of clinical outcome on MRI (103). ASPECTS has proven reliable among physicians of different clinical backgrounds and experience (101).

The ASPECTS score examines the middle cerebral artery territory and is an ordinal score out of 10 with a score of 10 indicating a normal CT brain scan. It is determined from the evaluation of the MCA territory. A point is deducted for each

region within the brain affected by ischaemic changes (101). These regions include M1, M2, M3, M4, M5, M6, Insula, Caudate Nucleus, Lentiform Nucleus, and posterior limb of the Internal Capsule, refer to figure below.

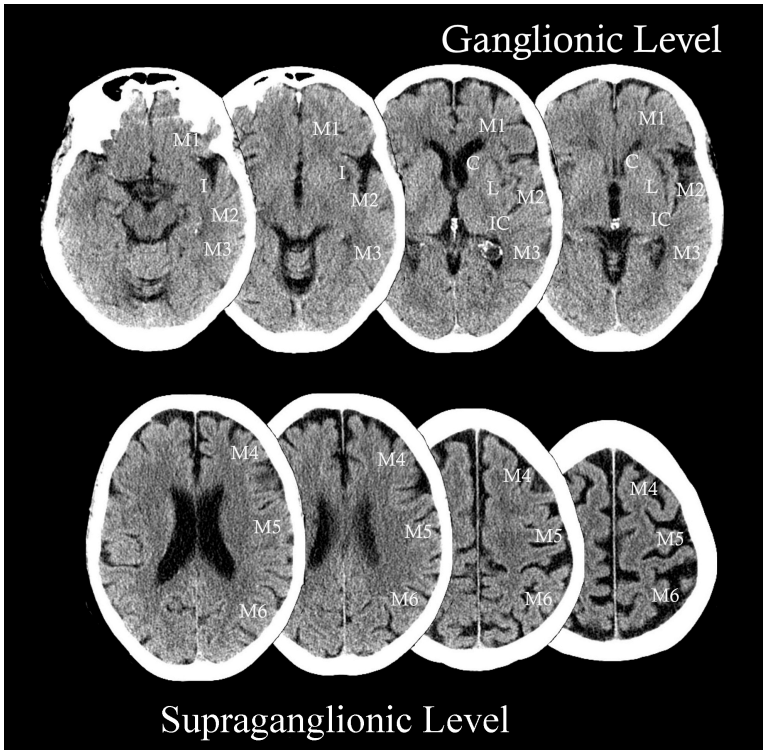

Figure 2: Labeled ASPECTS regions on a non-contrast CT Scan. Regions include M1, M2, M3, Insula, Caudate Nucleus, Lentiform Nucleus, and posterior limb of the Internal Capsule at the level of the ganglia and M4, M5, M6 at the supraganglionic level. (http://www.spencercolecollier.com)

The relevant ischaemic changes include hypointense areas and loss of grey/white demarcation and should be visible across 2 axial thick slices. Early swelling and sulcal effacement should not contribute to ASPECTS scoring as this can represent

increased CBV in salvageable penumbra (99). A score >7 indicates a high probability of good functional outcome (modified Rankin score), and a score ≤7 is likely to be associated with a poor functional outcome following tPA treatment (101).

The significance of early ischaemic changes on CT remains unclear. Uncertainty remains over whom to treat; for instance, the elderly with severe stroke or with early ischaemic changes on CT (70). For patients with thrombolytic treatment, as the ASPECTS value decreases, the probability of functional dependence, death and sICH increases (70). It has been shown that the ASPECTS score can identify patients with MCA occlusions that will receive particular benefit from thrombolysis treatment, when they have a baseline ASPECTS of >7 (9). The ASPECTS also has high sensitivity and good specificity for sICH. In the original study conducted by Barber et al, for patients with an ASPECTS of 7 or less, the risk of sICH with tPA treatment is 14-times greater than a patient with an ASPECTS above 7, with baseline ASPECTS value a significant predictor of sICH; OR=14, 95% CI (2-117), p=0.012 (101). However, this could not be reproduced in studies based on the data from the ECASS-II or NINDS-stroke trials (104, 105).

Early ischaemic change on NCCT is strongly time dependent. The earlier the scan is performed, the more difficult it is to detect early signs of ischemia and many studies report low inter-rater agreement for presence and extent of early ischaemic

change, (k=0.20-0.88) (106). Within 4.5 hours, the therapeutic window for IV-tPA the rate of detection is < 67% for images within 3 hours of stroke onset, increasing to >80% at 6 hours, outside the therapeutic window for thrombolysis (106-108). It has been shown that the ASPECTS score at 24 hours after stroke onset is a better predictor of clinical outcome at 3-months than the baseline ASPECTS (103). This likely relates to early insensitivity of CT to ischaemic injury and infarct growth in those without reperfusion that can be detected at 24h.

Computed Tomography Perfusion (CTP)

Brain imaging with CT Perfusion (CTP) has been shown to provide pathophysiological information in acute ischaemic stroke that non-contrast CT does not. NCCT changes represent irreversibly injured ischaemic core (109). CTP cerebral blood volume (CBV) reduction or severely reduced cerebral blood flow (CBF) also estimate ischaemic core but, unlike NCCT, visual conspicuity of the abnormality is not dependent on development of ionic edema, which takes time to evolve (110). This does, however, depend on an assumption that the visualized low flow has been consistently present for some time prior to imaging in order for the ischemic injury to become permanent (and in general this assumption holds for clinical purposes). CTP also has the added advantage of distinguishing potentially salvageable tissue (penumbra) from ischaemic core, as represented by the Tmax (time to maximum of the tissue residue function after deconvolution) parameter which represents delayed arrival of blood flow via collateral pathways

(76). Given that acute stroke treatments such as thrombolysis remain underutilized, reducing procrastination and increasing diagnostic confidence through knowledge of cerebral blood volume (CBV), cerebral blood flow (CBF) and Tmax may influence the decision for thrombolytic treatment for more marginal patients (111), with recent work showing CTP can be used to predict favourable outcome after thrombolysis (77, 78).

Assessment of ASPECTS regions has also been applied to CTP and studies have suggested greater correspondence with follow-up MRI and greater inter-rater agreement than NCCT ASPECTS, kappa on NCCT between 4 raters=0.219 versus kappa on CTP =0.980 (112, 113).

Computed Tomography Angiogram (CTA) and Digital Subtraction Angiography (DSA)

Methods for obtaining information on collateral status can be ascertained using advanced neuroimaging techniques. Both angiographic and perfusion information can be used to accurately measure collateral flow and structure. Among the 4 common clinical methods for imaging collateral status, digital subtraction angiography (DSA) is considered gold standard (114). DSA allows for the measurement of collateral status and number in all collateral circulations with both temporal and spatial resolution (114). It has good inter-observer reliability and thus is a good modality for collateral scoring techniques. However, DSA is invasive in nature, with a contrast dye injection into the femoral artery preventing

it from being performed routinely. It is therefore now chiefly reserved for therapeutic purposes and the time critical nature of thrombectomy means that full collateral imaging (requiring injection of both carotids and a vertebral artery) is not performed routinely prior to thrombectomy. Therefore, in MCA occlusion the anterior cerebral artery leptomeningeal contributions can be assessed with ipsilateral carotid injection, but posterior cerebral artery contributions are unknown. In ICA T-occlusion no collateral information is derived from an ipsilateral injection. Computed Tomography Angiography (CTA) also provides good inter-observer reliability, is non-invasive and is rapidly available making it ideal for assessing the collateral status in patients with acute stroke (114). However, CTA is often timed to peak arterial phase meaning that collateral flow, which arrives later, can be underestimated using this approach. Dynamic CTA derived from perfusion data or multiphase CTA provides non-invasive and accurate assessment of all collateral pathways (115).

The Collateral Circulation

In the setting of acute ischaemic stroke, arterial revascularization to restore antegrade perfusion to the ischaemic territory remains the principal therapeutic approach (116). Collateral circulation refers to a subsidiary network of vascular channels that compensate during arterial insufficiency due to thromboembolism, compromised hemodynamic factors, or a combination of both (80). Brain at risk of infarction during an acute ischaemic stroke can remain potentially salvageable

beyond the accepted time window of 4.5 hours from thrombolysis. Collaterals have been recognized to influence recanalization, reperfusion, haemorrhagic transformation and subsequent neurological outcomes after stroke (81). Both extracranial sources of cerebral blood flow and intracranial routes of compensatory perfusion contribute to collateral circulation. These sources can be divided into primary and secondary collateral pathways. The primary intracranial collateral circulation is largely recognised as the Circle of Willis, which may provide arterial anastomoses between the two anterior cerebral arteries (ACA) via the anterior communicating artery and connect the anterior and posterior circulations via the posterior communication artery via the internal carotid artery (ICA) and the posterior cerebral artery (PCA) (114). There is considerable inter-individual variation in the calibre and existence of these communicating segments. Secondary to the Circle of Willis, retrograde flow via ophthalmic artery and leptomeningeal anastomoses constitute secondary collateral circulations. Leptomeningeal collateral circulation provides crucial nutritional support to the penumbra, potentially reducing the degree of infarction post stroke (114). The leptomeningeal anastomoses across the cortical surface connect the ACA and middle cerebral artery (MCA), the MCA and PCA and the PCA and ACA. Robust leptomeningeal collaterals have been linked with rapid recanalization of MCA occlusion and possible prevention of larger infarcts and also predictive of improved long-term clinical outcome in patients with MCA occlusions (80). Furthermore, with a greater degree of collaterals after an ischaemic stroke, there are reduced haemorrhagic complications after thrombolysis and endovascular

therapy, improved recanalization rates, smaller infarct size, smaller infarct growth and improved clinical outcome (114).

Collateral status is recognized to vary across individuals and populations depending on baseline risk factors, time course of ischaemic injury, severity of ischaemic injury, imaging findings, treatment options and management of secondary outcomes. For example, the anatomy of the Circle of Willis varies between individuals, with approximately 50% of individuals with an incomplete Circle of Willis (117). The presence of these variations compromises the ability for collateral compensation during an arterial occlusion. Similarly, other variability has been noted across populations. For example, Moyamoya cerebral angiography is characterized by a progressive stenosis or occlusion of the ICA and/or the proximal portion of the ACA and MCA (118). This steno-occlusive pattern on conventional angiography appears as a "puff of smoke" (Moyamoya in Japanese) (118). Ischaemic stroke is the most common presentation in young adults with Moyamoya disease but it also associated with an increased risk of haemorrhagic stroke (119). It is rare in Western ethnicities and occurs approximately at a ten-fold increase in East-Asian countries (118). Furthermore, it is known that the level of leptomeningeal reserves in humans demonstrates substantial variation in their distribution, size, number and compensatory capacity, with the suggestion that they are also modifiable depending on factors such as age and metabolic syndrome, for example (120). Older age, chronic

hypertension, hyperlipidaemia and the diameter of blood vessels and their pressure gradients may also influence the degree of collateral circulation (120).

Studies have examined the effect of genetic variation in mice on the influence of native pial collateral circulation and severity of stroke. The findings show that the extent of the native pial collateral circulation and collateral remodelling after occlusion in the MCA territory vary widely with genetic background and that this variability is a major contributor to differences in final infarct volume (121). Other studies in mice have shown there are genetic factors (such as VEGFA and CLIC4 expression) that may regulate collateral circulation, including collateral vessel density and diameter (122).

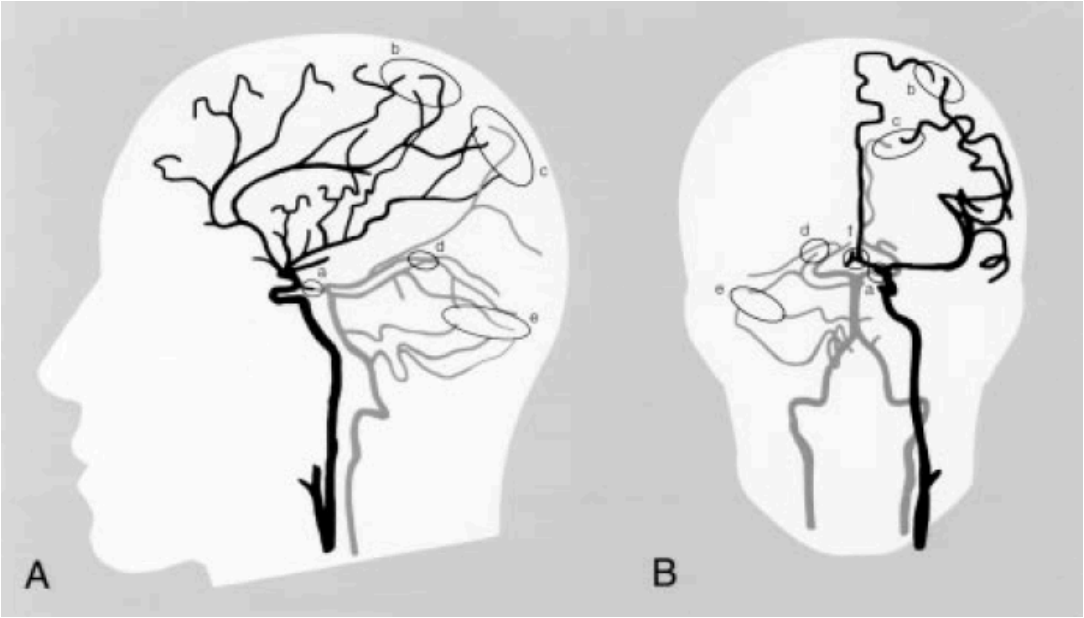

Figure 3: Diagrammatic representation of the intracranial arterial collateral circulation in lateral (A) and frontal (B) views.

Shown are posterior communication arteries (a); leptomeningeal anastomoses between anterior and middle cerebral arteries (b) and between posterior and middle cerebral arteries (c); tectal plexus between posterior cerebral and superior cerebellar arteries (d); anastomoses of distal cerebellar arteries (e); and anterior communicating artery (f). (80)

These anatomical differences and potential genetic variations between ethnic groups may account for the differences in incidence of cerebrovascular diseases, incidence of secondary neurological sequelae, and overall patient outcomes.

Measuring acute stroke outcomes

The modified Rankin Scale

The modified Rankin Scale (mRS) is a popular and widely used assessment of global functional outcome in stroke (123). It is the predominant primary endpoint for measure functional outcome in acute stroke clinical trials (123). The mRS is a 7 point ordinal scale (0-6), providing a measure of an individual's independence or dependence post stroke: Grade 6, denotes a deceased patient; grade 3, denotes moderate disability requiring some help; and grade 0, denotes no residual symptoms (123). The mRS meets the criteria for a satisfactory clinical assessment with a reasonable inter-rater agreeability. Many studies consider a good outcome (favorable or independent outcome) as mRS 0-2 and a poor outcome (unfavorable or "death and disability" outcome) as a mRS score of 3-6 (123).

Table 2: Summary of the modified Rankin Scale (mRS)

0	No symptoms
1	No significant disability, despite symptoms, able to perform all usual duties and activities
2	Slight disability; unable to perform all previous activities but able to look after own affairs without assistance
3	Moderate disability; requires some help but able to walk without assistance
4	Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance
5	Severe disability; bedridden, incontinent, requires constant nursing care and attention
6	Deceased

The Barthel Index

The Barthel Index (BI) is a scoring technique that measures the patient's performance in ten activities of daily living. It is considered a reliable scale for measuring a patient's disability, including feeding, grooming, bathing, ambulation, stair climbing. A maximum score of 100 indicates the patient is fully independent in physical functioning and a score of 0 represents a totally dependent bedridden state (124).

Symptomatic Intracranial Haemorrhage (sICH)

One of the most feared complications of reperfusion therapies in stroke is the risk of symptomatic intracerebral haemorrhage (sICH), which occurs in approximately 1.7%-6% of patients (depending on sICH definition and treatment type) and carries a mortality rate of close to 50% (125). Haemorrhagic transformation of ischaemic brain tissue occurs as a result of extravasation of blood into the brain tissue. Some degree of haemorrhagic transformation is part of the natural history of large infarcts. However, if the haematoma exerts mass effect beyond the original infarct it is likely to worsen the functional outcome. The rates of sICH remain quantitatively similar whether treatment with IV-tPA is administered within 3 hours or 3-4.5 hours post stroke onset (126, 127). Haemorrhagic events are classified according to clinical and CT criteria. Haemorrhagic infarction 1 (HI1) is defined as small petechiae along the margins of the infarct; haemorrhagic infarction 2 (HI2) as confluent petechiae within the infarcted area but no space-occupying effect; parenchymal haemorrhage (PH1) as blood clots in 30% or less of the infarcted area with some slight space-occupying effect; and parenchymal haemorrhage 2 (PH2) as blood clots in more than 30% of the infarcted area with substantial space-occupying effect. However, the definition of symptomatic ICH has differed over the decades.

Table 3: CT criteria for the definition of symptomatic intra-cerebral haemorrhage

Haemorrhage Classification	Definition
HI1 (Haemorrhagic infarction)	Small petechial haemorrhage without space-occupying effect
HI 2	More confluent petechial haemorrhage without space-occupying effect
PH 1 (Parenchymal Haematoma)	Haemorrhage in <30% of the infarcted area with mild space-occupying effect
PH 2	Haemorrhage in >30% of the infarcted area with major space-occupying effect

In the NINDS tPA trials, symptomatic ICH (sICH) was defined as any haemorrhagic transformation temporally related to any worsening in neurological condition, not necessarily altering the patient's NIHSS (7). The incidence of sICH in this trial was reported at 6.4%, with incidence rates not significantly differing between experienced and inexperienced centres (7).

This definition of sICH has been criticized as including patients whose ICH was minimal relative to the extent of ischaemic injury and unlikely to have altered long-term patient outcome. Consequently, other definitions of sICH have been introduced. The SITS MOST study defined sICH as a local or remote Type 2

parenchymal haemorrhage on imaging within 36 hours after treatment combined with a neurological deterioration of >=4 NIHSS points from baseline or from the lowest NIHSS score between baseline and 24 hours. (128) Within the SITS registry, the overall rate of sICH is ~1.8%. (128) The rates of sICH using the SITS-MOST definition were no higher with endovascular thrombectomy (4.4%) than with best medical therapy alone (4.3%), with mortality risk at 90 days not differing significantly between the groups (9).

In the ECASSII trial, the definition of sICH was blood at any site in the brain on the CT scan and documentation of clinical deterioration or adverse events indicating clinical worsening (drowsiness, increase in hemiparesis) or causing a decrease in the NIHSS score of 4 or more points. Within the ECASSII study, the rate of sICH in the IV-tPA treated patients was 8.8% (72).

In a comparison of the ECASSII, NINDS and SITS-MOST definitions of sICH within the SITS registry (n=31,627), the rate of sICH per the SITS-MOST definition was 1.8%, per ECASSII was 5.1% and per NINDS was 7.4% (128). The ECASSII and SITS-MOST sICH definitions, which combine radiological features and occurrence of substantial early neurological deterioration, best identify sICH that alter the patient's outcome (129).

Table 4: Clinical and radiological criteria for the definitions of sICH in the NINDS-tPA, ECASSII and SITS-MOST trials.

	Clinical	Radiological
NINDS tPA trials	Any worsening	Any ICH
ECASSII	Only >=4 NIHSS	Any ICH
SITS-MOST	Only >=4 NIHSS	PH only

Many factors are thought to contribute to an increased risk of sICH following thrombolytic treatment. These include hypertension, atrial fibrillation, increasing age, delayed time to reperfusion and high NIHSS (130). Additionally, imaging tools can be used to predict the risk of sICH. These include early ischaemic changes on CT, large diffusion MRI lesions, profound cerebral blood volume reduction or severe hypoperfusion on perfusion MR or CT and early blood brain barrier disruption on permeability imaging (87). Scoring systems can be used to help predict the risk of haemorrhage in stroke patients eligible for thrombolytic treatment.

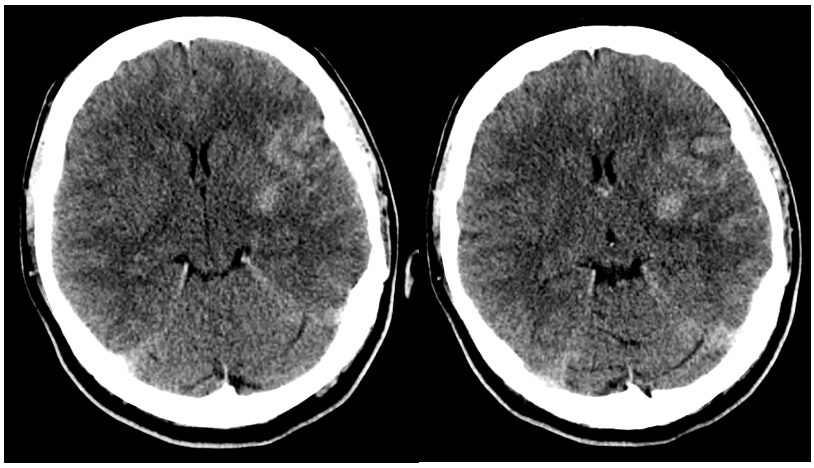

Figure 4: Non-contrast CT scan of 43-year old female with a left frontoparietal middle cerebral artery stroke. The multifocal higher density areas are regions of petechial haemorrhage.

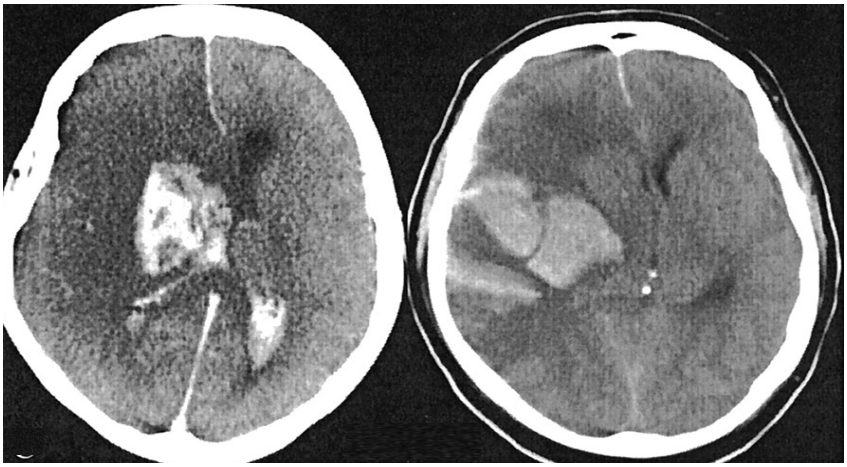

Figure 5: Non-contrast CT scan showing an example of PH-1 with a haemorrhage occupying <30% of the infarcted area and only mild space-occupying effect (left) and PH-2 with a haemorrhage occupying >30% of the lesion volume with significant mass effect (right).

Reperfusion therapies for acute ischaemic stroke

Thrombolytic drugs lyse blood clots by activating plasminogen. Tissue plasminogen activator (tPA) is a naturally occurring enzyme that is manufactured using recombinant technology to produce the drug alteplase. IV-tPA plays a major role in maintaining homeostatic control in the blood coagulation cascade (131). It acts by cleaving the precursor molecule plasminogen into its active form of plasmin, which can then lyse fibrin-based clots in focal cerebral ischemia (131). Plasmin is a proteolytic enzyme that is capable of breaking the links between fibrin molecules, which provide the structural integrity of the clots. IV alteplase treatment, within 4.5 hours of stroke onset, is associated with a significant reduction in disability at 3-months post stroke onset (132). This benefit has been shown to be independent of stroke severity (104). Various other thrombolytics have been trialled for stroke. Streptokinase was initially used but had an unacceptable risk of intracerebral haemorrhage and problems with allergic reactions (133). Intravenous urokinase is sometimes used in China and intra-arterial pro-urokinase was used in the PRO-ACT studies (134, 135). Tenecteplase has theoretical advantages over alteplase in increased fibrin specificity and resistance to the inhibitor PAI-1 and has shown promise in phase 2 trials (136, 137). Phase 3 trials are ongoing. Desmoteplase, derived from vampire bat saliva, has the highest fibrin specificity of thrombolytics trialled in humans but was unsuccessfully trialled in delayed time windows (138).

Intra-arterial therapy (IAT) for acute stroke refers to mechanical thrombectomy and intra-arterial thrombolysis. Mechanical thrombectomy devices for acute ischaemic stroke are designed to navigate cerebral arteries, capturing and retrieving thrombi through the use of microcatheters and guidewires to reestablish cerebral blood flow. IAT may be combined with IV-tPA, a rationale developed from the Emergency Management of Stroke (EMS) Bridging Trial (2006), which showed higher rates of recanalization, blood flow restoration, in patients receiving both IV-tPA and IAT. These endovascular treatments have been shown to restore blood flow effectively (139, 140). However in 2013, multiple randomized controlled trials (MR RESCUE, SYNTHESIS, and IMSIII) failed to show improved clinical outcome in patients treated with endovascular therapies (141-143), reporting neutral results comparing IV thrombolysis therapy with intra-arterial therapy in acute ischaemic stroke. These trials had important shortcomings, including imaging protocols that did not require a proven target vessel occlusion, less effective device technologies and selective recruitment due to limited equipoise to randomize among enrolling physicians. Perhaps the main limitation of these trials was the use of first-generation thrombectomy devices such as MERCI and early Penumbra aspiration catheters. The emergence of stent retrievers and highly trackable large bore aspiration catheters set the stage for a new generation of trials (144).

In 2015, five randomized controlled trials demonstrated the superiority of stent retriever thrombectomy over standard treatment (including intravenous alteplase

treatment in eligible patients) for acute ischaemic stroke patients with large artery occlusion, (145-149). This is now considered standard acute stroke therapy.

MR CLEAN (147) was a phase 3, multi-centre randomized controlled trial comparing IAT within 6 hours of stroke (including IA thrombolysis, mechanical thrombectomy, or both) plus usual care (IV alteplase in 90% of patients) to usual care (control group) in patients with acute ischaemic stroke with a proximal intracranial arterial occlusion, confirmed on CT angiogram (CTA), MR angiogram (MRA) or digital-subtraction angiography (DSA). The primary outcome for the trial was the modified Rankin score (mRS) at 90 days, and secondary outcomes including the National Institutes of Health Stroke Scale (NIHSS) at 24 hours, 5-7 days or discharge, Barthel Index (activities of daily living) and quality of life questionnaire at 90 days. This trial showed that IAT, within 6 hours of stroke onset to patients with a proximal intracranial occlusion of the anterior circulation, had clinically significant increase in functional status and independence at 90 days without increasing mortality.

EXTEND-IA (149) was a multi-centre, randomized controlled trial comparing IV-alteplase within 4.5 hours of stroke onset with IV alteplase within 4.5 hours plus stent retriever thrombectomy (commenced within 6 hours of stroke onset) in patients with an anterior circulation acute ischaemic stroke and proximal arterial occlusion, evidenced on CTA, and an ischaemic core <70 mL on CT perfusion imaging. The co-primary outcomes were neurological improvement, defined as NIHSS reduction of 8 or more points or a score of 0 or 1 at 3 days, and

reperfusion as the percentage reduction in the perfusion-lesion volume between baseline imaging and imaging at 24 hours. Both primary outcomes favored the IAT group with increased reperfusion and greater early neurological recovery and the mRS was also significantly improved in the thrombectomy group.

ESCAPE (148) was a randomized controlled trial comparing IAT within 12 hours (IA thrombolysis, mechanical intervention, or both) plus usual care (which included IV alteplase in eligible patients), to usual care in patients with an anterior circulation acute ischaemic stroke and proximal arterial occlusion, evidenced on vessel imaging and an Alberta Stroke Program Early CT Score (ASPECTS) of 6-10 on initial non-contrast CT (NCCT). The primary outcome was a shift in the mRS at 90 days. The primary end-point favoured intervention with a common OR (odds of improvement of 1 point on the mRS) of 2.6 (95% CI, 1.7-3.8), a median 90-day mRS score of 2 in the intervention group compared to 4 in the control group ($P < .001$), and a higher rate of functional independence (90-day mRS score 0-2) in the intervention group, 53.0% versus 29.3%. Additionally, the mortality rate at 90 days was lower in the intervention group compared to control group (10.4% vs 19.0%, $P = 0.04$), and there was no significant difference in the occurrence of symptomatic intracranial haemorrhage (sICH) between the 2 groups.

SWIFT PRIME (145) was a multi-centre, randomized controlled trial comparing IV alteplase with stent retriever thrombectomy (commenced within 6 hours of onset) to IV alteplase alone in patients with an anterior circulation acute

ischaemic stroke and proximal arterial occlusion, as evidenced on CTA or MRA. The primary outcome was the degree of disability at 90 days post stroke as measured by the mRS. There was a similar pattern of reduction in disability and favourable 90-day outcomes in the endovascular intervention group with 60.2% having an mRS ≤2 compared to 35.5% in the IV rt-PA only group, p =0.0002.

REVASCAT (146) was a multi-centre, randomized controlled trial comparing thrombectomy (within 8 hours) with medical therapy alone in patients who had received intravenous alteplase within 4.5 hours from symptom onset without revascularization after 30 minutes of alteplase infusion. The primary outcome was the mRS at 90 days. Thrombectomy reduced the severity of disability over the range of the modified Rankin scale (adjusted odds ratio for improvement of 1 point, 1.7; 95% confidence interval (CI), 1.05 to 2.8) and led to higher rates of functional independence (a score of 0 to 2) at 90 days (43.7% vs. 28.2%; adjusted odds ratio, 2.1; 95% CI, 1.1 to 4.0).

Published in 2016, two randomized controlled trials (THERAPY, PISTE) that were terminated early due to loss of equipoise following publication of the initial 5 trials reported trends to improved functional outcome that did not reach statistical significance. The PISTE trial was a multi-centre, randomised controlled clinical trial comparing intravenous thrombolysis alone with IVT and intra-arterial mechanical thrombectomy in patient who had acute ischaemic stroke with large artery occlusive anterior circulation stroke confirmed on CTA. The primary

endpoint was the proportion of patients achieving functional independence at 90 days (mRS 0-2). There was no significant difference between treatment groups for this endpoint (absolute difference 11%, adjusted OR 2.12, 95% CI 0.65- 6.94, p=0.2) but the magnitude of effect was consistent with the larger completed trials. (150) THERAPY was an international, multicentre, prospective, randomized (1:1), open label, blinded end point evaluation, concurrent controlled clinical trial of aspiration thrombectomy using the Penumbra device after intravenous alteplase compared to intravenous alteplase alone for patients with large vessel ischaemic stroke due to a thrombus length of >=8mm. In an attempt to identify the poorest prognosis patients, the THERAPY trial targeted patients with a thrombus length of 8mm or longer and failed to show any significant difference in the primary outcome of functional independence mRS 0-2 at 90 days, 38% IAT verses 30% intra-venous thrombolysis, p=0.52. (151)

A third randomised controlled trial published in 2016 (THRACE) randomly assigned patients with proximal cerebral artery occlusion to receive either intravenous thrombolysis alone or intravenous thrombolysis plus mechanical thrombectomy. The primary outcome was the proportion of patients achieving functional independence at 3 months, defined by mRS 0-2 although this outcome assessment was unblinded. There were 202 patients in the IVT group, of whom 42% achieved mRS 0-2 and there were 200 patients in IV-thrombolysis plus mechanical thrombectomy, of whom 53% achieved mRS 0-2, OR 1.55, 95% CI 1.05-2.30, p=0.028. (152)

In 2016, the HERMES collaborators published a meta-analysis of the five positive RCTs published in 2015 (MR CLEAN, EXTEND IA, SWIFT-PRIME, ESCAPE, REVASCAT). For the primary outcome, the degree of disability on the mRS, the adjusted combined odds ratio for reduced disability at 90 days was 2.49, 95% CI 1.76-3.53, $p<0.0001$. The number needed to treat for one patient to have reduced disability of at least 1 point on the mRS was 2.6. The adjusted odds ratio for patients achieving functional independence (mRS 0-2) at 90 days was 2.71, 95% CI 2.07-3.55, $p<0.0001$. Numerous clinical and imaging subgroups were examined and no significant heterogeneity in treatment effect was found across age, severity (NIHSS), site of arterial occlusion or eligibility for alteplase. It was therefore concluded that endovascular thrombectomy is of benefit to most patients with acute ischaemic stroke caused by occlusion of the proximal anterior circulation within 6 hours of stroke onset, regardless of patient characteristics or geographical location (9). Subsequent meta-analysis of the time to treatment data suggested this benefit extended to at least 7.3 hours (64).

In early 2018, the DAWN trial published their results on the effect of endovascular therapy performed after 6 hours of stroke onset. Patients with occlusion of the intracranial internal carotid artery or proximal middle cerebral artery who had last been known to be well 6 to 24 hours earlier and who had a mismatch between the severity of the clinical deficit and the infarct volume, with mismatch criteria defined according to age (<80 years or ≥80 years) were

included. A total of 107 patients were assigned to the thrombectomy group (thrombectomy plus standard care) and 99 patients to the standard care only group. The mean score on the utility-weighted modified Rankin scale at 90 days was 5.5 in the thrombectomy group as compared with 3.4 in the standard care group, adjusted difference (Bayesian analysis), 2.0 points; 95% credible interval, 1.1 to 3.0; and the rate of functional independence at 90 days was 49% in the thrombectomy group as compared with 13% in the control group, adjusted difference, 33 percentage points; 95% credible interval, 24 to 44. The rate of sICH did not differ significantly between the two groups (6% in the thrombectomy group and 3% in the control group, p=0.50), nor did 90-day mortality (19% and 18%, respectively; p=1.00) (153). Similarly, in early 2018 the DEFUSE 3 investigators published their results on the thrombectomy in patients 6 to 16 hours after they were last known to be well and who had remaining ischemic brain tissue that was not yet infarcted. Included patients were those with proximal middle-cerebral-artery or internal-carotid-artery occlusion, an initial infarct size of less than 70 ml, and a ratio of the volume of ischemic tissue on perfusion imaging to infarct volume of 1.8 or more. There were 92 patients randomly assigned to endovascular therapy (thrombectomy) plus standard medical therapy (endovascular-therapy group) and 90 patients in the standard medical therapy alone (medical-therapy group). Endovascular therapy plus medical therapy, as compared with medical therapy alone, was associated with a favourable shift in the distribution of functional outcomes on the mRS at 90 days (OR=2.77; p<0.001) and a higher percentage of patients who were functionally independent,

defined as a score on the mRS (0 -2) (45% vs. 17%, p<0.001). There was no significant difference in 90-day mortality or sICH rates between the two groups (154).

The Concept of Time is Brain

The goal of acute stroke treatment with reperfusion therapies (intravenous thrombolysis and intra-arterial therapies) is to rescue the penumbral tissue at risk of becoming core infarct. Reperfusion success and the time from symptom onset to reperfusion are known to be critical for the salvation of the penumbra (155). Irrespective of age or stroke severity, rapid treatment with intravenous thrombolysis drugs (such as IV-tPA), within 4.5 hours of stroke onset, increases the probability of blood flow restoration to the penumbral area and decreases the risk of future disability and dependence (132). This benefit gradually diminishes towards 4.5 hours from stroke onset, however is not associated with any increased risk of symptomatic intracerebral haemorrhage or increase mortality rate with treatment delay (63).

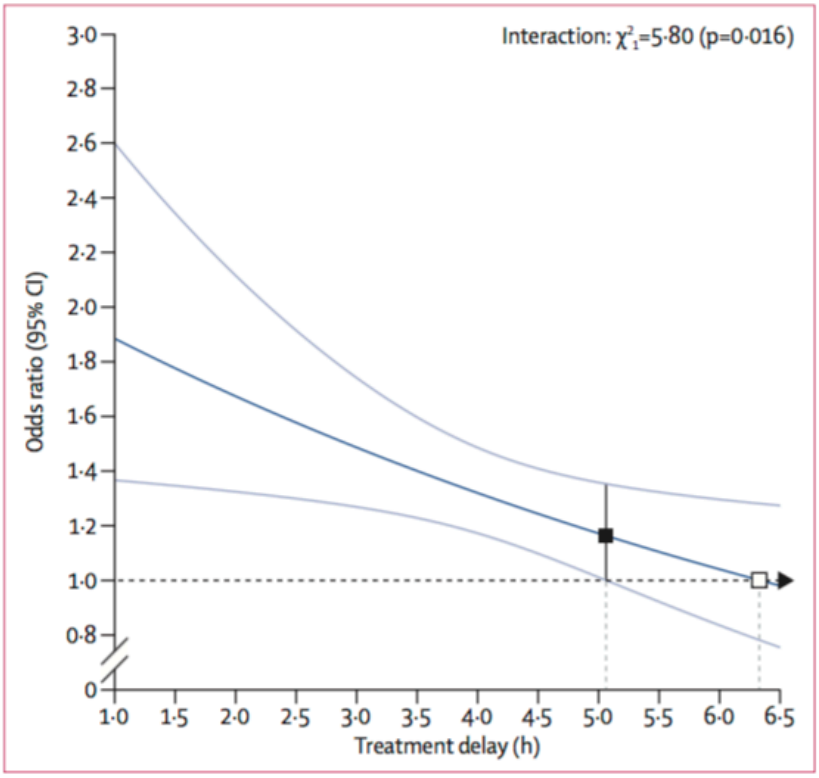

Figure 6: Effect of time to treatment with alteplase on good stroke outcome, as measured by the modified Rankin score (0-2) at 3 months

The solid line is the best linear fit between the log odds ratio for a good stroke outcome for patients given alteplase compared with those given control, p-value for interaction = 0.016. (63)

Recent studies have provided additional evidence regarding the association between treatment time and the benefit of reperfusion therapies. Compared to best medical therapy alone, treatment with endovascular thrombectomy, has been associated with lower degrees of disability at 90 days, becoming non-significant if performed after 7.3 hours from stroke onset. Within this period, functional outcomes were better if the procedure was performed earlier (within 2 hours) after stroke onset (64).

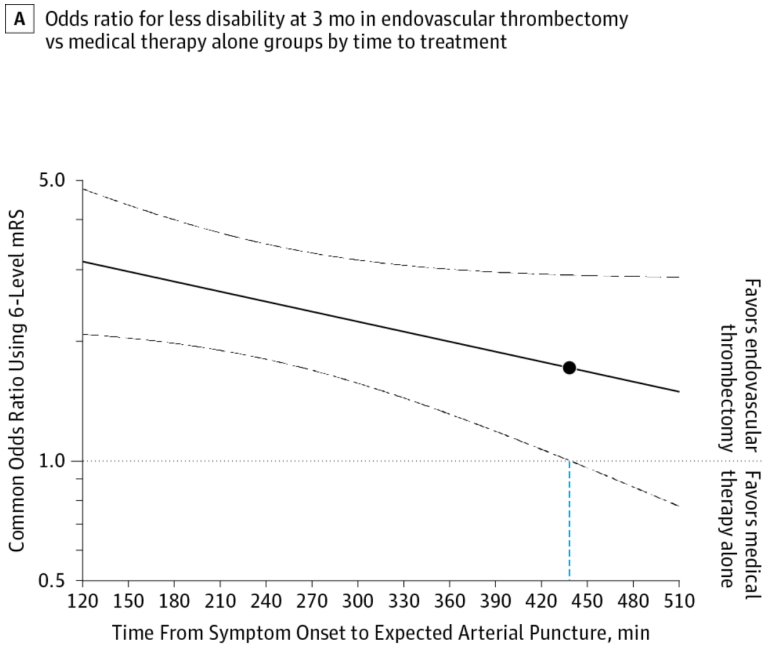

Figure 7: Association of time from stroke onset to expected time of endovascular thrombectomy procedure start (Arterial Puncture) with disability levels (mRS) at 90 days in endovascular (n = 633) vs medical therapy (n = 645). The p-value for interaction was .07. The lower bound of the 95% CI crosses 1.0 at 438 minutes (7.3 hours), as represented by the vertical blue dashed line. (64)

More recent studies have assessed the efficacy of endovascular thrombectomy between 6 and 24 hours after stroke onset in patients with a proximal middle cerebral artery stroke who had a mismatch between the severity of the clinical deficit and the infarct volume. DAWN found that patients with a mismatch between clinical deficit and infarct had better stroke outcomes with thrombectomy plus standard care when treated with thrombectomy within 24 hours after stroke

onset than standard care alone (153). DEFUSE3 assessed the efficacy of thrombectomy for stroke between 6-16 hours after stroke onset in patients who had remaining ischaemic brain tissue that was not yet infarcted and confirmed by perfusion imaging. They found using this inclusion criteria that endovascular therapy plus standard medical therapy for ischaemic stroke treated between 6-16 hours from stroke onset resulted in better functional outcomes than standard medical therapy alone (154).

The extent of ischaemic injury due to stroke can also be largely attributed to degree of reperfusion and the collateral circulation (a subsidiary network of vascular channels that compensate during an arterial occlusion), which have been shown to strongly change the time course of ischaemic injury after stroke onset (81). Advanced neuroimaging techniques have allowed for the identification and assessment of cerebral blood flow, including the status of collateral blood vessels and reperfusion success. It has been shown that in patients with good collaterals, the patient responds to reperfusion therapies better, has a lower risk of haemorrhagic complications and reduces the ischaemic burden by sustaining and perfusing the penumbral region (81). In a covariance analysis of the ratio of penumbral loss, it was shown that the ratio of penumbral loss was influenced by the quality of collaterals ($p=0.021$), the quantity of reperfusion ($p=0.003$) and an interaction between these two factors ($p=0.031$) (156). Similar results were also seen in an analysis that found that greater quality of collaterals was associated with reduced infarct growth (157). These results are also in line with studies that

have associated greater degree of collaterals with reduced final infarct growth (121, 158, 159).

The “time is brain” concept has highlighted the crucial importance of time in treating acute ischaemic stroke and has led to system improvements to ensure timely care and treatment is provided to acute stroke patients. For example, the Target Stroke in the United States of America was a national quality improvement initiative focused on improving acute ischaemic stroke care by reducing door-to-needle times for eligible patients being treated with intra-venous thrombolysis drugs. This included promoting prenotification of hospitals by emergency medical services, activating the entire stroke team with a single call, rapid acquisition and interpretation of brain imaging and premixing IV-tPA for high-likelihood candidates. Impacts from this initiative has seen the average door-to-needle time droop from 74 minutes to 59 minutes, an increase in the percentage of patients treated within 60 minutes from less than 30% to more than 50% and of these patients, lower in-hospital mortality and reduced long-term disability. (160) The Helsinki Model represents another example of improvements to systems of care for faster stroke thrombolysis, initiated at the Helsinki University Central Hospital in Finland and replicated at the Royal Melbourne Hospital in Australia. Hospital process improvements (including ambulance prenotification, direct triage of patient to CT scanner and administration of IV-tPA directly in the CT suite) reduced the median (IQR) door-to-needle time from 61 (43-75) minutes to 46 (24-79) minutes, p=0.040 in patients treated with IV-tPA in the 8 months after the

protocol change at the Royal Melbourne Hospital (161). There have been more recent efforts in reducing the time from stroke onset to hospital arrival, such as the introduction of Mobile Stroke Units, which will be discussed further on.

Secondary Stroke Prevention

Antiplatelet Therapy

In patients with acute ischaemic stroke, both aspirin and clopidogrel are antiplatelet agents that act to reduce the risk of recurrent ischaemic stroke. Two large, randomized trials, the International Stroke Trial (IST) and the Chinese Acute Stroke Trial (CAST), together enrolled more than 40,000 patients to hospital within 48 hours of the onset of stroke symptoms, randomizing patients within 48 hours of the onset of symptoms to 2 to 4 weeks of daily aspirin therapy or placebo. Results from both trials suggested that aspirin therapy decreased the risk of recurrent stroke and death without significantly increasing the risk of haemorrhagic stroke (162, 163).

In the CAPRIE trial, a randomised, blinded, international trial designed to assess the relative efficacy of clopidogrel and aspirin, it was shown that clopidogrel had a relative risk reduction of 7.3% for the primary endpoint (ischaemic stroke, myocardial infarction, or vascular death) in comparison to aspirin, although non-significant (164). Other randomized trials, such as CHARISMA, randomized

15603 patients to either dual antiplatelet therapy (clopidogrel plus low-dose aspirin) or placebo and followed them for 28 months. The primary endpoint was a composite of myocardial infarction, stroke, or death from cardiovascular causes. The rate of the primary efficacy end point was 6.8% with clopidogrel plus aspirin and 7.3% with placebo plus aspirin (relative risk, 0.93; 95% CI (0.83 to 1.05); p=0.22). Thus, it was shown that clopidogrel plus aspirin was not significantly more effective than aspirin alone in reducing the rate of myocardial infarction, stroke, or death from cardiovascular causes (165).

A double-blind, randomized, placebo-controlled trial (MATCH) compared aspirin with placebo in 7599 high risk patients with recent ischaemic stroke or TIA who were all already on clopidogrel 75 mg/day. The primary endpoint was a composite of ischaemic stroke, myocardial infarction, vascular death, or rehospitalisation for acute ischaemia. They showed that by adding aspirin to clopidogrel in high-risk patients with a recent ischaemic stroke, there is a non-significant difference between the groups in reducing major vascular events. However, the risk of life-threatening or major bleeding is significantly increased by the addition of aspirin, relative risk 1.26 (0.64-1.88, p<0.0001) (166).

Aspirin has consistently been found to be substantially less effective than anticoagulation in reducing thromboembolic risk in patients with AF (167-169). Additionally, it has been shown that oral anticoagulation therapy is superior to clopidogrel and aspirin for prevention of vascular events in patients with AF (RR

1.44, 95% CI 1.18-1.76, p=0.0003) (170). This was further demonstrated in the European AF trial, which found the superior efficacy of anticoagulation over aspirin for stroke prevention in patients with AF and a recent TIA or minor stroke (171).

Anticoagulant Therapy

Anticoagulation therapy is an established therapeutic strategy to reduce the risk of stroke in AF patients (172-175). Up until recently, oral anticoagulants, including warfarin and other vitamin K antagonists, have been the main therapies recommended for long-term stroke risk reduction in AF patients. They work by reducing the synthesis of the vitamin K-dependent coagulation factors. Warfarin has been shown to reduce the risk of stroke by two-thirds (66%) when compared with no treatment (167, 176).

The two phases of the Stroke Prevention in Atrial Fibrillation (SPAF-1) and (SPAF-2) studies compared aspirin with placebo, and warfarin with placebo in patients with atrial fibrillation. Results confirmed a substantial relative risk reduction (40%) by warfarin over aspirin for the primary end point of thromboembolism, and no significant increase in major haemorrhages in the age group below 75 years (177, 178). These results have been replicated in further trials comparing warfarin with aspirin for stroke prevention (179).

Safety of Anticoagulant Treatment

In patients with AF, all combinations of warfarin, aspirin and clopidogrel are associated with increased risk of nonfatal and fatal bleeding. The anticoagulation decision for a physician treating a patient with AF depends on the expected reduction in ischaemic stroke risk from anticoagulation therapy verses the expected increase in risk of intracranial haemorrhage. ICH in the elderly is associated with a mortality rate of 60% (61).

The haemorrhagic risk of warfarin depends on age and the International Normalized Ratio (INR), the blood test used to assess the effect of warfarin on the time it takes for blood to clot is referred to as the prothrombin time (PT). The PT evaluates the extrinsic pathway of coagulation and is used to determine how long it takes for the blood to clot. It is also used to check whether medicines, such as warfarin, are preventing blood clotting. The PT can also be referred to as an INR test, which is a standardized PT result and is most commonly used by physicians. The use of INR testing devices and attendance at anticoagulation clinics that provide INR testing as part of coordinated care have been associated with better outcomes from warfarin therapy (180). The recommended INR target in patients with AF to prevent further thrombotic events is between 2.0 and 3.0. For patients receiving therapy with warfarin, the proportion of time spent in the INR range is strongly associated with reduced risk of both bleeding and thromboembolism (181, 182).

Warfarin is problematic to use. The response to oral anticoagulation therapy is affected by gut flora, variations in hepatic functions, interactions with several drugs and diet and requires regular monitoring (183). They are associated with high levels of discontinuation, with many patients remaining on warfarin receiving inadequate anticoagulation (184-186). It is because of these issues that new anticoagulant agents were developed to reduce drug interactions and improve therapeutic consistency without the need for drug monitoring.

Direct (non-vitamin K) Oral Anticoagulants

The new paradigm for anticoagulation is based on the identification of molecules that more directly block the coagulation cascade. The main groups of novel oral anticoagulants act as direct thrombin inhibitors or as activated factor X inhibitors (187). Several new oral anticoagulants have been developed that dose-dependently inhibit thrombin or activated factor X with rapid onset and offset of actions and fewer drug interactions (188). This allows for the administration of fixed doses without the need for routine coagulation monitoring. Dabigatran, rivaroxaban and apixaban have all been approved for use by regulatory authorities.

Dabigatran

Dabigatran etexilate is a novel, potent, competitive, and reversible direct thrombin inhibitor, comparable to warfarin for prevention of thromboembolic

events in patients with AF. It is a low molecular pro-drug that converts to its active form, dabigatran, after oral administration.

In the Randomized Evaluation of Long-term anticoagulant therapy (RE-LY) trial, at a dosage of 110mg twice daily, dabigatran had similar efficacy to warfarin in preventing stroke and systemic embolism with lower rates of major haemorrhage. At a dosage of 150mg twice daily, dabigatran was associated with lower rates of stroke and systemic embolism than warfarin (1.69% per year in the warfarin group compared with 1.53% per year in the 100mg dose of dabigatran), RR for dabigatran: 0.91, 95% CI (0.74-1.11), $p<0.001$ for non-inferiority. Additionally, the incidence of intra-cerebral haemorrhage in atrial fibrillation patients was significantly lower with dabigatran, when compared with warfarin: 0.12% 110mg dose versus 0.38%, $p<0.001$ (188).

Rivaroxaban

Factor Xa is a coagulation factor that catalyzes the cleavage of prothrombin, critical for thrombin generation. Rivaroxaban is a small-molecule factor Xa inhibitor, demonstrated to have effective anticoagulant effects in human plasma. The phase III randomized controlled trial, ROCKET-AF, randomized 14264 patients with atrial fibrillation to rivaroxiban 20mg or dose-adjusted warfarin. The primary endpoint was occurrence of stroke or nonsystemic embolism. It showed that rivaroxaban produced a significant reduction in the risk

of stroke or systemic embolism (1.7% in the rivaroxaban versus 2.2% in the warfarin group, hazard ratio for Rivaroxaban 0.79, 95% CI (0.66-0.95), p<0.001 for non-inferiority). In comparison to warfarin, rivaroxaban was associated with a significant reduction in intracranial haemorrhages (0.5% vs. 0.7%, p=0.02) and fatal bleeding (0.2% vs. 0.5%, p=0.003) in the rivaroxaban group (189).

Apixaban

Apixaban is another novel compound used in patients with atrial fibrillation. It is reversible and administered orally as a factor Xa inhibitor with good oral bioavailability. The ARISTOTLE randomized controlled trial, randomized 18206 patients to warfarin or apixaban 5 mg twice daily. The primary end point was the occurrence of stroke or embolism. In comparison with warfarin, apixaban significantly decreased the risk of stroke or systemic embolism (1.27% in the apixaban group versus 1.60% in the warfarin group, hazard ratio 0.69, 95% CI (0.60-0.80), p<0.001 for non-inferiority and p=0.01 for superiority). It also reduced the risk of haemorrhagic stroke (0.24% per year in the apixaban group, as compared with 0.47% per year in the warfarin group (hazard ratio, 0.51; 95% CI, 0.35 to 0.75; p<0.001)) (190).

Timing of Anticoagulation Therapy

The risk of recurrent ischaemic stroke or transient ischaemic attack (TIA) following cardioembolism to the brain is high (between 10% and 20%) during the 90 days post initial event, with 50% of that risk occurring in the first week (191). Because early haemorrhagic transformation is a major concern, the optimum time to start oral anticoagulation remains a controversial issue. The absence of evidence-based guidelines to address this issue has led to wide variations in restarting anticoagulation after stroke. A meta-analysis of the pivotal clinical trials of DOACs (including dabigatran, apixaban and rivaroxaban) has identified a favourable risk-benefit ratio compared to warfarin and other vitamin K antagonists. They have been associated with significant reductions in stroke, reduced ICH, reduced mortality and similar bleeding rates. Of notability, the reduction in ICH in patients on DOACs in comparison to vitamin K antagonists suggests that the early initiation of DOACs may be associated with a more postitive risk-benefit ratio, reduced incidence of recurrent ischaemic and lower incidence of HT (192). Thus, the optimal timing of DOAC initiation after stroke to best balance the risk of haemorrhagic transformation versus recurrent stroke is of vital clinical importance.

Statin therapy for hyperlipidemia

Statins reduce the risk of both initial ischaemic stroke and recurrent ischaemic stroke. The pivotal SPARCL (Stroke Prevention by Aggressive

Reduction in Cholesterol Levels) randomized 4731 patients with a history of stroke or TIA to atorvastatin 80mg per day (n = 2365) or placebo (n = 2366) and were followed for an average of 4.9 years. The primary study endpoint, fatal or nonfatal stroke, was significantly less frequent in the atorvastatin group (11.2%) versus placebo (13.1%) and represented a relative risk reduction (RRR) of 16% (p = 0.03, 95% CI (0.71–0.99). (193)

Since then, the relationship between statin adherence and reduced recurrent stroke risk has been studied in patients with stroke caused by atrial fibrillation (patients which were excluded from the SPARCL trial), with results indicating the relationship between statin adherence and reduced recurrent stroke risk is as strong among patients with atrial fibrillation as it is in patients without atrial fibrillation. (194)

According to the 2017 Australian Stroke Guidelines from the Australian Stroke Foundation, all patients with ischaemic stroke or TIA with possible atherosclerotic contribution and reasonable life expectancy should be prescribed a high-potency statin, regardless of baseline lipid levels (strokefoundation.org.au).

Antihypertensive Therapy

Hypertension is a major risk factor for acute ischaemic stroke, with the risk increasing with every rise in systolic blood pressure (195). It is also a major

risk factor for recurrent ischaemic stroke and trials have shown that antihypertensive therapies can reduce the rate of recurrent stroke, independent of baseline blood pressure. The PROGRESS trial included over 6100 patients with either ischaemic stroke or less often a haemorrhagic stroke or TIA in the previous five years (mean baseline blood pressure was 147/86 mmHg). Patients were assigned to perindopril (antihypertensive) or placebo. They found a reduction in blood pressure of 9/4 mmHg in the perindopril compared with placebo decreased the rate of the primary endpoint of fatal or nonfatal stroke (10% vs 14% with placebo, relative risk reduction 28%, 95% CI (17% to 38%). (196)

In the PATS trial (post stroke antihypertensive study), 5665 Chinese patients with a history of stroke (mostly ischaemic) were randomly assigned to treatment with indapamide or placebo, (average blood pressure at randomisation was 154/93 mmHg). At a median follow up of 2 years, there were significantly fewer strokes in the active treatment compared with placebo (143 vs 219, hazard ratio 0.69, 95% CI (0.54-0.89) (197). This trial was limited because of its premature termination and 28% dropout rate.

The largest trial however was the PRoFESS trial (Prevention Regimen for Effectively Avoiding Second Strokes) which randomly assigned 20332 patients with noncardioembolic ischaemic stroke to receive either telmisartan or placebo, (average blood pressure was 144/84 mmHg). At an average follow-up of 2.5 years, there was no significant difference between the active and placebo groups

in the primary outcome of recurrent stroke (8.7% vs 9.2%, hazard ratio 0.95, 95% CI (0.86-1.04)), however significant benefit compared with placebo would not have been expected, since telmisartan therapy only reduced the blood pressure by an average of 3.8/2.0 mmHg more than placebo (198). This study was limited because of a high rate of cessation of therapy because of side effects in some patients treated with telmisartan as well as the initiation of antihypertensive therapy in some patients in the placebo group because of the development of hypertension.

According to the 2017 American College of Cardiology/American Heart Association guidelines, initiation of blood pressure therapy for previously untreated patients with ischemic stroke or TIA who, after the first three days, have an established blood pressure of ≥140mmHg systolic or ≥90mmHg diastolic (or ≥130mmHg systolic or ≥80mmHg diastolic in patients with lacunar stroke) is recommended. The guidelines also recommend resumption of blood pressure therapy for previously treated patients with known hypertension for both prevention of recurrent stroke and prevention of other vascular events in those who have had an ischemic stroke or TIA and are beyond the first three days after stroke onset. The goal blood pressure of<130/<80mmHg is reasonable for secondary stroke prevention in all patients. (199)

The evolution of stroke medicine

According to the Executive Summary from the American Heart Association, between 1999-2009 the relative rate of stroke death fell by 36.9% and the actual number of stroke deaths declined by 23% despite approximately 800 000 new strokes each year (200). Similarly in China, stroke mortality is decreasing despite 2.5 million new stroke cases each year and 7.5 million stroke survivors. The annual cost of stroke care in China is approximately 40 billion RMB (approx. $8 billion AUD), 10 times the cost of cardiovascular diseases (27).

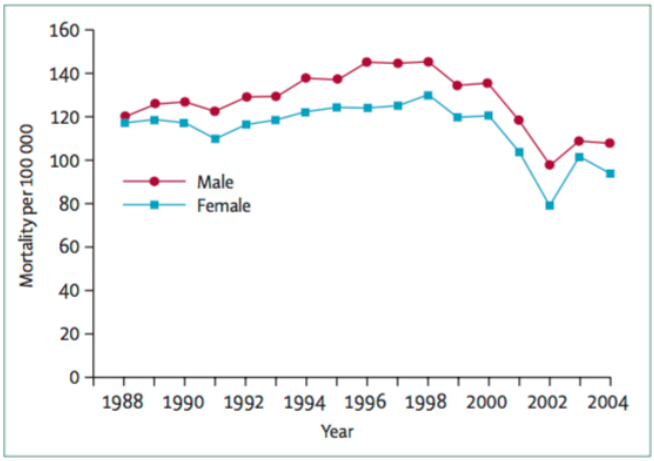

Figure 8: Trends of stroke mortality in urban Chinese populations (201)

In a recent pooled analysis of the five RCTs, it was concluded that modern endovascular thrombectomy added to best medical therapies (such as IV-tPA) more than doubles the odds of a higher mRS score compared to best medical therapy alone in patients with anterior circulation acute ischaemic strokes (9).

The findings from these trials have had and continue to have global implications for restructuring systems of care to provide timely treatment to patients, particularly those with acute ischaemic stroke due to large vessel occlusions. Given that these stroke interventions are time critical and patients treated earlier after onset have the greatest chance of benefitting from reperfusion therapies (11), it has been clinically crucial to develop systems, tools and technologies to optimize the number of patients receiving treatment earlier (12). To do this, there has been a focus on education in recognising stroke symptoms, implementing efficient code stroke methods and the introduction of telemedicine which have all led to an increase in the proportion of stroke patients arriving at hospital earlier (13). Additionally, the preliminary application of mobile stroke units has allowed the use of pre-hospital treatment to reduce the median time from stroke onset to therapy decision to as little as 35 minutes (14).

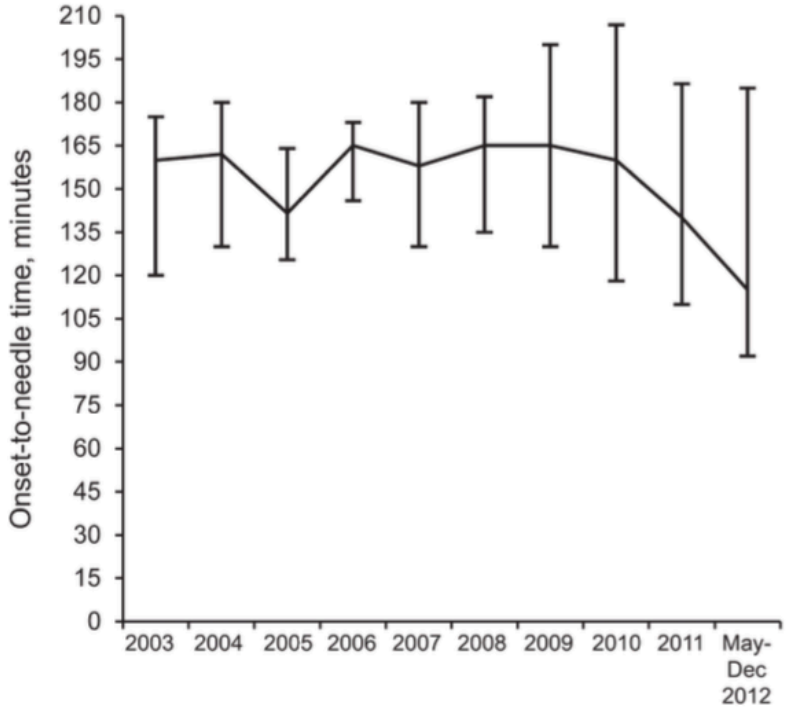

Figure 9: The Royal Melbourne Hospital annual onset to treatment time with thrombolysis, in minutes with interquartile range (161)

The onset to needle time delay decreased from 140 minutes in 2011 to 115 minutes in 2012 after implementation of hospital pre-notification by ambulance paramedics and transport direct to CT on the ambulance stretcher.

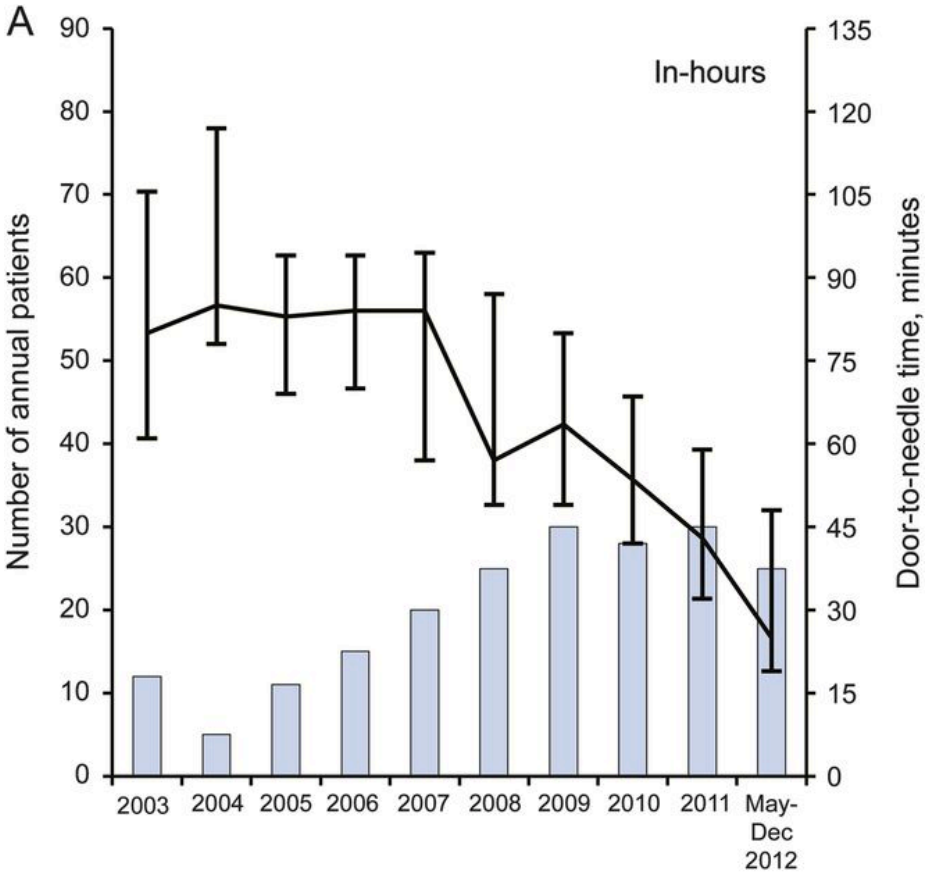

Figure 10: A breakdown of the number of annually in-hours treated patients with thrombolysis and the median door-to-needle times at the Royal Melbourne Hospital. (161)

The in-hours median (IQR) door-to-needle time was 43 (33-59) minutes in 2011, decreasing to 25 (19-48) minutes in 2012, a significant (p=0.009) decrease in treatment delay after the implementation of the code stroke protocol at the Royal Melbourne Hospital.

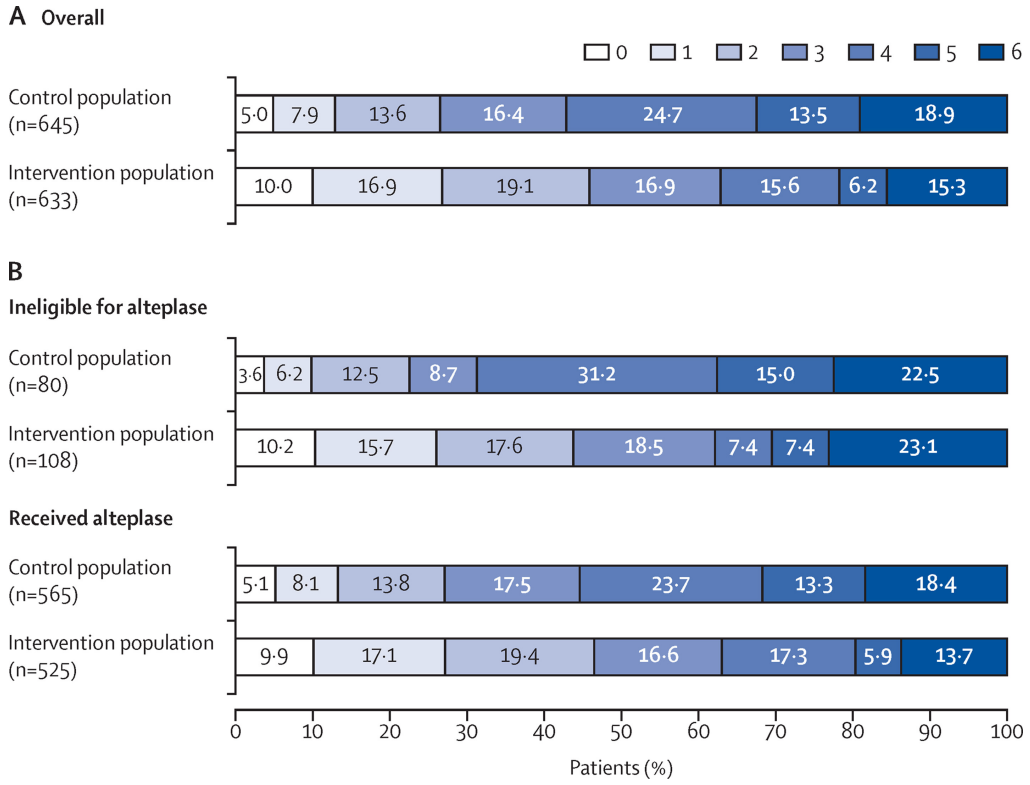

Figure 11: Distribution of modified Rankin scores by treatment population at 90 days (9)

Pooled data from MR CLEAN, ESCAPE, REVASCAT, EXTEND-IA and SWIFT PRIME highlighting reduced disability (mRS) at 90 days post thrombectomy versus controls (adjusted OR=2.49, 95% CI (1.76-3.53), p<0.0001). Analysis was adjusted for age, baseline stroke severity NIHSS, IV-tPA treatment, ASPECTS at baseline, site of occlusion and time from stroke onset to randomisation.

SUMMARY

A Lancet paper published in 2014, a year prior to the 5 RCTs, suggested that if these trends in stroke incidence, mortality and disability-adjusted life years lost continue, by 2030 there will be almost 12 million stroke deaths, 70 million stroke survivors and more than 200 million disability adjusted life years lost globally (1). Stroke outcome is not entirely represented by mortality rates and level of disability - there are a range of other neurological sequelae that are increasingly recognised and contribute an important burden with reduced quality of life. Given the improved immediate outlook of acute stroke, there is a need to understand whether the interventions will also affect these longer-term complications.

Post stroke sequelae

Stroke is not only an acute condition. Little attention has been paid to the long-term consequences and complications post stroke. Post stroke sequelae are highly varied and include, but are not limited to: depression, post-traumatic stress disorder, seizures and epilepsy, recurrent stroke, obstructive sleep apnoea, cognitive impairment, disability, recurrent strokes, fatigue, infections, falls and spasticity (202-205). Consequently, with the prevalence of stroke survivors

forecasted to substantially grow, so too will the associated health care costs and socioeconomic burdens. This will create a greater need for comprehensive post stroke rehabilitation services. The fundamental issue with post stroke sequelae and complications is understanding and predicting *which* patients are more susceptible to their development. Through determining markers for post stroke sequelae, higher risk patients can be targeted for enhanced monitoring, prolonged specialist follow up and potential prophylaxis treatment.

This thesis will focus specifically on determining markers of epileptogenesis post ischaemic stroke. The following sections will outline what post stroke seizures are and why this is an important area for further study.

Post stroke Epilepsy

The development or tendency towards recurrent post stroke seizures is termed post stroke epilepsy (PSE), and is considered one of the major complications of stroke. Post stroke epilepsy poses a considerable burden to stroke survivors and, even when well-controlled with medications, negatively impacts quality of life (206). Seizures develop in 2–14% of patients who have had an ischaemic stroke (207), with stroke the most common cause of seizures in the elderly population, accounting for 30-50% of epilepsy in the age group above 60 (208, 209). Given the recent evolution of stroke reperfusion treatments (with intra-arterial therapies and intravenous tissue plasminogen activator), knowledge

on pathophysiology, epidemiology, risk factors and consequences of acute stroke management is incomplete. Through determining markers for post stroke seizures and epilepsy, higher risk patients can be targeted for enhanced monitoring and prolonged specialist follow up. These findings may assist with selection of high-risk patients for trials to determine the efficacy of antiepileptogenic therapies following strokes.

Terminology and classification

Inconsistencies in terminology and ambiguities in seizure identification and classification have contributed to difficulty in interpreting the results of various studies as well as the lack of clarity in the research (210). This confusion is partly due to numerous changes in the definition of seizures over the years. Epilepsy is a disease of the brain characterised by an enduring predisposition to generate epileptic seizures. Epileptic seizures are a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain. As such, seizures are a 'symptom' of epilepsy (211, 212). Post stroke epilepsy is diagnosed when a patient develops a seizure at least one month after the stroke with a likelihood of a lowered seizure threshold and a recurrence risk (211). However, seizures associated with strokes can be also classified by early seizures (acute symptomatic seizures) and late seizures (unprovoked). Early seizures are those occurring within seven days of the stroke, while late seizures are those that develop after seven days (213). According to the International

League Against Epilepsy (ILAE) 2015 revised report, an early seizure post stroke refers to a seizure that has occurred during the first week post stroke, with the implication that these seizures are not suggestive of an underlying predisposition of epileptic seizures. A late onset seizure occurs after one-week post stroke and is required for a diagnosis of PSE. Thus, PSE is defined as recurrent late seizures, or a single seizure occurring more than one month after stroke (214). Early onset seizures usually present with a focal onset while generalised tonic-clonic seizures are more common with late onset seizures (206).

Prior to 2014, PSE was defined as two or more unprovoked late seizures after the onset of stroke (213). Unprovoked epileptic seizures occur in the absence of precipitating factors, a defining feature of epilepsy, whereas acute symptomatic seizures occur at the time of a systemic insult or in close temporal association with a documented brain insult (215). Unless otherwise stated, this review will refer to the older definition, given its ubiquity across the literature.

The burden of post stroke epilepsy

As discussed, strokes carry a significant burden of disease globally, especially in the elderly population (1, 17). Such seizures, termed post stroke seizures, have been shown to exacerbate the medical complications of strokes and increase the associated disability, morbidity, hospitalizations and resource utilization (216, 217). PSE has a negative effect on stroke recovery with a

decreased survival rate at both 30-days and one-year post stroke. Psychosocial issues such as driving, dependency and total compliance of medication, employment, income, depression, fears, anxiety, misconceptions and social stigma, education, lifestyle considerations, life insurance and child bearing potential have major impacts on a patient's quality of life (218, 219). Although appropriate management with anti-epileptic medication can result in seizure remission, some literature suggests that 30-40% still remain incompletely controlled (219). Thus, they require appropriate management and support in the long term. In the only study that has specifically and prospectively evaluated response to AED therapy in post stroke epilepsy patients, 81% of patients were seizure free at a median follow up of 30 months (220). With an increasingly ageing population, and age itself being an independent risk factor for stroke, the incidence and prevalence of PSE is increasing (206). Economically, post stroke seizures and PSE are associated with greater direct medically related costs, mainly attributed to increased CT/MRI scans, increased length of stay in hospital, more consultations and increased number of days in the ICU with additional costs associated with unemployment and ongoing support and management (221).

Epidemiology

Risk factors for PSE include late-onset seizures, stroke severity, stroke size in both volume and diameter, cortical involvement, persistence of stroke disability and genetic factors (210, 222). Stroke severity has been consistently

agreed upon as the strongest risk factor for PSE, with total anterior circulation infarct the major involved circulation (223). Second to this, extent of involvement of cortex is a risk factor for PSE, particularly in the parieto-temporal cortex, supramarginal gyrus and superior temporal gyrus (223). Interpretation of previous literature is subject to several caveats, as mentioned this is primarily due to differing inclusion/exclusion criterions, definitions of seizures and epilepsy and different follow-up times.

Previous studies have reflected an incidence of the various definitions of PSE between 2-12% (210, 222, 224-244) with studies suggesting an increased risk of developing post stroke seizures over time (207, 225, 230), with the highest risk of developing PSE during the first year (226). Long-term population-based follow-up studies have revealed that the 10-year estimate of development of PSE after total anterior circulation infarcts was 28.7%, partial anterior circulation infarcts 13.4%, and posterior circulation infarcts was 4.8%, with the greatest risk of seizure after ischaemic stroke in the first year with a mean incremental risk of seizure development at 1.5% per year after the first year (223). The manifestation of PSE has been reported in one study to be 50% simple partial seizures, 30% generalized tonic-clonic seizures, 10% complex partial seizures, 5% status epilepticus and unknown in a further 5%. Other studies have reported generalised tonic-clonic post stroke seizures as high as 77% of those who experienced seizures (223). However, in 2015, a meta-analyses of 34 cohort studies revealed that the incidence of the new definition of post stroke epilepsy was 6.93% (245).

Pathophysiology

Epileptogenesis is a process that describes the mechanism of a previously normal brain network initiating an epileptogenic process, ultimately culminating in a greater susceptibility to an unprovoked seizure (246). This is very relevant to post stroke epilepsy, an architype of 'acquired epilepsies'. Numerous animal models of post stroke epilepsy have been performed assessing the process of epileptogenesis after experimental stroke (247-251). These studies have been problematic for a number of reasons including the inability to account for stroke comorbidities (such as hypertension and hyperlipidemia), the majority of models were young adulthood animals when stroke is most prominent in the elderly, the follow up times for seizure development were often too long (20 months in some studies) and there was a large variability in seizure incidence between the studies which poses questions about variability in the animal models used (246). However, in most cases with stroke, the process of epileptogenesis appears to be slow, occurs in only a subset of animals and has a relatively low frequency (246). Targeting this epileptogenic process may prevent the occurrence of unprovoked seizures.

The pathophysiologic mechanisms are not completely understood, but there are several suggested mechanisms for seizures after ischaemic stroke. Post stroke seizures can occur soon after the onset of ischemia or can be delayed, classified as early onset seizures (EOS) and late onset seizures (LOS). Many studies make this distinction based on differences in the presumed pathophysiology.

Early onset seizures are often viewed as markers of the underlying severity of brain injury; resulting in biochemical dysfunction leading to an increase in glutamate levels, resulting in electrically excitable tissue (207, 210, 244). An increase in intracellular Ca^{2+} and Na^{+} leading to cellular biochemical dysfunction, electrically irritable tissue and a lower threshold for depolarisation has been one suggested mechanism (252). Alternatively, acute ischemia leads to increased extracellular concentrations of the excitatory neurotransmitter glutamate, which has been associated with secondary neuronal injury (253). These progressive changes in neuronal networks can lead to spontaneous recurrent seizures. Early seizures are an independent risk factor for late seizures, which usually develop weeks to months after brain insult (254).

These late seizures (LOS) are also thought to be the result of chronic changes in the neuronal networks leading to epilepsy. These chronic changes are possibly attributable to gliosis leading to changes in membrane properties, selective neuronal loss, axon sprouting, hyper-excitability and neuronal synchrony in the setting of acute ischemia (254). In particular, axon sprouting is a well-established response to brain injury and has a strong correlation with epileptogenesis. However, much work remains on the mechanisms by which axon sprouting leads to seizures (246). In human imaging studies, it has been suggested that the ischaemic penumbra or the potentially salvageable brain tissue within the cortical infarct is the region involved in PSE, reporting that only partly destroyed tissue that borders critically decreased cerebral blood flow are the areas of neuronal instability and seizure activity (223). Other studies have also reported

haemorrhagic transformation as a risk factor for both acute symptomatic seizures and epileptogenesis, with the pathophysiological mechanism related to disruption of the blood brain barrier (237). Animal experimental models of post stroke epilepsy in rats have revealed the epigenetic mechanisms involved in the regulation of post stroke gene expression, particularly miRNAs, and their participation in brain damage, neuro-protection, synaptic plasticity and glial scar formation (223). The findings suggest similarities between changes in gene expression after stroke and those after other brain injuries that trigger epileptogenesis.

Aetiology

It is difficult to predict who is likely to develop a seizure after the stroke. However, decades of research have suggested that there are a several risk factors in the development of PSE. The more notable ones include cortical involvement (210, 213, 228, 229, 231, 232, 234-236, 238, 239, 241, 242, 245), haemorrhagic lesions (both primary haemorrhage and haemorrhagic transformation of the ischaemic infarct) (216, 224-228, 231-234, 237, 239, 241, 245), infarct size (225, 229, 231, 232, 234, 238, 239, 241, 245) and stroke severity (222, 226, 227, 230, 231, 240, 243). Younger age has also been shown to have an association with developing PSE, however, to a lesser degree (231, 233, 241, 242). It is important to note that most of these studies were performed using different seizure classifications and follow-up times.

Table 5: Summary of studies illustrating the risk factors for different seizure populations

Author	Year	Type of Study	Stroke Population* (n=)	Seizure Population	Risk Factors for Seizure Population	Mean Follow up Time
Burn et al.	1997	P	675	11.5% #	Haemorrhagic Lesion, Lesion Size	5 Years
Reith et al.	1997	P	1197	4.2% ES	Stroke Severity	14 Days
Bladin et al.	2000	P	1841	8.9% #	Stroke Severity, Cortical Involvement, Haemorrhagic Lesion, LS	9 Months
Berges et al.	2000	R	3205	3.2% LS	^^Stroke Severity, Occipital Involvement, Haemorrhagic Lesion, LS	Min. 2 Years
Labovitz et al.	2001	R	904	3.1% ES	Cortical Involvement, Haemorrhagic Lesion	7 Days
Lamy et al.	2003	P	581+	5.5% LS	Early Seizures, Cortical Involvement, Lesion Size	3 Years
Lossius et al.	2005	P	484+	3.1% PSE	Stroke Severity	7 Years
Kammersgaard et al.	2005	P	1197	3.2% PSE	Stroke Severity, Younger Age, Haemorrhagic Lesion, Lesion Size, ES	7 Years
Alberti et al.	2008	P	638	4.8% ES	Cortical Involvement, Haemorrhagic Lesion, Lesion Size	7 Days
Szaflarski et al.	2008	R	6044	3.1% ES	Younger Age, Haemorrhagic Lesion, Pre-morbid Function	24 hours
Leone et al.	2009	P	440	11% #	Cortical Involvement, Haemorrhagic Lesion, Lesion Size, Previous Lesion	5 Years
Chiang et al.	2010	R	143+	7.6% LS	Cortical Involvement	6 Years
Strzeleczyk et al.	2010	P	264	3.8% PSE	Cortical or cortical/subcortical Involvement, Supratentorial Stroke, Haemorrhagic Lesion involving Cortical Ischaemia, Ischaemia with Neurological Deficit, Neurological Deficit, ES, LS	1 Year
Beghi et al.	2011	P	714	6.3% ES	Cortical Involvement, Haemorrhagic Lesion	7 Days
Okuda et al.	2012	R	448	4% LS	Cortical Involvement, Lesion Size	8 Months
Procaccianti et al.	2012	P	2053	3.2% ES	Cortical Involvement, Haemorrhagic Lesion, Lesion Size, Hyperglycaemia in Patients without Diabetes	14 Days
Jungehulsing et al.	2013	P	1020	8.2% PSE	Stroke Severity, Hypertension	2 Years
Graham et al.	2013	P	3310	6.4% PSE	Cortical Involvement, Younger Age, Haemorrhagic Lesion, Lesion Size	3.8 Years
Serafini et al.	2015	P	782	2.2% PSE^	Cortical Involvement, Younger Age	2 Years
Tanaka et al.	2015	R	2150	6.6% LS	^^ Stroke Severity, Frontal Cortical Involvement, VPA monotherapy, Convulsions on Admission	1 Year
Leung et al.	2016	P	2805	4.1% ES	Cardioembolism, Transient Complete Occlusion Recanalisation	6 Years

P = Prospective, R = Retrospective
* All Stroke Subtypes unless otherwise indicated, + Ischemic Strokes
ES Early Seizures, LS Late Seizures, PSE Post-Stroke Epilepsy, # All Post-Stroke Seizures
^ New Definition of PSE (2014 ILAE Guidelines)
^^ Study involves Late Seizure Recurrence

Infarct Size

A larger infarct was consistently shown to increase the risk of developing early and late seizures as well as PSE (225, 229, 231, 232, 234, 238, 239, 241, 245). There have been numerous studies showing the association between lesion size by clinical diagnoses and the development of post stroke seizures. A large, multi-centre study suggested the link between a larger infarct and the risk of PSE,

can be shown with the Oxford Community Stroke Project Classification (OCSP). They found that compared with Total Anterior Circulation Infarcts (TACIs, the reference category), Partial Anterior Circulation Infarcts (PACIs), Posterior Circulation Infarcts (POCIs) and Lacunar Infarcts (LACIs) all had significantly lower risks of developing PSE, with hazard ratios of 0.62, 0.27 and 0.46 respectively (241). Likewise other studies have classified infarct size using the OCSP classification and demonstrated that patients presenting with TACIs are at increased risk of seizures (225, 239). One study defined a large lesion as a cerebral infarct involving half a hemisphere, with the hazard ratio for developing PSE 31.9 compared to a small lesion (229).

Other studies have contributed to these findings with the use of neuroimaging. Numerous studies have found that cortical involvement is an independent predictor of seizure development as determined through neuroimaging (210, 213, 228, 229, 231, 232, 234-236, 238, 239, 241, 242, 245). Others have used imaging tools and quantitative measures to categorize small, medium and large lesions. One study reported an increase in risk of developing PSE by 16% for every increase in lesion diameter size by 10mm (18). Another study looked at computerised measurement of lesions that were manually delineated. Small infarcts (<10cm^3) had a hazard ratio of 0.31, medium lesions (10-100cm^3) had a hazard ratio of 0.42 and large lesions (>100cm^3) had a hazard ratio of 0.94 in developing post stroke seizures (226).

Stroke Severity

Numerous studies have associated stroke severity scales, such as the Scandinavian Stroke Scale (SSS), the mRS, the NIHSS, Barthel Index and the Canadian Neurological Score, with the development of post stroke seizures. Despite the differences in the stroke severity scales used, all studies reliably associated increased stroke severity with an increased risk of developing post stroke seizures (222, 226, 227, 230, 231, 243).

Less studied has been the link between leptomeningeal collaterals and the development of post stroke seizures. As discussed, robust leptomeningeal collaterals have been linked with rapid recanalization of MCA occlusion and possible prevention of larger infarcts and also predictive of improved long-term clinical outcome in patients with MCA occlusions (80). Furthermore, with a greater degree of collaterals after an ischaemic stroke, there is reduced risk of haemorrhagic complications after thrombolysis and endovascular therapy, improved recanalization rates, smaller infarct size, smaller infarct growth and improved clinical outcome (255-257). Understanding of the role collateral status has on longer-term stroke complications is limited.

Combination of risk factors

The validated CAVE score was developed in 2014 for the use in intracerebral haemorrhage, estimating the risk of late seizures using several

variables. The CAVE score (0–4 points) consists of cortical involvement of ICH (1 point), age <65 years (1 point), lesion volume >10mL (1 point), and early seizure within 7 days of ICH (1 point). The corresponding risk of late seizures during follow-up was 0.6%, 3.6%, 9.8%, 34.8%, and 46.2% for CAVE score points 0 to 4, respectively (258). Similar to these ICH patients, Strzelczyk et al (236) developed a PoSERS scale (post stroke epilepsy risk scale) with 10 risk factors to predict PSE in an ischaemic and haemorrhagic population. Seven of these factors on the scale were determined to be statistically significant, including early onset seizures (within 14 days), late onset seizures (after 14 days), poor stroke outcome (mRS=>3), cortical involvement, primary ICH and supratentorial stroke.

More recently in June 2018, a multivariable prediction model and validation study for late seizures (after 7 days) after ischaemic stroke was published. The SeLECT (259) score was based on five clinical predictors in 1200 participants who had an ischaemic stroke in Switzerland and was externally validated on 1169 participants from three independent cohorts in Austria, Germany and Italy. Overall, the risk of late seizures was 4% (95% CI 4–5) 1 year after stroke and 8% (95% CI 6–9) 5 years after stroke. The final model included five variables - severity of stroke, large-artery atherosclerotic aetiology, early seizures, cortical involvement, and territory of middle cerebral artery involvement. The lowest SeLECT value (0 points) was associated with a 0·7% (95% CI 0·4–1·0) risk of late seizures within 1 year after stroke (1·3% (95% CI 0·7–1·8) within 5 years), whereas the highest

value (9 points) predicted a 63% (42–77) risk of late seizures within 1 year (83% (62–93) within 5 years). The model had an overall concordance statistic of 0·77 (95% CI 0·71–0·82) in the validation cohorts. Of interest, a subgroup analysis of people receiving intravenous thrombolysis (n=186) and endovascular thrombectomy (n=28) in the validation cohort was performed. The SeLECT model showed good discrimination in participants receiving intravenous thrombolysis or endovascular thrombectomy in the contemporary (recruitment until 2014) validation cohort from Austria. However, additional data from novel stroke cohorts might be needed to address fully the effect of new treatments on post stroke epilepsy (259).

Acute stroke reperfusion therapies and post stroke seizures

Whether the recent advances in reperfusion therapies for acute ischaemic stroke have influenced the incidence of seizure development has not been well studied. Intuitively, the most direct way to prevent PSE would be to prevent or mitigate the initial stroke. To date, there remains little evidence that improved stroke care reduces the incidence of PSE. In fact, two studies have found that thrombolysis increases the likelihood of acute symptomatic seizures, within 7 days, post ischaemic stroke (260, 261), whilst others have either shown no association or that the association is likely confounded by worse stroke severity (262). Few have examined post stroke seizures following intra-arterial therapies (IAT). These will be discussed in detail.

Much can be inferred from the underlying physiological consequences of improved revascularisation. By preventing the growth of the ischaemic core and salvaging the penumbra (263), it is plausible that mechanical thrombectomy can result in reduced infarct sizes and severity post stroke, however converse hypotheses exist.

In ischaemic stroke, haemorrhagic transformation has been consistently associated with increased risk of seizure development (224-227, 231-234, 237, 239, 241, 245, 264, 265). Given that there are observable trends towards increased HT in patients who reperfuse (whether spontaneously, pharmacologically or mechanically with intra-arterial therapies) (266), understanding the impact on seizure outcome is of importance for stroke management and detection of late onset sequelae, such as seizures and epilepsy.

SUMMARY

Despite extensive knowledge of the risk factors for seizure development, predicting higher risk patients is still relatively difficult. As indicated, the forecasted increase in prevalence of stroke survivors portends substantial growth in health care costs and socioeconomic burdens with a greater need for comprehensive post stroke rehabilitation services. The fundamental issue with post stroke seizures is understanding and predicting *which* patients are more susceptible to the development. Through determining markers for post stroke seizures, higher risk patients may be targeted for enhanced monitoring, prolonged specialist follow up and potential prophylaxis treatment.

Genetic factors and post stroke epilepsy

In most epilepsies there is no known environmental cause (i.e. stroke, tumour, CNS infections, head trauma) of the epilepsy (i.e. non-acquired epilepsies). Most of the genetic findings thus far have been identified in this patient group. However, even in patients with a known risk factor for epilepsy such as ischaemic stroke, only a subpopulation of patients develops epilepsy (223). Studies of epilepsy patients with ischaemic strokes suggest that, in addition to the primary ischaemic insult, there may be other underlying epileptogenic pathologies present. Factors that contribute to this risk for epileptogenesis in a given individual generally remain unknown. No antiepileptic treatments are available at present for patients at risk of developing epilepsy after brain insult,

emphasising the need to understand cause-specific mechanisms that can be targeted towards epileptogenesis in individual patients (223).

It has been estimated that approximately 30% of all epilepsies are of genetic origin with more than 500 loci linked to epilepsy in human beings and mice (223), however the likelihood of a genetic cause of epilepsy is easily underestimated. This is because some epilepsies (10-30% depending on epilepsy type) are Mendelian (single gene) disease whereas most of the common epilepsies have a complex pattern of inheritance, thus are less likely to be identified in a clinical setting (267). However, even when a cause for epilepsy is apparently found (e.g. a stroke) there is clear evidence that epilepsies develop only in some susceptible individuals, suggesting the presence of genetic modifiers and susceptibility alleles. According to some studies assessing post-traumatic epilepsies, the incidence of family history of epilepsy in patients with closed head trauma (i.e. stroke) who develop late seizures is 6-17%, compared with only 3-4% of those who do not (268). These findings strongly suggest that factors that are presumably genetically determined, predispose these patients to the development of post-traumatic epilepsies (268).

Only two studies have explored the genetic influence on PSE or the contribution of genetics to the response to injury and consequent epileptogenesis in patients with stroke. The first paper published in March 2014, found evidence of an association between the functional polymorphism of CD40 (T allele) and PSE

development. Carriers of the T allele showed increased plasma sCD40L levels, reflecting an oxidative stress state and a pro-thrombotic state (269). The second paper published in October 2014, showed a significant difference in the distribution of the allele and genotype frequencies of the rs671 polymorphism (A allele) on the ALDH2 gene between PSE patients and ischaemic stroke patients. They also showed that the presence of ALDH2*2 significantly increased the risk of PSE in patients without a history of alcohol consumption, suggesting that alcohol consumption may have an effect on the development of cardiovascular disease (270). The interaction between genetics, epilepsy risk factors and post stroke epilepsy development is an area that is largely under investigated, with only the ALDH2 gene investigated in this manner to our knowledge.

Although there have not been any genome-wide allelic association studies designed to identify genetic variants associated with post-traumatic epilepsies, several candidate genes have been studied using allelic association (268). Inheritance of the apolipoprotein E (ApoE) 4 allele is associated with increased risk of Alzheimer's disease, progression to disability in multiple sclerosis, and poor outcome after traumatic brain injury (268). One study assessed the incidence of seizures in patients who suffered severe traumatic brain injury. Six months after injury, 22 patients (20%) had at least one late post-traumatic seizure, RR=2.41 (271). Additionally, the ApoE genotype was investigated in 322 patients with severe traumatic brain injuries. Although no statistical associations were

found, two out of the four individuals (50%) with the E4/E4 had late post-traumatic seizures (272).

Many studies to date have examined the genetic contribution to stroke recovery, stroke outcome and the development of stroke comorbidities, with few polymorphisms identified to be associated with outcome after ischaemic stroke. For example, the APOE4 gene is associated with impairment of neuronal repair processes and has been associated with significantly poorer recovery over the first month post stroke with a lower proportion of patients with minimal or no disability at 3 months post stroke (273). Similarly, the IGF1 locus affects brain plasticity after brain injury and the Rs7136446 polymorphism has been associated with good functional outcome at 3 months post stroke (223). As stroke severity and the persistence of stroke disability are primary risk factors for the development of PSE, genes such as IGF1 and APOE4 could be potential candidate genes for further exploration for the development of PSE. So far, no literature has been published on the influence of genetics on stroke progression and the development of subsequent epilepsy.

Is there a treatment for post stroke seizures?

Currently, the most efficient ways to prevent epileptogenesis are genetic counselling or prevention of primary insult (for example, by minimising your risk for stroke) (274). Developments in the past decade of potentially antiepileptogenic

compounds in animal models emphasised the need for an early intervention within the initial weeks after stroke, before the epileptogenic cascade sets in (259). However to date, no indications for primary or secondary treatment with antiepileptic drugs in preventing post stroke seizures and epilepsy exist and as such, only weak recommendations have been made against the use of AED prophylactic treatment in acute ischaemic stroke patients for prevention of seizures (275). This is because undertaking clinical anti-epileptogenesis trials is difficult. Contributory reasons for this include the difficulty in identifying people at high risk of seizures after a stroke and the need for prolonged follow up (259). Trials with unselected populations would require large sample sizes and thus be costly. However, identifying a higher risk target population would better allow for designing and developing trials that explore the anti-epileptogenic properties of specific agents, including AEDs. Defining prognostic biomarkers of seizures after acute ischaemic stroke is of clinical imperative to advance the development of anti-epileptogenic treatments and guide the clinical management of stroke survivors (259).

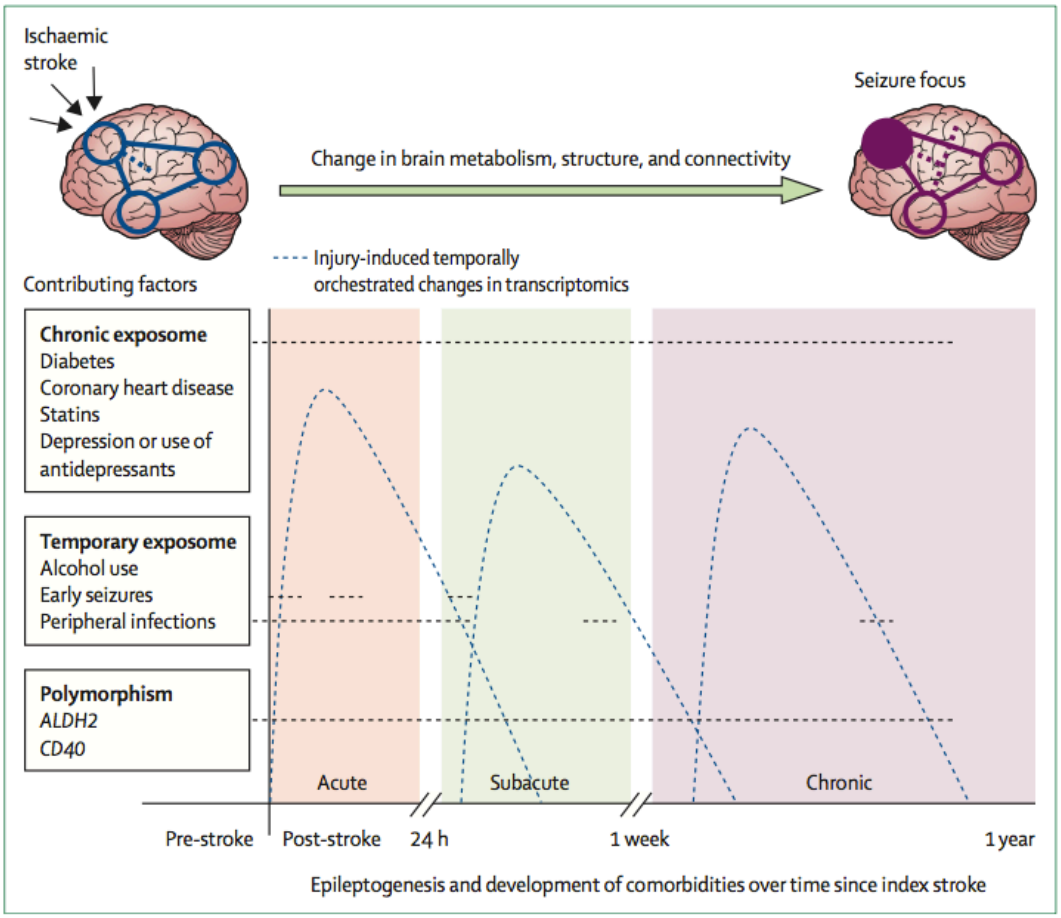

Figure 12: Evolution of epileptogenesis and the development of comorbidities after an index stroke and contribution of genetic factors and the exposome (223)

One theory of the contributing factors of epileptogenesis post ischaemic stroke

SUMMARY

As indicated, in some studies both acute seizures and post stroke epilepsy have been associated with higher mortality, longer hospitalization and greater disability after stroke (2). Prophylactic antiepileptic drugs are not indicated in stroke patients, but some of these drugs have proven useful and safe in the management of post stroke seizures (3). The identification of the patients at highest risk to suffer epileptic disorders could be of interest for the design of future clinical trials. To date, no blood biomarkers and only few genetic biomarkers have been associated with the risk of epilepsy after ischaemic stroke. Their identification would be clinically useful to select high-risk patients for future trials.

Rationale for this thesis

With greater public awareness in early stroke recognition and more efficient treatment delivery (e.g. code stroke, telemedicine), there has been a welcome increase in the proportion of patients arriving at hospital within the timeframe to be eligible for reperfusion therapies (13). Numerous randomized controlled trials have demonstrated the superiority of reperfusion therapies, including intra-arterial therapies (IAT) and intra-venous tissue plasminogen activator (IV-tPA), over standard treatment for acute ischaemic stroke patients with large artery occlusion (145-149). These advances have contributed to a 68% increase in the number of stroke survivors between 1990 and 2010 (1). These

stroke reperfusion treatments are time critical with improved outcomes associated with even a few minutes reduction in onset to treatment time (63, 64). However, individual variation in stroke pathophysiology means that the appearance of brain imaging can provide a more accurate prognosis than time alone (65). As such, and with a shift to earlier stroke treatment times, it is currently unknown whether current imaging tools will persist as simple and reliable methods for assessing early ischaemic changes. Additionally, given the improved immediate outlook of acute stroke with reperfusion therapies, there is need to understand whether such reperfusion therapies affect longer term complications of stroke, and whether there is a role for imaging biomarkers in not just acute stroke outcomes but also the longer-term complications of stroke.

Currently, the Alberta Stroke Program Early CT Score (ASPECTS) on non-contrast computed tomography (NCCT) represents a commonly used imaging tool for detecting early ischaemic change (EIC). However, the use of ASPECTS is highly time dependent, with optimal detection on NCCT hours to days from stroke onset (108, 276, 277). As such, and with a shift to earlier stroke treatment times, it is currently unknown whether the utility of ASPECTS will persist as a simple and reliable method for assessing ischaemic change. Thus, there is need to optimise the role of imaging in hyperacute stroke and assess the potential use of acute stroke imaging in longer term complications of stroke, such as epilepsy.

Epilepsy is one of the major complications of stroke. Post stroke epilepsy poses a considerable burden to stroke survivors and, even when well-controlled with

medications, negatively impacts their quality of life (206). Seizures develop in 2–14% of patients who have had an ischaemic stroke (207). Such wide variation in the reported incidence and prognostic factors (278) has been attributed to differences in follow-up times, epilepsy and seizure definitions, heterogeneity in study designs, methodology for seizure classification, stroke populations investigated and the potential underlying genetic differences between populations (230). Whether the recent advances in reperfusion therapies for acute ischaemic stroke have influenced the incidence of seizure development has not been well studied. Two studies have found that thrombolysis increases the likelihood of acute symptomatic seizures, within 7 days, post ischaemic stroke (260, 261), whilst others have shown no association (262) and few studies have examined post stroke seizures following intra-arterial therapies (IAT). A better understanding of the relationship between acute stroke therapies and the development of seizures may lead to improved post stroke monitoring and follow-up. Similarly, factors that contribute to seizure development post stroke in a given individual generally remain unknown. Genetic mutations play a major role in the development of certain epilepsies; it is not understood, however, whether those patients who develop epilepsy post stroke are genetically predisposed to developing epilepsy or if it is purely determined by the nature and extent of brain ischaemia.

Thesis aims

The aim of this dissertation was the investigation into clinical, radiological and genetic markers of ischaemic stroke outcome.

The specific aims of this thesis were:

1. How can we optimise the role of imaging in hyperacute stroke and assess the potential use of acute stroke imaging in longer-term complications of stroke, in particular epilepsy?
2. Given the improved immediate outlook of acute stroke, how do these novel stroke interventions affect longer-term complications of stroke, in particular epilepsy?
3. In a population of acute ischaemic stroke patients, is there an association between genetic variants and the development of post stroke epilepsy?

General Methods

Overview

This chapter outlines the general methodology utilized for the studies within this thesis. Whilst specific methodologies are provided in each study, they are typically briefer due to the word limit restrictions imposed by journals. As such, this chapter will elaborate and provide additional information and examples concerning specific study designs and aims, ethics, recruitment, participant information, radiological methods and post stroke seizure follow up.

To answer the 3 objectives, 7 studies were undertaken. The results of these objectives will be presented according to the following overview.

Table 6: Overview of studies undertaken

Study	Objective 1: Radiological	Objective 2: Clinical	Objective 3: Genetic
1	Reliability and utility of the Alberta Stroke Program Early CT Score in hyperacute stroke		
2	Reliability and utility of the Alberta Stroke Program Early CT Score on CT perfusion and non-contrast CT in hyperacute stroke		
3	A comparison of the influence of hemorrhagic transformation on post ischaemic stroke seizure development in patients receiving reperfusion therapies		
4		The association between different acute stroke therapies and development of post stroke seizures	
5		Does ethnicity affect the association between atrial fibrillation and post stroke seizure development?	
6		A registry of clinical and MRI outcomes following early versus late initiation of anticoagulation after ischaemic stroke or transient ischaemic attack in patients with atrial fibrillation	
7			Is there a genetic association on the development of post stroke seizures?

Objective 1- Radiological markers: *How can we optimise the role of imaging in hyperacute stroke and assess the potential use of acute stroke imaging in longer-term complications of stroke, in particular epilepsy?*

Study 1: Reliability and utility of the Alberta Stroke Program Early CT Score in hyperacute stroke

Study Overview: The aim of this chapter was to evaluate whether hyperacute time from ischaemic stroke onset to initial non-contrast CT influences inter-rater variability and prognostic accuracy of the Alberta Stroke Program Early CT Score for 3-month functional outcome, as determined by the modified Rankin scale (mRS). This was a retrospective, single center, cohort study including all patients admitted to the Royal Melbourne Hospital with an anterior circulation, acute ischaemic stroke between December 2007 and April 2014. These patients must have received intravenous tissue-plasminogen activator (IV-tPA) on arrival to the hospital. All patients receive a NCCT as part of routine clinical care. Patients treated with IV-tPA have baseline demographic and follow-up information (including 3-month modified Rankin scale) recorded as part of routine clinical care. All such information is recorded into the *'IV-tPA stroke database'*, stored on the hospital CORTEX server and password protected. Patients were only excluded if their initial NCCT was severely movement degraded resulting in unattainable radiological information being collected.

Study 2: Reliability and utility of the Alberta Stroke Program Early CT Score on CT perfusion and non-contrast CT in hyperacute stroke

Study Overview: Given the results of study 1, this chapter aimed to assess and compare the practical evaluation (including reliability, prognostic accuracy and reproducibility) of ASPECTS on CT perfusion and NCCT in early versus later times after stroke onset. This was a retrospective, single centre, cohort study including all patients admitted to the Royal Melbourne Hospital with an anterior circulation, acute ischaemic stroke between December 2009 and April 2014. Similar to study 1, these patients must have received intravenous tissue-plasminogen activator (IV-tPA) on arrival to the hospital and have baseline demographic and follow-up information (including 3-month modified Rankin scale) recorded as part of routine clinical care. All such information is recorded into the *'Stroke database'*, stored on the hospital CORTEX server and password protected. Only patients with additional CT perfusion scans on arrival to the hospital were included. Patients were excluded if their initial NCCT or CTP was severely movement degraded resulting in unattainable radiological information being collected.

Study 3: A comparison of the influence of hemorrhagic transformation on post ischaemic stroke seizure development in patients receiving reperfusion therapies

Study Overview: This chapter aimed to compare the influence of hemorrhagic transformation on post ischaemic stroke seizure development in patients receiving reperfusion therapies (intra-venous thrombolysis (IV-tPA) and intra-arterial therapy (IAT) (with/without IV-tPA). This was a retrospective, single centre cohort study conducted at the Royal Melbourne Hospital. We included patients with anterior circulation, acute ischaemic stroke admitted between 2008-2015 treated with either IAT and/or IV-tPA on arrival. All patients receiving reperfusion therapies have follow up 24-hour NCCT as part of routine clinical care. These scans were acquired for assessing haemorrhagic transformation. Patients with a history of epilepsy or seizures prior to their stroke were excluded.

Objective 2- Clinical markers: *Given the improved immediate outlook of acute stroke, how do these novel stroke interventions affect longer-term complications of stroke, in particular epilepsy?*

Study 4: The association between different acute stroke therapies and development of post stroke seizures

Study Overview: This was a retrospective, multi-centre cohort study conducted at the Royal Melbourne Hospital and Jinling General Hospital, Nanjing. This study aimed to establish whether there is an association between different acute stroke treatments and post stroke seizure development. We included patients with anterior circulation ischaemic stroke admitted to the two hospitals between 2008 and 2015. Patients included were divided into 4 groups based on the type of acute reperfusion treatment received: 1. IV-tPA only, 2. IAT only, 3. IAT+IV-tPA and 4. stroke unit care only (i.e. no IV-tPA or IAT).

Study 5: Does ethnicity affect the association between atrial fibrillation and post stroke seizure development?

Study Overview: This chapter assessed whether ethnicity affects the association between atrial fibrillation and post stroke seizure development. There have been suggestions that patients with cardioembolic stroke are at a greater risk of developing seizures than other stroke subtypes. However, the incidence of atrial

fibrillation and cardioembolic stroke varies considerably across countries, generally higher in Western populations than in Asian populations. We hypothesised that Royal Melbourne Hospital (Melbourne) patients will have significantly higher incidence of AF-related PSS than in the Jinling Hospital (Nanjing) population. This was a retrospective, multi-centre cohort study including patients with anterior circulation ischaemic stroke admitted between 2008-2015. Occurrences of post stroke seizures were ascertained by reviewing medical records or telephone follow-up. To test the hypothesis of an interaction between ethnicity and atrial fibrillation for post stroke seizure occurrence, a logistic regression model with atrial fibrillation and ethnicity together with an ethnicity-by- atrial fibrillation interaction term was used.

Study 6: A registry of clinical and MRI outcomes following early versus late initiation of anticoagulation after ischaemic stroke or transient ischaemic attack in patients with atrial fibrillation

Study Overview: This is a protocol/methods chapter, which follows on from the previous study. This is a prospective, observational clinical trial aiming at assessing clinical and MRI outcomes following early versus late initiation of anticoagulation after ischaemic stroke in patients with atrial fibrillation. Included patients will be patients who present with an acute ischaemic stroke of cardioembolic (atrial fibrillation -related) origin and who have an MRI following their primary ischaemic event, and are deemed suitable for initiation of

anticoagulation therapy. Subjects must be enrolled within 30 days of symptom onset. A secondary outcome of this study is the development of post stroke seizures within two years of stroke onset. Occurrences of post stroke seizures will be ascertained by reviewing medical records or telephone follow-up.

Objective 3 – Genetic markers: *Is there a potential genetic association to the development of seizures post stroke or is it a complication of the stroke itself?*

Study 7: Is there a genetic association on the development of post stroke seizures?

Study Overview: This chapter aims to examine whether there is a potential genetic component to the development of seizures post stroke or whether it is a complication of the stroke itself. Post stroke epilepsy occurs in approximately 10% of patients, posing a considerable extra burden on both the patient and physician and even in the mild or well-controlled epilepsies, quality of life is significantly lower. The interaction between genetics, epilepsy risk factors and post stroke epilepsy development is an area that is largely under investigated. To our knowledge, only two papers have assessed the influence of genetics on post stroke epilepsy development in humans and no literature has been published on the influence of genetics on stroke progression and the development of subsequent epilepsy. To answer this question, a case-controlled, multicentre study was performed. Cases were ischaemic stroke patients who developed post stroke

seizures within two years of stroke onset. Controls were ischaemic stroke patients who did not develop post stroke seizures after two years from stroke onset. Included patients had DNA collected or accessed either through blood samples or saliva kits or if the patient had previously stored blood samples at retrospective hospitals. A sample size of 285 controls (ischaemic stroke and no epilepsy) and a further 285 cases was calculated. This was based off the two primary papers on the genetic influence on post ischaemic stroke epilepsy patients, which will be discussed in further detail within the chapter. The differences in the genotype and allele frequencies between the groups will be compared using a Chi-square distribution test, with a significance level set at $p<0.05$.

Ethics

For studies 1 and 2, ethical approval was granted by the Melbourne Health Human Research Ethics Committee **(project number QA2010089)** and due to the nature of these projects, patient consent was waived.

For studies 3-5, ethical approval was granted by the Melbourne Health Human Research Ethics Committee (**project number QA2010089)**. Due to the nature of these projects, patient consent was waived.

For study 6, a retrospective clinical trial, multicentre ethical approval was granted by the Hunter New England Human Research Ethics Committee (**HNEHREC16/02/17/4.01).** In accordance with the Helsinki declaration, all participants will provide informed consent, with independent witness authentication as part of the recruitment procedure.

For study 7, ethical approval was granted by the Melbourne Health Human Research Ethics Committee **(HREC/17/MH/35, site specific number RMH 2017.028).** In accordance with the Helsinki declaration, all participants provided informed consent, with independent witness authentication. All participants were capable of providing their informed consent at the time of recruitment.

Clinical Information

Patient populations and data extraction

Royal Melbourne Hospital, Melbourne, Australia

The Royal Melbourne Hospital (the primary site), located in Victoria, Australia, provides intravenous thrombolysis therapy to acute ischaemic stroke patients who arrive to the hospital within 4.5 hours of stroke onset. It also serves as the state-wide referral centre for intra-arterial therapies, including endovascular thrombectomy and intra-arterial urokinase. All patients were recruited from the *Stroke database*, an ongoing registry of all stroke patients (ischaemic, haemorrhagic and transient ischaemic attack) admitted to the acute stroke ward since 2009. Patients treated with either IV-thrombolysis and/or IAT are additionally enrolled into a databank containing further stroke information, risk factors, imaging data and follow up information. Information collected in both registries includes baseline stroke demographics (age and sex), stroke risk factors (diabetes, hypertension, systolic and diastolic blood pressure, hyperlipidaemia, atrial fibrillation, previous stroke or transient ischaemic attack and smoking) and treatment (IV-thrombolysis/intra-arterial therapies). Information exclusive to the treated patients includes clinical follow-up information (modified Rankin Scale at 3 months post stroke onset, haemorrhagic transformation assessed using the ECASS classification on follow up CT brain imaging and two-year seizure data collection. The information from this database was used in studies 1-7.

Jinling Hospital, Nanjing, China

Participants recruited from this centre were derived from the *Nanjing Stroke Registry Program*. This program began in 2002 and represents the first hospital-based stroke registry program conducted in mainland China. It is based at the Jinling Hospital, Nanjing University School of Medicine, which is located in Nanjing, a city in southeast China. All stroke patients registered will have had a stroke and have presented to the hospital within 7 days of stroke onset. Patients with transient ischaemic attack are not included into the registry. Stroke was defined by World Health Organisation criteria as a rapid onset of clinical signs of a local or global disturbance of cerebral function, lasting more than 24 hours or leading to death, with no apparent vascular cause.

It is an ongoing, observational and prospective registry collecting demographic, clinical, neuroimaging, laboratory data and collected and stored blood samples of registered patients. Information collected includes demographic characteristics, risk factors for cardiovascular disease, major clinical manifestations, significant personal and family history, laboratory results, neuroimaging findings, stroke subtypes and stroke risk factors (hypertension, hyperlipidemia, smoking and diabetes). An initial non-contrast CT is performed on all patients if a stroke is suspected. Further, computerized tomography angiography, magnetic resonance angiography and digital subtraction cerebral angiography are performed if indicated. Similarly, an electroencephalography and 24-hour electroencephalography (Holter) monitor are performed if indicated. The 3-month modified Rankin Scale and any new findings or revisions are documented in the

patient follow up appointment, or via telephone interviewing. Written informed consent was obtained from all patients registered. This program was approved by the Review Board of Jinling Hospital.

Data was extracted from Nanjing over two site visits (2015, 2016). The first site visit was for a month and involved assessing feasibility, application of funding through the Endeavour Fellowship Australia Program, site-specific details (organisation of personnel, ethics) and extracting the stroke baseline data from the registry. Once funding was secured through the Endeavour Program, the second site visit was undertaken and involved the collection of the post stroke epilepsy data, accessing the biobank for stored blood samples and organisation of DNA testing. The information from this centre was used in studies 4, 5 and 7.

John Hunter Hospital, Newcastle, Australia

Patients at this centre were recruited exclusively for Study 7. Patients were recruited from the *Australian Stroke Genetics Collaborative (METASTROKE collaboration)*, a National Health and Medical Research Council funded collaborative lead by Professor Christopher Levi at the John Hunter Hospital in Newcastle Australia. This project aimed at examining the relevance of genetic variations linked to atherothrombosis, in the context of environmental risk factors. ASGC stroke cases comprised stroke patients of European ancestry who were admitted to four clinical centres across Australia (The Neurosciences Department at Gosford Hospital in Gosford; the Neurology Department at the John Hunter Hospital in Newcastle; the Queen Elizabeth Hospital in Adelaide and

the Royal Perth Hospital in Perth between 2003 and 2008. Stroke was defined by World Health Organisation criteria as sudden focal neurological deficit of vascular origin, lasting more than 24 hours and confirmed by imaging (non-contrast CT and/or magnetic resonance imaging). Other investigative tests such as electrocardiogram, carotid Doppler and trans-oesophageal echocardiogram were conducted to confirm ischaemic stroke mechanism as clinically appropriate. Cases were excluded from participation if they were aged <18 years old, diagnosed with haemorrhagic stroke/transient ischaemic attack or were unable to undergo brain imaging on arrival to hospital. A total of 1230 patients were included into this study across the four centres. However, for the purposes of the study performed in study 7, only patients enrolled at the John Hunter Hospital in Newcastle were included. Information collected in this study included baseline demographics (age, sex), stroke subtypes (as determined by the TOAST classification), cardiovascular risk factors (hypertension, hypercholesterolaemia, diabetes mellitus, atrial fibrillation, myocardial infarction, smoking status), baseline National Institute of Health Stroke Scale, treatment with intravenous thrombolysis, follow-up information (3-month modified Rankin Scale and intracerebral haemorrhage) and DNA collection and storage.

Data was extracted from John Hunter over a single site visit (2017). This site visit was for two weeks and involved accessing the *METASTROKE* patient database and reviewing medical records/clinical notes/follow-up correspondence for the occurrence of post stroke seizures. New South Wales has an electronic medical records system that records any inpatient/outpatient or emergency visit to a

hospital within the region. All living patients were then followed up for post stroke seizures with a letter containing the validated epilepsy questionnaire (discussed below). This enabled the identification of stroke patients with and without post stroke seizure occurrence.

University of Campinas, Sao Paul, Brazil

Patients at this centre were enrolled into the *Joinville Stroke Registry*, a collaboration between four Brazilian centres aiming to contribute to studies that use detailed clinical and genomic information of biobanks. The biobank originated from two sequential initiatives to study epidemiological aspects of stroke in Brazil, initiating in 2005. In 2010, control data, blood extractions and DNA storage were initiated. Controls were matched by age and sex to patients and individuals with a previous history of stroke or blood relatives to patients with a history of stroke were excluded. This constitutes a taskforce of the Brazilian Consortium of Stroke Research, which is sponsored by the Brazilian Ministry of Health and the National Council for Scientific and Technological Development, with the aim of defining stroke incidence trends and case-fatality proportions. Information collected within the registry includes demographic socio-economic data (years of education, type of work, family history of stroke, cardiovascular risk factors, biochemical tests and functional status (modified Rankin Scale and National Institute of Health Stroke Scale), stroke subtype (as determined by the TOAST classification), follow-up information at 3, 6, 9 and 12 months in the first

year and once a year for four years post stroke diagnosis (death, modified Rankin Scale, physician visits, blood pressure control, cholesterol levels and smoking habits. Patients at this centre were recruited exclusively for Study 7. Data extraction of patients from this centre occurred in 2017 and was undertaken by personnel onsite. As baseline stroke data and patient blood samples were previously collected within the registry, patients were followed up for post stroke seizures via the same validated post stroke seizure questionnaire.

Prince of Wales Hospital, Hong Kong, China

Patients from this centre were enrolled in a larger collaboration assessing genome wide associations between variants and epilepsy in Chinese patients, supported by the Research Grants Council of the Hong Kong Special Administrative Region in China (project numbers HKU7623/08M, HKU7747/07M and CUHK4466/06M). Epilepsy patients of Han Chinese ethnicity aged between 2 and 91 years old were recruited from neurology clinics of five regional hospitals in Hong Kong covering a combined catchment population of approximately 3 million. Syndromic classification was adapted from the revised international organization of phenotypes in epilepsy. Patients were excluded if they had significant psychiatric comorbidity, history of pseudoseizures, alcohol or illicit drug abuse, and presence of progressive or degenerative neurological or systemic disorders. Within this registry, patients were already identified and recorded as having epilepsy due to a previous ischaemic stroke. Because of this, baseline stroke data was limited to age

at stroke and sex at this centre. Patients at this centre were recruited exclusively for study 7.

Data was extracted from this site in 2016 during and after a site visit. The site visit was organized during the Endeavour Fellowship and allowed for the onsite organization, screening of post stroke epilepsy data, accessing stored blood samples of patients and organization of DNA testing.

Clinical Measures

Post stroke seizure follow up

Post stroke seizure follow up was required for studies 3-8. Occurrence of post stroke seizures was ascertained by reviewing a seizure questionnaire modified from that used in our center previously and from a validated screening questionnaire (Keezer MR, et al. Epilepsia 2014, 55:1763-1771), corroborated with the patient's hospital medical records and records from their primary physicians. Events were recorded as seizures if the symptoms included motor or autonomic components, with or without impairment of consciousness, as defined by the International League Against Epilepsy (211). If discrepancies were found between patient recall and medical records/notes, final determination was made by an epileptologist. This questionnaire was translated into Chinese for use at the centres in China and also into Portuguese for centres in Brazil. Patients with post stroke seizures were identified if they experienced seizures up to two years from stroke onset. This cut-off was chosen based on previous findings that suggested

that the highest risk of seizure development was within the first year (226). A copy of the questionnaire is provided in the appendix.

Radiological measures

This section pertains to studies 1, 2 and 3 and will outline the use of the Alberta Stroke Program Early CT Score (ASPECTS) used on initial non-contrast CT and CT perfusion. This will then discuss the use of radiological biomarkers in longer-term post stroke sequelae, in particular post stroke seizures.

ASPECTS on NCCT

Royal Melbourne Hospital Picture Archiving and Communicating System was used to assess the patient's initial CT scan (Somatom 16 slice and Definition FLASH 128 slice scanners, Siemens, Erlangen, Germany) on radiological workstations. The first CT scans to be uploaded onto Synapse commenced in December 2007. Information on the initial CT date and time was located on the *Stroke Database*. The ASPECTS score was assessed using 5mm axial thick slices as per standard recommendations. The regions included M1, M2, M3, M4, M5, M6, Caudate Nucleus, Lentiform Nucleus, Insula and the posterior limb of the Internal Capsule. The ASPECTS provides a semi-quantitative score out of 10 with a score of 10 indicating a normal CT scan. A point was deducted for each region of the brain affected by ischaemic changes. These ischaemic changes included hypointensity visible on 2 adjacent slices or loss of grey/white demarcation. A

lesion that was straddling 2 regions only conceded a 1-point deduction. Furthermore, the assessor was allowed to know the side of the brain that was infarcted, but the CT scan was initially read blinded. A consensus was achieved between a neuro-interventionist (Dr. Neil Rane) and a student researcher (Jill Naylor). The initial CT scans were simultaneously assessed using Synapse. These scores were recorded and entered into the corresponding *Stroke Database*.

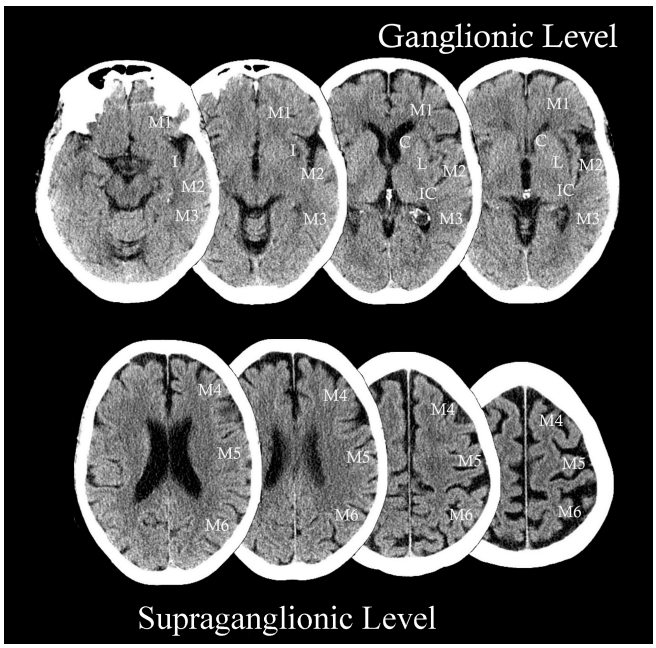

Figure 13: The ASPECTS demarcations

This image shows the demarcations of the ASPECTS on plain CT at the level of the ganglia and at the level of the supraganglia. There are 10 regions. M1, M2, M3, M4, M5, M6, Insula (I), Internal Capusle (IC), Lentiform Nucleus (L) and Caudate (C). A mark is deducted for each region that is affected by ischaemic change.

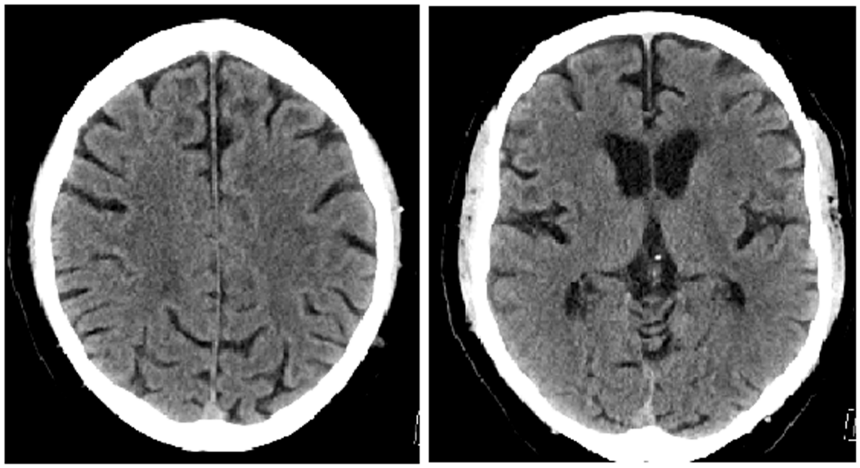

Figure 14: Example of ASPECTS 10 on NCCT

This image shows a normal non-contrast CT brain scan. This image would score an ASPECTS of 10. Note that all slices were used for ASPECTS assessment.

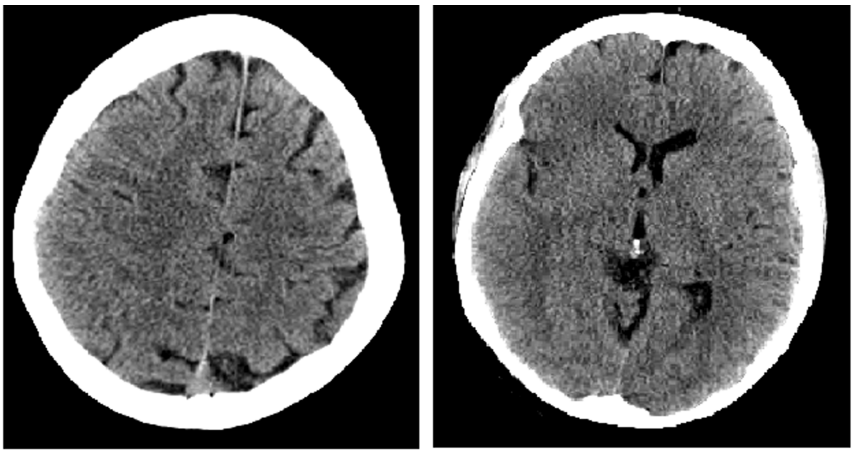

Figure 15: Example of ASPECTS 5 on NCCT

Areas of ischaemic change are evident in regions M5, M6 at the supraganglionic level and regions M1, M2 and Insula at the level of the basal ganglia. Note that all slices were used for ASPECTS assessment.

ASPECTS on CT Perfusion

Prior to 2013, a 16-slice Somatom CT scanner (Siemens, Erlangen, Germany) was used and acquired two separate CTP slabs at the ganglionic and supraganglionic levels, each with 2x12 mm slices. Images were acquired every second for 40s following an initial 4s delay. Subsequently a 128-slice Definition FLASH CT Scanner (Siemens) was used and acquired full brain CTP (10x10mm slices) with 30 time-points acquired over 60s. Perfusion maps were generated using RAPID fully automated software (RAPID, non-commercial research version, Stanford University). In all cases, changes needed to be visible on at least one slice. The same two assessors were aware of the affected hemisphere, but the CTP scans was read independently and blinded to time to scan.

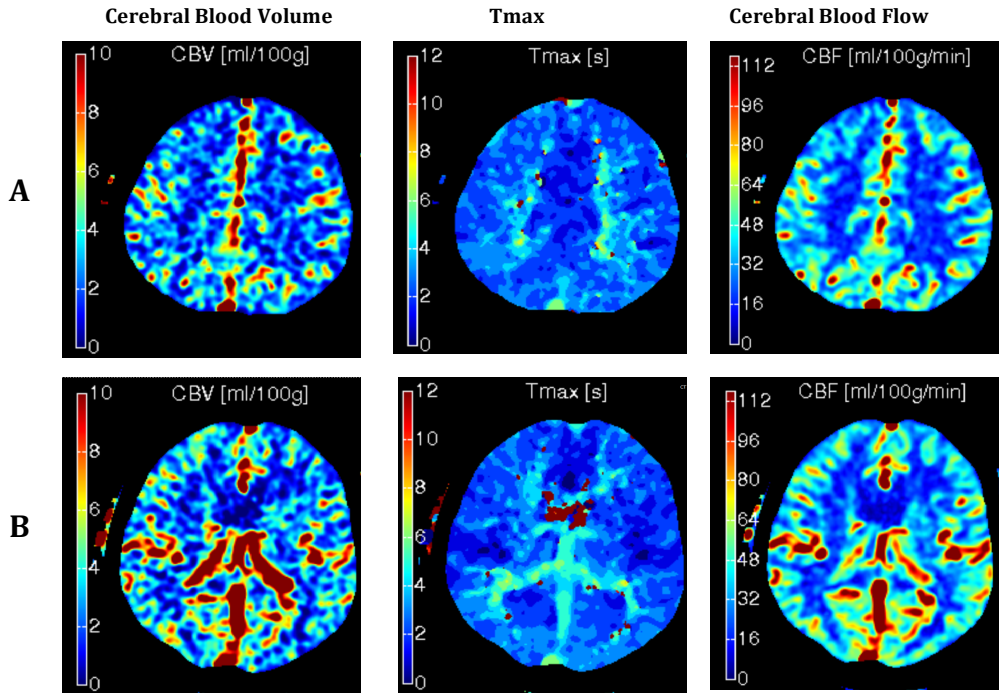

Figure 16: Example of ASPECTS 10 on CT Perfusion

This image shows a normal CT Perfusion brain scan. This image would score an ASPECTS of 10.

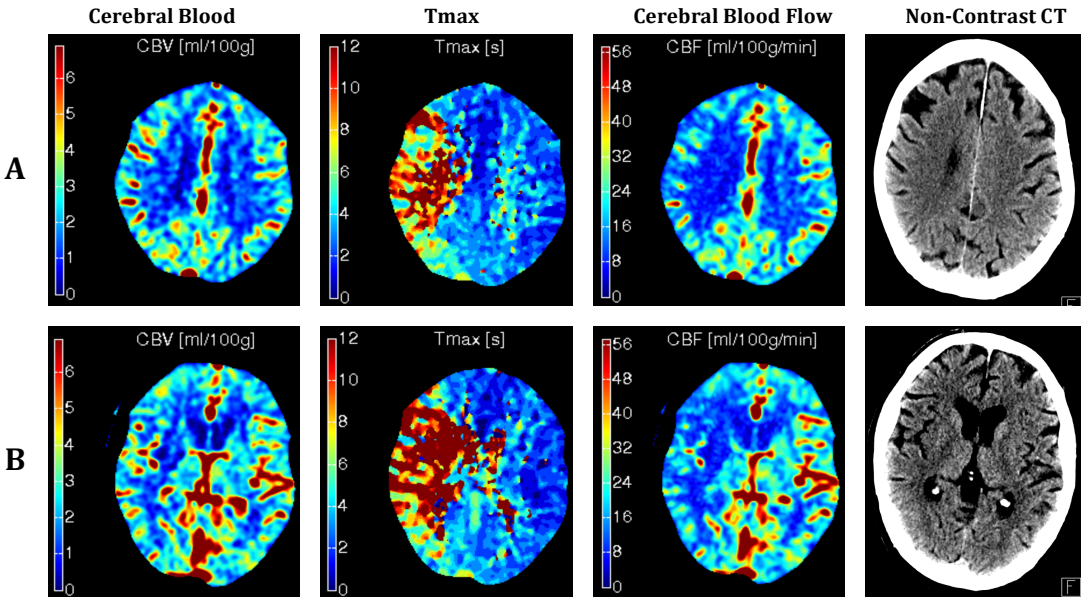

Figure 17: Example of ASPECTS scoring on CT Perfusion

Tmax, CBF and CBV maps were assessed using the same regional ASPECTS template as for NCCT. Only Tmax >6s delay was considered abnormal as per previous validation of the threshold for potentially salvageable brain. Abnormal CBV or CBF was defined as unequivocal reduction compared to the corresponding region in the unaffected hemisphere. This is a patient with right middle cerebral artery territory ischaemic stroke. Row A highlights ischaemic change at the supra-ganglionic level. Row B highlights ischaemic change at the level of the ganglia. This patient received an ASPECTS of 5 on CBV, indicating ischaemic changes in regions M1, M2, M5, Insula and Lentiform nucleus. On CBF this patient received an ASPECTS of 4, indicating ischaemic changes in regions M1, M2, M4, M5, Insula and Lentiform nucleus. On Tmax, this patient received an ASPECTS of 2, indicating ischaemic changes in regions M1, M2, M3, M4, M5, Caudate nucleus, Lentiform nucleus and insula. Additionally

provided is the baseline NCCT of the patient. This patient received an ASPECTS of 5 on NCCT, indicating ischaemic changes in regions M5, M6, M2, Insula and the Lentiform nucleus.

Assessment of haemorrhagic transformation of the ischaemic infarct

Patients routinely undergo neuroimaging between 24 and 48 h after endovascular intervention. In general, patients underwent noncontrast computed tomography (NCCT) using a Siemens Somatom multidetector scanner (Siemens, Erlangen, Germany) with 5 ‑ mm ‑ thick slices at 24 hours post onset. Thus, hemorrhagic transformation (HT) on follow-up 24hr CT brain imaging was assessed by three neurointerventionists and determined by consensus and blinded to the patient's seizure status. HT was recorded as any amount of blood present in the treated infarcted area, observed in at least 2 adjacent slices. The Hounsfield unit scale was employed to distinguish between angiographic contrast extravasation, hemorrhage, or a mixture of both. The degree of HT was classified into HI (petechial haemorrhage without space-occupying effect) or PH (haemorrhage with mass effect) in accordance with The European Cooperative Acute Stroke Study (ECASS) (279), with further sub-classification based on size of the haemorrhage.

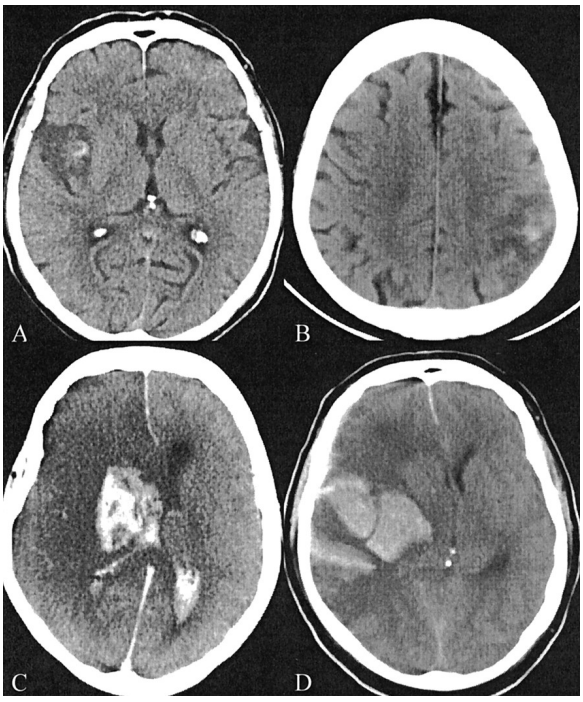

Figure 18: Example of ECASS definition for defining haemorrhagic transformation on CT (A) HI1, (B) H12, (C) PH1, (D) PH2

Genetic Measures

This section pertains to Study 7 and will outline phenotypic data collection across the centres, genetic sample collection, sample processing and sample sequencing.

Phenotypic data collection

This was a case-controlled, multicentre study. Cases were ischaemic stroke patients who developed post stroke seizures within two years of stroke onset. Controls were ischaemic stroke patients who did not develop post stroke seizures after two years from stroke onset. These two populations were age/sex matched. Post stroke seizure patients were identified through the same process discussed in section *Clinical Information: Post stroke seizure follow up.*

Sample Collection

All centres, excluding the Royal Melbourne Hospital, had a biobank of patient blood samples. Information on these patient registries is available in section *Clinical Information: Patient Populations.* Patient samples at the Royal Melbourne Hospital were collected either through two methods (1. collection of blood during patient hospital visit or 2. collection of saliva through delivery of a saliva kit to patient address of residence).

Blood Collection

Identified case patients from the Royal Melbourne Hospital who returned to the hospital for follow-up appointments or visits were consented to having a whole blood sample taken during their visit. Four times 4mL K2E EDTA (Greiner Bio-One, Kremsmunster, Austria) Vacuette® Tubes were obtained and de-identified samples were subsequently stored in an -80°C freezer until sample processing.

Saliva Kits

An Oragene-DNA (OG-575) saliva kit was chosen for use in this study as it is convenient for patients, can be sent in the standard postal system, is minimally invasive and patient samples can stay stable for years at room temperature. Patients who verbally consented (telephone interview) to participating in this study were sent via mail a package containing:

- A letter of introduction
- A patient information and consent form
- An Oragene-DNA (OG-575) saliva kit
- Instruction manual

Once completed saliva kits were sent back to the Royal Melbourne Hospital, the de-identified sample was stored in -80°C freezer until sample processing.

Sample preparation and quality assessment

This section pertains exclusively to the centres that did not have genotyped patient blood samples (Royal Melbourne Hospital, Jinling Medical Hospital and Univeristy of Campinas).

Genomic deoxyribonucleic acid (DNA) was extracted from whole blood samples using Gentra Puregene Kits (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Frozen blood samples were thawed in a 37°C water bath with mild agitation. RBC lysis solution (9mL) was mixed with whole blood (3mL) and centrifuged (Allegra^{TM} X-12R Centrifuge, Beckman Coulter, Brea, CA, USA) for 2 minutes at 2000*g* following incubation for 5 minutes at room temperature. The supernatant was discarded, and cell lysis solution (3mL) and protein precipitation solution (1mL) was added before vortexing the tube 20 seconds followed by centrifuging for 5 minutes at 2000*g*. This formed a dark brown pellet of precipitated proteins, which was discarded. The supernatant was mixed with isopropanol (3mL) and gently inverted 50 times until the DNA became visible as threads or a clump. At this point, the tube was centrifuged again at 2000*g* for 3 minutes, after which the DNA was visible as a small white pellet, and the supernatant was discarded from the tube. Ethanol (70%, 3mL) was added and the tube inverted several times to wash the DNA pellet, before centrifuging for 1 minute at 2000*g*. The supernatant was discarded, and the DNA pellet was air dried for 5-10 minutes, before adding DNA hydration solution (300μL). This mixture was incubated at 65°C for 1 hour to dissolve the DNA, and then at room

temperature overnight. Lastly, the DNA samples were briefly centrifuged and transferred to a storage tube ready for genotyping. The DNA extraction process yielded purified DNA that typically had an A_{260}/A_{280} ratio between 1.7 and 1.9, and was up to 200kb in size. The absorbance A_{260}/A_{280} ratios were calculated to assess the purity of the DNA samples. DNA samples that failed to achieve an A_{260}/A_{280} ratio of at least 1.7 were re-purified, or re-extracted from stored whole blood samples.

Selected 10 SNPS

Table 7 – List of selected 10 SNPs

Gene	SNP/Allele	Human/Animal	Effect	Reference
PCDH7- encodes a calcium-dependent adhesion protein	rs28498976 G (reference) allele A (alternate) allele	GWAS Human	Genome-wide association study (lancet 2014) – Identified the rs2849876 SNP to be the most strongly associated variant with all epilepsies. P=5.44X10^{-9}, OR=0.9, 95% CI 0.87-0.94)	Genetic determinants of common epilepsies: a meta-analysis of genome-wide association studies. The Lancet Neurology. 2014;13(9):893-903.
CAMSAP1L1: Chromosome: 1q32.1– encodes a cytoskeletal protein	rs2292096 A (reference) allele G (alternate) allele rs6660197 C (reference) allele	GWAS Human	GWAS association study identified the rs2292096 common variant as highly correlated to epilepsy development (P=0.00038) OR 95%CI: (0.75, 0.60-0.94) and the rs6660197 reaching genome-wide	Guo Y, Baum L, Sham P, Wong V, Ng P, Lui C et al. Two-stage genome-wide association study identifies variants in CAMSAP1L1 as susceptibility loci for epilepsy in Chinese. Human Molecular Genetics. 2011;21(5):1184-1189.

	T (alternate) allele		significance on the 1q32.1 chromosome (P=0.00599) OR 95%CI: (0.67, 0.53-0.85).	
ALDH2 – recent studies have demonstrated a protective effect of ALDH2 in cardiovascular diseases. ALDH2 is considered a marker of oxidative stress, affecting 4-HNE levels (involved in cerebral and mitochondrial ischemia)	rs671 G (reference) allele A (alternate) allele	Case Control Human	Protective effect: frequency of rs671 SNP is significantly higher in the IS group compared to the PSE group (31.8% vs 24.3%, P=0.00036, OR=1.98, CI=1.36-2.87). The presence of ALDH2*2 significantly increased the risk of PSE in subjects without a history of alcohol consumption, suggesting that alcohol consumption may have an effect on the development of cardiovascular diseases Method: The ALDH2 rs671 polymorphism was genotyped using polymerase chain reaction-restriction fragment length polymorphism	Yang H, Song Z, Yang G, Zhang B, Chen M, Wu T et al. The ALDH2 rs671 Polymorphism Affects Post stroke Epilepsy Susceptibility and Plasma 4-HNE Levels. PLoS ONE. 2014;9(10):e109634.
CD40-1C/T – involved in the progression of multiple disease states: up-	rs1883832 T (reference) allele C (alternate)	Human	The frequency of the T allele in PSE patients was significantly higher than in IS patients (50.5%	Zhang B, Chen M, Yang H, Wu T, Song C, Guo R. Evidence for involvement of the CD40/CD40L system in post stroke

regulation of pro-inflammatory and pro-atherogenic genes, produces ROS.	allele		vs 38.5%, P=0.0000017, OR=1.628, CI: 1.335-1.986) and the carriers of this allele were over-represented in the PSE group than in the IS group (70.4% vs 58.8%, P=0.00058, OR=1.671, CI: 1.246-2.241) Method: Genotyped for the CD40-1C/T polymorphism using PCR-RFLP.	epilepsy. Neuroscience Letters. 2014;567:6-10.
APOE 4 polymorphism- is associated with impairment of neuronal repair processes.	rs7412 C (reference) allele T (alternate) allele rs429358 T (reference) allele C (alternate) allele	Human	Presence of the APOE4 polymorphism was associated with significantly poorer recovery over the first month post stroke. (P=0.023) and with a lower proportion of subjects with minimal or no disability (mRS 0-1, P=0.01) at 3 months post stroke. Method: PCR-RFLP APOE4 polymorphism has also been identified in patients who are carriers of the APOE4 allele as having an increased risk of epilepsy after	Cramer S, Procaccio V. Correlation between genetic polymorphisms and stroke recovery: Analysis of the GAIN Americas and GAIN International Studies. European Journal of Neurology. 2012;19(5):718-724. Diaz-Arrastia R, Gong Y, Fair S, Scott K, Garcia M, Carlile M et al. Increased Risk of Late Posttraumatic Seizures Associated With Inheritance of APOE ϵ4 Allele. Arch Neurol. 2003;60(6):818.

			traumatic brain injuries.	
IGF1 locus: Insulin-like growth factor 1 affects brain plasticity after brain injuries. Circulating serum IGF has been associated with better stroke recovery after ischaemic stroke.	rs7136446 C (reference) allele T (alternate) allele	Human	The SNP rs7136446 is associated with good functional outcome (mRS 0-2) 24 months after ischaemic stroke with correction for age, sex, smoking, hypertension and diabetes.	Pitkänen A, Roivainen R, Lukasiuk K. Development of epilepsy after ischaemic stroke. The Lancet Neurology. 2016;15(2):185-197.
PITX2 gene **Chromosome Intergenic 4q25:**	rs7136446 C (reference) allele T (alternate) allele	Human GWAS		Gore-Panter, S., Hsu, J., Hanna, P., Gillinov, A., Pettersson, G., Newton, D., Moravec, C., Van Wagoner, D., Chung, M., Barnard, J. and Smith, J. (2014). Atrial Fibrillation Associated Chromosome 4q25 Variants Are Not Associated with PITX2c Expression in Human Adult Left Atrial Appendages. *PLoS ONE*, 9(1), p.e86245.

Genotyping

A minimum of 100ng of each DNA sample was organised into 96 well plates (Axygen Scientific, Union City, CA, USA) at a concentration of 10ng/μL, with

water used as the buffer. At the Royal Melbourne Hospital, the samples were couriered on dry ice to the Australian Genome Research Facility (AGRF) for custom SNP genotyping. At the Jinling Medical Hospital and at the University of Campinas genotyping was performed onsite.

Genotyping for the selected SNPs was completed using the MassARRAY® platform (Agena Bioscience™, San Diego, CA, USA). The rs numbers are the accession numbers in the National Centre for Biotechnology Information (NCBI) Single Nucleotide Polymorphism Database (dbSNP). DNA samples were de-identified so that genotypes were determined without knowledge of the patients' seizure outcome.

MassARRAY® uses iPLEX® Gold chemistry (Sequenom, Hamburg, Germany), a leading application for targeted genotyping. The iPLEX® assay is a primer extension process to detect sequence differences at the single nucleotide level. The MassARRAY® Assay Design Suite (Agena Bioscience™, San Diego, CA, USA) software was used to automatically design the iPLEX® single base extension primers for each SNP of interest from dbSNP rs numbers. The starting point was PCR amplification, followed by the addition of shrimp alkaline phosphatase to inactive remaining nucleotides in the reaction. Following a brief incubation period, a mixture of the extension primers, extension enzyme, and mass-modified dideoxynucleotide terminators was added. The extension primers annealed directly adjacent to each SNP site, and are extended and terminated by a

single complementary base into the genotyping target site. Finally, the extension products were desalted with Clean Resin (Agena Bioscience™, San Diego, CA, USA) before transfer via an automated nanodispenser onto a 384-well SpectroCHIP® Array (Agena Bioscience™, San Diego, CA, USA). The SpectroCHIP® enabled automated data acquisition by a matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass-spectrometer.

Statistical Methodology

The statistical analysis plans will be discussed in each chapter.

Chapter 4

Objective 1- Radiological Markers

Chapter 4 investigates the use of the Alberta Stroke Program Early CT Score in hyperacute times from stroke onset.

Currently the Alberta Stroke Program Early CT Score (ASPECTS) represents a commonly used imaging tool for detecting ischaemic change. It is also used as a tool to predict patients who will respond best to IV-tPA therapy. However, early ischaemic change on NCCT is strongly time dependent. The earlier the scan is performed, the more difficult it is to detect early signs of ischemia. Thus, the goal of this chapter was to evaluate whether time from stroke onset to initial non-contrast CT influences the inter-rater variability and prognostic accuracy of ASPECTS for 3-month functional outcome. This was a retrospective, single centre, cohort study of acute anterior circulation ischaemic stroke patients admitted to the Royal Melbourne Hospital, who received thrombolysis with IV-tPA between 2007 and 2014.

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Reliability and Utility of the Alberta Stroke Program Early Computed Tomography Score in Hyperacute Stroke

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Goal: The Alberta Stroke Program Early Computed Tomography Score (ASPECTS) on non-contrast computed tomography (NCCT) is dependent on the visibility of early ischemic change. The goal of our study was to evaluate whether time from ischemic stroke onset to initial NCCT influences the inter-rater variability and prognostic accuracy of ASPECTS for a 3-month functional outcome. *Materials and Methods:* Ischemic stroke patients treated with intravenous tissue plasminogen activator (IV-tPA) from 2007 to 2014 at the Royal Melbourne Hospital were included. ASPECTS were blindly assessed by 2 independent raters with inter-rater agreement determined by weighted kappa. Onset time to computed tomography time was dichotomized at the median (≤100 and >100 minutes). Outcome was assessed using the modified Rankin Scale. Logistic regression and receiver operating characteristic analysis were used to assess the prognostic utility of ASPECTS in the early and later time periods. *Results:* There were 379 patients included. Inter-rater agreement was significantly lower in the early time period: kappa = .75 (95% confidence interval (CI), .59-.84) ≤ 100 minutes versus .92 (95% CI, .91-.93) > 100 minutes, $P < .001$. The distributions of absolute inter-rater differences in ASPECTS differed significantly between time epochs ($P = .03$). The prognostic accuracies of ASPECTS across time epochs were area under the receiver operating characteristic curve ≤ 100 minutes = .57 (95% CI, .50-.64) and >100 minutes = .66 (95% CI, .59-.73), $P = .055$. *Conclusions:* This study demonstrated a significantly lower inter-rater agreement and a trend toward reduced prognostic accuracy of ASPECTS in earlier time periods. The use of ASPECTS to select patients for revascularization in early time windows may be unreliable. **Key Words:** Ischemic—stroke—CT—ASPECTS—time.

Introduction

Stroke interventions are time critical, and patients who present to the hospital within the first 60 minutes of onset have the greatest chance of benefitting from revascularization therapies.[1] Thus, it has been clinically crucial to develop systems, tools, and technologies to optimize the number of patients receiving treatment earlier.[2] For example, education in recognizing stroke symptoms, efficient code stroke methods, and the introduction of telemedicine have led to an increase in the proportion of stroke patients arriving at the hospital earlier.[3] The preliminary implementation of mobile stroke units has allowed the use of pre-hospital treatment to reduce the median time from stroke onset to therapy decision to as little as 35 minutes.[4] Currently, the Alberta Stroke Program Early Computed Tomography Score (ASPECTS) on non-contrast computed tomography (NCCT) represents a commonly used imaging tool for detecting early ischemic change

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(EIC). However, the use of ASPECTS is highly time dependent, with optimal detection on NCCT hours to days from stroke onset.[5-7] As such, and with a shift to earlier stroke treatment times, it is currently unknown whether the utility of ASPECTS will persist as a simple and reliable method for assessing ischemic change.

Five randomized controlled trials demonstrated the superiority of intra-arterial and intravenous tissue plasminogen activator (IV-tPA) treatment over standard treatment for acute ischemic stroke patients with large artery occlusion.[8-12] For most of these trials, the ASPECTS was used to grade the degree of EIC, allowing the prediction of irreversible ischemic injury.[13] Similarly, for patients only eligible for IV-tPA, the ASPECTS on NCCT has proven a simple and reliable method to identify stroke patients unlikely to make an independent recovery despite this treatment.[14,15] Important advantages of ASPECTS on NCCT are its geographical and temporal ubiquity and its high sensitivity for the detection of intracerebral hemorrhage.[14] EIC on NCCT is measured by a reduction in Hounsfield units (HU), which are time dependent. Parenchymal hypoattenuation is due to an increase in brain tissue water content as a result of ischemic injury, leading to ionic or vasogenic edema.[16,17] Thus, the visibility of ischemic change on NCCT varies from hours to days later depending on the magnitude of water uptake in ischemic tissues.[5-7] As it is established that time affects the visibility of ischemic change, we hypothesized that the ASPECTS in hyperacute times is not a reliable measure for selecting patients for reperfusion therapies. Consequently, the objective of our study was to evaluate whether hyperacute time from ischemic stroke onset to initial NCCT influences the inter-rater variability and prognostic accuracy of ASPECTS for a 3-month functional outcome.

Methods

Study Population

This was a retrospective, single-center, cohort study of acute anterior circulation ischemic stroke patients admitted to the Royal Melbourne Hospital between December 26, 2007, and April 20, 2014, who received thrombolysis. Patients were excluded if baseline demographic or follow-up clinical data were unavailable during the patients' time in the ward or if a 3-month follow-up modified Rankin Scale (mRS) was unavailable. Patients were also excluded if the NCCT was severely movement degraded. The study was approved by the Royal Melbourne Hospital Human Research Ethics Committee.

ASPECTS Assessment on NCCT

The initial NCCT (Somatom 16 slice and Definition FLASH 128 slice scanners, Siemens, Erlangen, Germany) was reviewed using the Picture Archiving and Communicating System on radiology workstations. NCCT ASPECTS were assessed using 5-mm axial slices as per standard recommendations (aspectsinstroke.com). The regions included M1, M2, M3, M4, M5, M6, Caudate Nucleus, Lentiform Nucleus, Insula, and the posterior limb of the Internal Capsule. The ASPECTS provides a semiquantitative score out of 10 with a score of 10 indicating a normal computed tomography (CT) scan. A score of >7 has been validated as being associated with a favorable prognosis for patients receiving thrombolytic therapy, and a score of ≤7 is generally associated with poor prognosis in thrombolysis patients.[18] A point was deducted for each region of the brain with loss of gray–white differentiation or hypointensity visible on at least 2 adjacent slices. Established, chronic infarction was ignored and not included in the analysis. The assessors (a neuroradiology fellow and a stroke research fellow) were aware of the affected hemisphere and rated ASPECTS independently with subsequent consensus for disagreements.

Statistical Analyses

Statistical analyses were performed using STATA (v13.1; StataCorp, College Station, TX). The time from stroke onset (time the patient was last known to be well) to initial NCCT was dichotomized at the median into 2 time epochs to achieve the most efficient estimates by balancing the groups when assessing the effect of time. The ultra-early phase of stroke was defined as the median or earlier time from stroke onset to initial NCCT. Fisher's exact tests were used to assess the differences in patients' baseline characteristics between 2 time epochs. The inter-rater agreement was estimated using a kappa with quadratic weights and cross-validated using Lin's concordance coefficient within each epoch. The distributions of absolute inter-rater differences in ASPECTS were compared over time epochs using a Fisher's exact test. The comparison across the time epochs of the utility of ASPECTS as a diagnostic tool for good functional outcome (scores of 0-2 on mRS at 3 months) was made using a Chi-squared test for respective areas under the receiver operating characteristic curve (AUCs). We also used multivariable logistic regression models with an appropriate interaction term to assess the effect of time epoch on the strength of association between ASPECTS and mRS 0-2 adjusted for age and the National Institutes of Health Stroke Scale (NIHSS).

Results

Subject Characteristics

There were a total of 379 participants who met the inclusion criteria and were included in this single-center, retrospective, observational study. The median (interquartile range (IQR)) time of acute stroke onset to CT scan (OCT) was 100 (73-142) minutes. Thus, 100 minutes was used as the time point to define the earlier (≤100 minutes) and later time periods (>100 minutes) of OCT. Baseline

Table 1. *Comparison of characteristics of study population across time epochs*

	Onset to CT (≤100 minutes), n = 190	Onset to CT (>100 minutes), n = 189	*P*-value
Age (years) median (IQR)	74 (64-82)	72 (63-80)	.23*
Male sex (n,%)	107 (56)	99 (53)	.47†
Diabetes (n,%)	48 (25)	57 (30)	.30†
Smoker (n,%)	48 (26)	41 (22)	.47†
Hypertension (n,%)	128 (67)	128 (67)	1.0†
Atrial fibrillation (n,%)	61 (32)	54 (29)	.50†
Structural heart disease (n,%)	3 (2)	6 (3)	.34†
Ischemic heart disease (n,%)	42 (22)	43 (23)	.90†
Peripheral vascular disease (n,%)	6 (3)	8 (4)	.60†
Dyslipidemia (n,%)	95 (50)	84 (44)	.30†
Previous stroke (n,%)	26 (14)	23 (12)	.76†
NIHSS baseline median (IQR)	13 (7-19)	10 (6-17)	.01*
OXFORD (n, %)			
TACI	69 (38)	66 (37)	.27†
PACI	97 (53)	85 (47)	
POCI	9 (5)	16 (9)	
LACI	8 (4)	13 (7)	
TOAST (n,%)			
Large artery	20 (11)	21 (12)	.61†
Cardioembolic	85 (45)	85 (47)	
Small vessel	4 (2)	1 (.6)	
Other	1 (.5)	3 (2)	
Unknown	77 (41)	71 (39)	
Intra-arterial therapy (n,%)	27 (14)	23 (12)	.65
ASPECTS median (IQR)	10 (9-10)	10 (8-10)	.27

Abbreviations: CT, computed tomography; LACI, lacunar infarct; PACI, partial anterior circulation infarct; POCI, posterior circulation infarct; TACI, total anterior circulation infarct.

*Two-sample Wilcoxon rank-sum (Mann–Whitney) test.

†Fisher's exact test.

characteristics were similar between time periods except for the higher NIHSS score in the early group (median 13 versus 10; $P = .01$; Table 1).

ASPECTS Assessment

Of the 379 initial NCCTs, 338 (89.2%) had ASPECTS > 7, suggesting good patient prognosis. The remaining 41 (10.8%) NCCTs had an ASPECTS of ≤7, suggesting poor patient prognosis. The median (IQR) for ASPECTS ≤100 minutes was 10 (9-10) and >100 minutes 10 (8-10), which did not differ ($P = .29$).

Inter-Rater Assessment on NCCT

Inter-rater agreement was lower in the earlier epoch: weighted kappa .75 (95% confidence interval (CI), .59-.84) versus .92 (95% CI, .91-.93) in the later window ($P < .001$). Similarly, Lin's concordance coefficient was lower: .75 (95% CI, .68-.81) versus .92 (95% CI, .90-.94). The differences in the magnitude of disagreement between the raters within 100 minutes compared with after 100 minutes (Q2) was statistically significant ($P = .031$), indicating increased disagreement in the earlier times (Table 1).

Prognostic Accuracy of ASPECTS on NCCT

The association of the ASPECTS with good 3-month functional outcome (mRS 0-2) in receiver operating characteristics (ROC) analysis tended to be weaker in the early (AUC = .57, 95% CI, .50-.64) versus the later epoch (AUC = .66, 95% CI, .59-.73), $P = .055$. However, the association of ASPECTS > 7 with good functional outcome (mRS 0-2) in ROC analysis did not reach significance despite an improvement in prognostic accuracy in the later time epoch: (AUC = .55, 95% CI, .51-.60) versus (AUC = .58, 95% CI, .54-.62), $P = .41$. In multivariable logistic regression adjusted for age and NIHSS score, the odds of a good functional outcome in the OCT epoch >100 minutes were significantly reduced compared with the OCT epoch ≤100 minutes (odds ratio (OR) = .55, 95% CI, .33-.91; $P = .02$). The association of ASPECTS > 7 with a 3-month good functional outcome, adjusted for NIHSS, was not significant (OR = 2.3, 95% CI, .74-7.3, $P = .15$) within the earlier time period. However, in the later time epoch,

Table 2. *Reliability and utility of the ASPECTS across time epochs*

Weighted kappa (95% CI)	.75 (.59-.84)	.92 (.91-.93)	<.001
Prognostic accuracy of ASPECTS for mRS 0-2 (AUC, 95% CI)	.57 (.50-.64)	.66 (.59-.73)	.055*
Prognostic accuracy of ASPECTS >7 for mRS 0-2 (AUC, 95% CI)	.55 (.51-.60)	.54 (.54-.62)	.41*
Odds ratio for mRS 0-2, per 1 point higher ASPECTS (95% CI)	1.46 (1.04-2.06)	1.45 (1.09-1.93)	.59†

Abbreviations: ASPECTS, Alberta Stroke Program Early Computed Tomography Score; AUC, area under the receiver operating characteristic curve; CI, confidence interval; CT, computed tomography; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale.

*AUC.

†Multivariable logistic regression model, adjusted for age and NIHSS. *P*-value represents interaction term for time from onset to CT and ASPECTS.

this association was stronger and reached statistical significance (OR = 3.8, 95% CI, 1.0-14.4, *P* = .048). However, no statistically significant time-by-ASPECTS interaction with good functional outcome was found in a multivariable logistic regression model adjusted for age and NIHSS (time epoch ≤100 minutes: OR (per ASPECT point) = 1.46 (95% CI, 1.04-2.06); time epoch >100 minutes: OR (per ASPECT point) = 1.45 (95% CI, 1.09-1.93); *P*-value for interaction =.59 (Table 2).

Discussion

This study has demonstrated that the NCCT ASPECTS in the early period after stroke is both more difficult to assess, as reflected in reduced inter-rater agreement, and less reliable for prognostication. This is a clinically important finding given the recommendation to use ASPECTS 6-10 as an eligibility criterion for thrombectomy in the recent American Heart Association guidelines update.[19]

When we assessed the prognostic accuracy of ASPECTS for a 3-month functional outcome, as measured by the mRS, the association strengthened as time from stroke onset to initial CT increased. There was a strong trend toward greater prognostic accuracy in the later time epoch >100 minutes (*P* = .055).

Unlike previous studies, we specifically examined the influence of earlier time on inter-rater agreement and the prognostic accuracy of the ASPECTS. The median of 100 minutes onset to NCCT was comparable with a meta-analysis of pre-hospital and in-hospital processing times, which reported a median onset to imaging time in 2 primary centers of 100 minutes (IQR: 67-175) and 108 minutes (IQR: 75-155).[3] More recently, 3 of the 5 randomized controlled trials demonstrating evidence for the superiority of endovascular thrombectomy over standard treatment for acute ischemic stroke reported median OCTs of within 135 minutes.[10-12] These fast onset to imaging times will be increasingly important as emergency systems evolve to a more efficient workflow, especially if CT-equipped mobile stroke units continue to proliferate.

In our data, we found that 89% of NCCT scans had an ASPECTS > 7, of which 63% had an entirely normal ASPECTS of 10. This is comparable with previous literature that reported ASPECTS > 7 on the initial NCCT in 90% of patients.[20] The recent endovascular trials also had similar ASPECTS, including MR CLEAN, in which 75% of patients had ASPECTS 8-10, despite a 6-hour inclusion window, pure large vessel occlusion (LVO) population, and no exclusion of patients based on ASPECTS or other markers of ischemic core volume.[10] EICs are often difficult to detect on plain CT, and many studies have reported a low inter-rater agreement for presence and extent of EIC, (k = .20-.88),[21] particularly in the earlier time window.[16] Plain CT reflects ionic edema, but it is not sensitive for recognizing the early cytotoxic edema visualized using diffusion magnetic resonance imaging.[17] Within the 4.5-hour window for intravenous thrombolysis, frank hypodensity is usually absent, and EICs are limited to loss of gray–white differentiation in the cortex and deep gray matter structures, if visible at all.[21,22] Early cortical swelling (loss of sulci) has been shown to be reversible in some cases with reperfusion as it often reflects increased cerebral blood volume in collateral vessels, rather than irreversible infarction.[23] The sooner these signs become evident, the more profound the degree of ischemia which is related to the quality of collateral blood flow.[24] However, the ability of observers to detect these signs on plain CT has been shown to vary among studies, with the detection rate appearing to be ≤67% in cases imaged within 3 hours.[5,6,25] An analysis of the National Institute of Neurological Disorders and Stroke (NINDS) CT data yielded a 31% prevalence for early infarct signs, with a weak correlation with acute NIHSS score but no effect on either clinical outcome or intracerebral hemorrhage (ICH) rate.[7] The prevalence increases to 82% at 6 hours, which is outside the therapeutic window for IV-tPA.[24] Our study demonstrated a significant increase in the frequency and magnitude of inter-rater differences in ASPECTS assessed within 100 minutes from stroke onset, highlighting the difficulty and variability of assessing ASPECTS early after stroke.

This study has potential implications in clinical practice. A key consideration in decision making is whether extensive irreversible ischemia may preclude treatment benefit. In this study, we identified that the ASPECTS tends to have greater prognostic accuracy as time passes. In

experimental models of cerebral ischemia, the visibility of early EICs on CT has been reported from approximately 1.3 hours post-onset.[26,27] In cases with a proximal vessel occlusion and poor collateral flow, EIC may occur earlier as a result of the more intense hypoperfusion.[28] However, reliance on NCCT alone early after stroke onset may fail to identify patients with evolving malignant middle cerebral artery (MCA) stroke. The addition of CT perfusion imaging within hyperacute times may help identify such patients and provide greater inter-rater agreement and prognostic accuracy for patients receiving recanalization therapies, provided this can be acquired and interpreted with minimal delay to treatment.[29]

Our study has limitations. The ASPECTS itself has limitations and is only applicable to the middle cerebral artery territory. ASPECTS assessment can be compromised by streak artifacts at the base of the skull, watershed infarcts, subcortical and age-related periventricular white matter changes, and poor-quality scans due to patient motion. In this study, only scans that were severely movement degraded were removed. The success of reperfusion therapies has a substantial impact on prognosis and was not assessed in the routine clinical care of these patients who received predominantly thrombolysis without thrombectomy. However, the literature on the prognostic significance of ASPECTS > 7 was developed in similar patient cohorts where reperfusion status was unknown. Our patient cohort was not exclusively composed of patients with LVO, which is the main context in which ASPECTS-based treatment selection has been proposed. However, this avoids selection bias, which would likely occur if only endovascular patients were included. The conspicuity of ASPECTS changes on NCCT over time is likely to be similar in the LVO and non-LVO patients, although this is something that can be examined further. Finally, as is the nature of this type of research, we are limited to two individual raters, and it is unclear how these results translate to inter-rater reliability in general.

Conclusions

This study confirms that ASPECTS in earlier times (within 100 minutes) have greater inter-rater variability, but it has additionally demonstrated that the prognostic accuracy of ASPECTS is weaker in the early window. Extra care should be exercised in adopting the ASPECTS on NCCT to ultra-early times as a tool for selecting patients for revascularization. We propose further investigation and validation of the ASPECTS as well as the role of alternative imaging modalities in hyperacute stroke.

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Chapter 5

Objective 1- Radiological Markers

Chapter 5 investigates the reliability, reproducibility and prognostic accuracy of the Alberta Stroke Program Early CT Score on CT perfusion and non-contrast CT in hyperacute stroke

In the previous chapter (Chapter 4), it was concluded that ASPECTS rated in earlier times from stroke onset (within 100 minutes) have greater inter-rater variability and reduced prognostic accuracy for 3-month functional outcome. CTP cerebral blood volume (CBV) reduction or severely reduced cerebral blood flow (CBF) also estimate ischaemic core but, unlike NCCT, visual conspicuity of the abnormality is not dependent on development of ionic edema, which takes time to evolve. Thus, the aim of this chapter was to evaluate and compare the utility of ASPECTS (including the reliability, reproducibility and prognostic accuracy) in earlier versus later times from stroke onset. The patient population from Chapter 4 was included in this study, however patients must have also had a CTP on admission.

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Reliability, Reproducibility and Prognostic Accuracy of the Alberta Stroke Program Early CT Score on CT Perfusion and Non-Contrast CT in Hyperacute Stroke

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Keywords
ASPECTS · Hyperacute stroke · CT Perfusion · NCCT

Abstract
Background: Alberta Stroke Program Early CT Score (ASPECTS) assesses early ischemic change on non-contrast CT (NCCT). We hypothesised that assessing ASPECTS regions on CT Perfusion (CTP) rather than NCCT would improve inter-rater agreement and prognostic accuracy, particularly in patients presenting early after stroke onset. ***Methods:*** Ischemic stroke patients treated with intravenous alteplase from 2009 to 2014 at our institution were included in this study. Inter-rater agreement and prognostic accuracy of ASPECTS across modalities were analysed by the time between stroke onset and initial NCCT, dichotomized 1st quartile versus quartiles 2–4, referred to as epochs. ASPECTS was assessed by 2 independent raters, blinded to stroke onset time, with agreement determined by weighted kappa (κ_w). Prognostic accuracy for favourable outcome (modified Rankin Scale 0–2) was assessed using the receiver-operating characteristic analysis. ***Results:*** A total of 227 participants were included. There was significant time-by-CT modality interaction for ASPECTS, $p < 0.0001$. The inter-rater agreement of ASPECTS on NCCT significantly increased as onset to CT time increased (κ_w epoch 1 = 0.76 vs. κ_w epoch 2–4 = 0.89, $p = 0.04$), whereas agreement using CTP parameters was stable across epochs. Inter-rater agreement for CTP-ASPECTS was significantly higher than NCCT in early epoch: Tmax $\kappa_w = 0.96$, $p = 0.002$; cerebral blood volume (CBV) $\kappa_w = 0.95$, $p = 0.003$; cerebral blood flow (CBF) $\kappa_w = 0.94$, $p = 0.006$, with no differences in the later epochs. Prognostic accuracy of ASPECTS on NCCT in epoch 1 were (area under the ROC curves [AUC] = 0.52, 95% CI 0.48–0.56), CBV (AUC = 0.55, 95% CI 0.42–0.69, CBF (AUC = 0.58, 95% CI 0.46–0.71) and Tmax (AUC = 0.62, 95% CI 0.49–0.75), $p = 0.46$ between modalities. ***Conclusions:*** CTP can improve reliability when assessing the extent of ischemic changes, particularly in patients imaged early after stroke onset.

Introduction

Stroke reperfusion treatments are time critical with improved outcomes associated with even a few minutes reduction in onset to treatment time [1, 2]. However, individual variation in stroke pathophysiology means that

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the appearance of brain imaging can provide a more accurate prognosis than time alone [3]. The Alberta Stroke Program Early CT Score (ASPECTS) is a commonly used approach to assess the early ischemic change on non-contrast CT (NCCT). More extensive ischemic changes (lower ASPECTS) have been associated with less favourable functional outcomes and increased occurrence of symptomatic intracerebral haemorrhages after reperfusion therapies [4–6]. On NCCT, early ischemic changes reflect ionic edema, which is associated with irreversible tissue injury [7, 8]. However, the visibility of early ischemic change is highly time dependent, with optimal detection possible hours to days from stroke onset. It has been shown previously that ASPECTS in hyperacute stroke, particularly within 100 min, has greater inter-rater variability and is less reliable as a prognostic tool for patient outcome [9].

CT perfusion (CTP) can be rapidly acquired immediately after NCCT and improves diagnostic confidence through detection of the delayed blood flow characteristic of vessel occlusion [10–12]. Assessment of ASPECTS regions has also been applied to CTP, and studies suggested greater correspondence with follow-up MRI than NCCT ASPECTS [13]. CTP has the added advantage of distinguishing potentially salvageable tissue from irreversibly injured ischemic core [14]. Knowledge of cerebral blood volume (CBV), cerebral blood flow (CBF) and Tmax may also influence decision making for reperfusion therapies [14], with recent work showing that CTP can be used to predict favourable outcome after thrombolysis [15–17]. We aimed to assess the practical evaluation (including reliability, prognostic accuracy and reproducibility) of ASPECTS on CTP and NCCT in early versus later times after stroke onset. We hypothesised that ASPECTS assessed using CTP parameters would have higher inter-rater agreement and greater prognostic accuracy than on NCCT, particularly in patients presenting early after stroke onset.

Methods

Study Population

This was a retrospective, single-centre cohort study at a comprehensive stroke centre. Consecutive anterior circulation ischemic stroke patients administered intravenous alteplase between December 26, 2009 and April 20, 2014 were included if both NCCT and CTP were performed.

ASPECTS Assessment on NCCT

The initial NCCT was reviewed using the Picture Archiving and Communicating Systems system on radiology workstations. ASPECTS were assessed using 5-mm axial slices as per standard recommendations (aspectsinstroke.com). The regions included M1, M2, M3, M4, M5 and M6, caudate nucleus, lentiform nucleus, insula and the posterior limb of the internal capsule. ASPECTS provides a semi-quantitative score out of 10 with a score of 10 indicating a normal CT scan. A score of >7 has been validated as being associated with a favourable prognosis for patients receiving thrombolytic therapy and a score of ≤7 is generally associated with poor prognosis in thrombolysis patients [8]. A point was deducted for each region of the brain with loss of grey-white differentiation or hypointensity visible on at least 2 adjacent slices. Established, chronic infarction was ignored and not included in the analysis. The assessors (stroke research fellows) were aware of the affected hemisphere but rated the scans independently and were blinded to the time of stroke onset and the other scans.

ASPECTS Assessment on CTP

Prior to 2013, a 16-slice Somatom CT scanner (Siemens, Erlangen, Germany) was used and 2 separate CTP slabs were acquired at the ganglionic and supraganglionic levels, each with 2 × 12 mm slices. Images were acquired every second for 40 s following an initial 4 s delay. Subsequently, a 128-slice Definition FLASH CT Scanner (Siemens) was used and acquired full brain CTP (10 × 10 mm slices) with 30 time points acquired over 60 s. Perfusion maps were generated using RAPID fully automated software (RAPID, non-commercial research version, Stanford University). Tmax, CBF and CBV maps were assessed using the same regional ASPECTS template as for NCCT. Only Tmax >6 s delay was considered abnormal as per previous validation of the threshold for potentially salvageable brain [18]. Abnormal CBV or CBF was defined as unequivocal reduction compared to the corresponding region in the unaffected hemisphere. In all cases, changes needed to be visible on at least one slice. The same 2 assessors were aware of the affected hemisphere, but the CTP scans was read independently and they were blinded to time to scan.

Statistical Analyses

The statistical analyses for this study were performed using STATA IC version 13.1 (StataCorp, College Station, TX, USA). Separate analyses were conducted for ASPECTS on the full ordinal scale as well as dichotomized at 0–7 vs. 8–10 for both inter-rater agreement and prognostic accuracy. Inter-rater agreement and prognostic accuracy of ASPECTS across modalities were described by quartiles of the stroke onset to NCCT time (OCT) and referred to as epochs. To assess the specific influence of early time on inter-rater agreement and prognostic accuracy within a given method, we dichotomized OCT at quartile 1 vs. 2–4. For sensitivity analysis, we also subsequently tested the dichotomy of OCT at the median. The differences in rates of change in ASPECTS over time across different modalities were investigated using generalized estimating equations modelling with ASPECTS as the dependent variable and imaging modality, time epoch, and modality-by-epoch interaction term as independent variables.

The inter-rater reliability of ASPECTS on different modalities was assessed using weighted kappa with quadratic weights and compared between modalities and/or time epochs using z-tests. Additionally, for every modality, the magnitude of inter-rater differences in ASPECTS between the hyper-acute epoch 1 and later time 2–4 epochs was compared using 2-sample Wilcoxon Mann-Whitney tests. A comparison of prognostic accuracy of ASPECTS for favourable functional outcome (defined as a modified Rankin Scale [mRS]

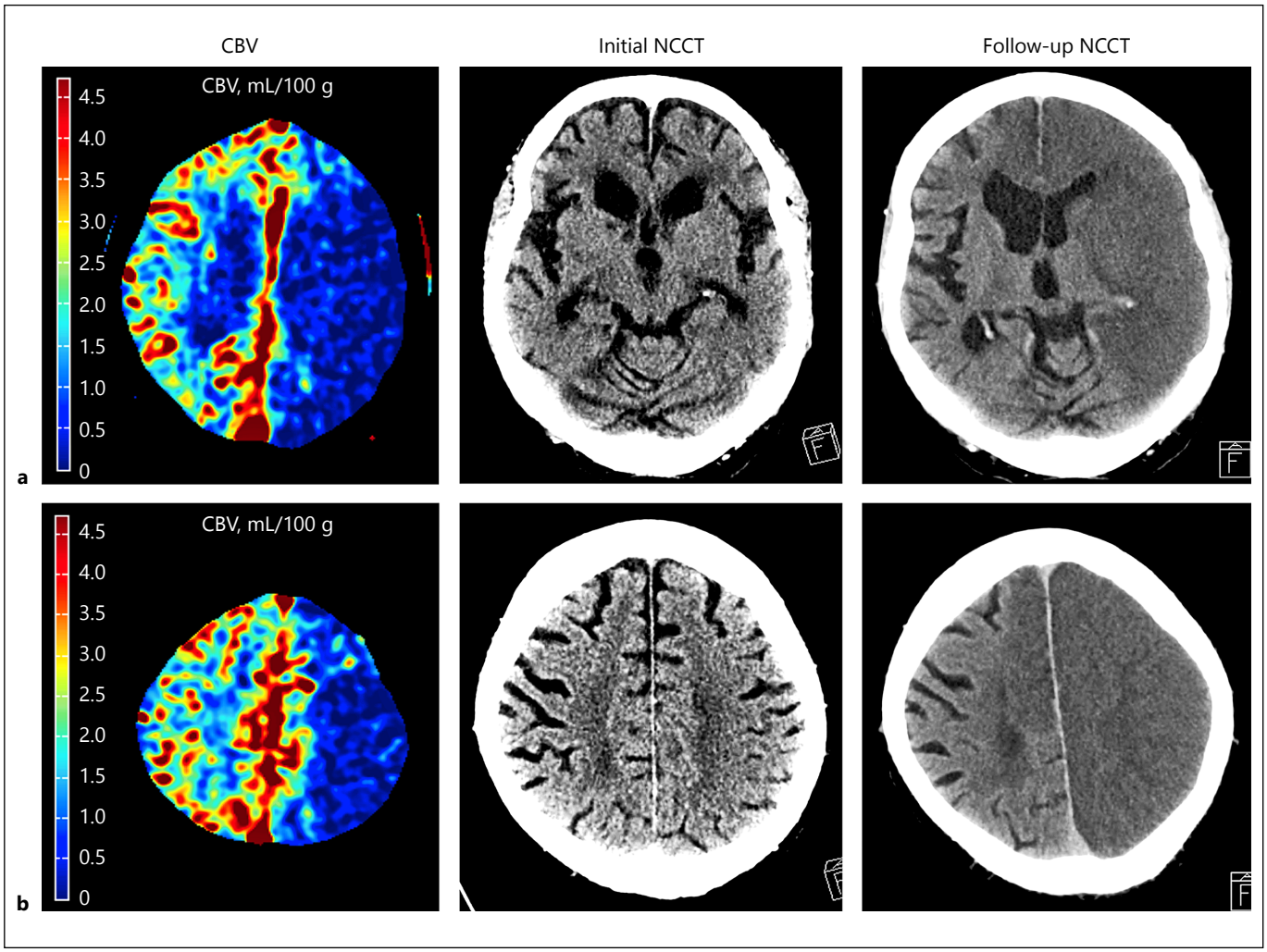

Fig. 1. Images of a patient with left middle cerebral artery territory ischemic stroke. **a** Ischemic change at the ganglionic level. **b** Ischemic change at the level of the supra-ganglia. Example shows visibility of ischemic change on initial CBV vs. the initial NCCT with comparison to the follow-up NCCT. These images were procured 90 min after stroke onset.

0–2 at 3 months) assessed on each modality was determined by assessing the combination of discrimination (ROC area) and calibration (Bayesian Information Criterion) for logistic regression models both adjusted for National Institute of Health Stroke Scale and ASPECTS >7 and also unadjusted for each modality. The area under the ROC curves (AUC) for prognostic accuracy across modalities were compared using a chi-square test. A BIC >10 points lower is regarded as very strong evidence of model superiority [19].

Results

Subject Characteristics

A total of 227 participants fulfilled the inclusion criteria. The time from stroke onset to NCCT (OCT) was a median value of 98 min and interquartile range (IQR) of 70–136 min. Thus, time epoch 1 was defined as ≤70 min and epoch 2–4 was defined as >71 min.

Distribution of ASPECTS Using NCCT and CTP Parameters across Time Epochs

The prevalence of ASPECTS >7 on the different parameters was NCCT 89%, Tmax 52%, CBV 76%, and CBF 66%. There was a significant time-by-CT modality interaction for ASPECTS, $p < 0.0001$, reflecting differences across modalities in rates of change in ASPECTS over time (Fig. 1). For a given time epoch, all CTP modalities had significantly lower average ASPECTS in comparison to NCCT, $p < 0.0001$. For a given rater, ASPECTS on

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Table 1. Relationship of inter-rater agreement of ordinal ASPECTS over time

Weighted kappa (95% CI)	Epoch 1 (≤70 min)	Epoch 2 (>70–≤98 min)	Epoch 3 (>98–≤136 min)	Epoch 4 (>136 min)	Epoch 2–4 (>70 min)	*p* value*	*p* value**
NCCT	0.76 (0.70–0.81)	0.85 (0.75–0.91)	0.90 (0.85–0.92)	0.93 (0.86–0.95)	0.89 (0.85–0.91)	0.047	0.029
CBV	0.95 (0.94–0.96)	0.94 (0.93–0.96)	0.94 (0.90–0.94)	0.94 (0.90–0.95)	0.94 (0.92–0.95)	0.41	0.12
CBF	0.94 (0.91–0.95)	0.95 (0.94–0.97)	0.91 (0.89–0.93)	0.93 (0.90–0.96)	0.92 (0.92–0.93)	0.30	0.66
Tmax	0.96 (0.94–0.98)	0.95 (0.94–0.96)	0.91 (0.88–0.94)	0.94 (0.92–0.95)	0.93 (0.92–0.94)	0.18	0.35

* *p* value represents the difference in inter-rater agreement between epoch 1 and epoch 2–4, as measured by standard error distribution.

** *p* value represents the magnitude of differences (≥1), as measured through a Wilcoxon test, between epoch 1 and epoch 2–4.

Table 2. Relationship of inter-rater agreement of dichotomized ASPECTS over time

Weighted kappa (95% CI)	Epoch 1 (≤70 min)	Epoch 2 (>70–≤98 min)	Epoch 3 (>98–≤136 min)	Epoch 4 (>136 min)	Epoch 2–4 (>70 min)	*p* value*
NCCT	0.49 (–0.1 to 1.0)	0.64 (0.54–0.74)	0.54 (0.47–0.61)	0.61 (0.50–0.72)	0.76 (0.61–0.91)	0.40
CBV	0.79 (0.64–0.95)	0.50 (0.36–0.63)	0.57 (0.44–0.71)	0.68 (0.55–0.81)	0.86 (0.78–0.94)	0.43
CBF	0.76 (0.58–0.94)	0.59 (0.48–0.71)	0.60 (0.48–0.72)	0.60 (0.48–0.72)	0.82 (0.72–0.92)	0.57
Tmax	0.83 (0.68–0.97)	0.52 (0.37–0.64)	0.52 (0.38–0.65)	0.58 (0.46–0.70)	0.83 (0.74–0.92)	0.25

* *p* value represents the difference in inter-rater agreement between epoch 1 and epoch 2–4, as measured by standard error distribution.

NCCT was significantly lower in epoch 2–4 than in epoch 1 (median difference –0.48 [95% CI 0.03 to –0.75], $p = 0.03$). In contrast, ASPECTS on all CTP modalities did not significantly change across epoch 1 vs. epoch 2–4 combined.

Reliability and Reproducibility of ASPECTS across Time Epochs

The inter-rater agreement for the full ordinal range of ASPECTS on NCCT significantly increased as onset to CT time increased for quartile 1 vs. later times on NCCT (κ_w epoch 1 = 0.76 [95% CI 0.70–0.81] vs. κ_w epoch 2–4 = 0.89 [95% CI 0.85–0.91], $p = 0.047$). Inter-rater agreement for CTP parameters was stable across time epochs: CBV (κ_w epoch 1 = 0.95 [95% CI 0.94–0.96] vs. κ_w epoch 2–4 = 0.94 [95% CI 0.92–0.95], $p = 0.41$), CBF (κ_w epoch 1 = 0.94 [95% CI 0.91–0.95] vs. κ_w epoch 2–4 = 0.89 [95% CI 0.92–0.93], $p = 0.30$), Tmax (κ_w epoch 1 = 0.96 [95% CI 0.94–0.98] vs. κ_w epoch 2–4 = 0.93 [95% CI 0.92–0.94], $p = 0.18$; Table 1). There was also a significantly greater magnitude of differences in score ("delta") between raters in time epoch 1 compared with epoch 2–4 on NCCT, $p = 0.003$, whereas all CTP parameters had similar delta across time epochs (Table 2). The greater magnitude of ASPECTS disagreements in epoch 1 vs. 2–4 on NCCT in comparison to CTP modalities led to a significant delta*time interaction ($p = 0.05$).

Reliability and Reproducibility of ASPECTS with NCCT versus CTP

The inter-rater agreement was significantly lower for NCCT than any CTP map type within time epoch 1 (NCCT vs. Tmax, $p = 0.003$; NCCT vs. CBV, $p = 0.006$; NCCT vs. CBF, $p = 0.002$). In sensitivity analysis combining time epochs 1 and 2, $\kappa_w = 0.83$ (95% CI 0.83–0.87) NCCT, $\kappa_w = 0.95$ (95% CI 0.95–0.96) Tmax, $\kappa_w = 0.94$ (95% CI 0.92–0.96) CBV, $\kappa_w = 0.94$ (95% CI 0.93–0.95) CBF; $p = 0.0008$ NCCT vs. Tmax; $p = 0.0002$ NCCT vs. CBV; $p < 0.0001$ NCCT vs. CBF (Table 2).

Prognostic Accuracy of ASPECTS within a Given CT Modality

As a function of onset-to-NCCT time, the prognostic accuracy of unadjusted ASPECTS >7 for good outcome, mRS (0–2), as measured by the AUC for NCCT was significantly lower in epoch 1 (AUC = 0.52, 95% CI 0.48–

Table 3. Relationship of prognostic accuracy of dichotomized ASPECTS for favourable functional outcome (modified Rankin Scale 0–2) over time

AUC (95% CI)	Epoch 1 (≤70 min)	Epoch 2 (>70–≤98 min)	Epoch 3 (>98–≤136 min)	Epoch 4 (>136 min)	Epoch 2–4 (>70 min)	*p* value*
NCCT	0.52 (0.48–0.56)	0.64 (0.54–0.74)	0.54 (0.47–0.61)	0.61 (0.50–0.72)	0.60 (0.54–0.65)	0.0351
CBV	0.55 (0.42–0.69)	0.50 (0.36–0.63)	0.57 (0.44–0.71)	0.68 (0.55–0.81)	0.58 (0.51–0.66)	0.71
CBF	0.58 (0.46–0.71)	0.59 (0.48–0.71)	0.60 (0.48–0.72)	0.60 (0.48–0.72)	0.60 (0.54–0.67)	0.78
Tmax	0.62 (0.49–0.75)	0.52 (0.37–0.64)	0.52 (0.38–0.65)	0.58 (0.46–0.70)	0.54 (0.46–0.61)	0.31

* *p* value represents the difference in areas under the curves between epoch 1 and epoch 2–4, as measured by a chi-square test.

Table 4. Relationship of prognostic accuracy of ordinal ASPECTS for favourable functional outcome (modified Rankin Scale 0–2) over time

AUC (95% CI)	Epoch 1 (≤70 min)	Epoch 2 (>70–≤98 min)	Epoch 3 (>98–≤136 min)	Epoch 4 (>136 min)	Epoch 2–4 (>70 min)	*p* value*
NCCT	0.59 (0.47–0.72)	0.56 (0.41–0.70)	0.65 (0.52–0.79)	0.65 (0.52–0.79)	0.62 (0.54–0.70)	0.76
CBV	0.64 (0.49–0.80)	0.55 (0.40–0.70)	0.65 (0.52–0.79)	0.65 (0.52–0.80)	0.61 (0.52–0.70)	0.67
CBF	0.60 (0.45–0.75)	0.61 (0.46–0.75)	0.69 (0.55–0.82)	0.59 (0.44–0.74)	0.63 (0.55–0.71)	0.75
Tmax	0.63 (0.48–0.78)	0.53 (0.38–0.68)	0.59 (0.44–0.74)	0.60 (0.44–0.76)	0.58 (0.50–0.67)	0.56

* *p* value represents the difference in areas under the curves between epoch 1 and epoch 2–4, as measured by a chi-square test.

0.56) vs. epoch 2–4 (AUC = 0.60, 95% CI 0.54–0.65), $p = 0.035$. A statistically significant association between time epoch and prognostic accuracy was not observed using any CTP parameter (Table 3).

As a function of onset-to-NCCT time, the prognostic accuracy of ordinal ASPECTS for good outcome, mRS (0–2), as measured by the AUC was lower in epoch 1 on NCCT vs. later times, was however not significant. Similarly, using CTP parameters, the prognostic relationship of ordinal ASPECTS for good outcome, mRS (0–2) did not significantly change over time (Table 4).

Prognostic Accuracy of ASPECTS between CT Modalities

Adjusted models containing ASPECTS >7 assessed using any of the CTP modalities were better calibrated than the model using NCCT ASPECTS: CBV BIC = –946.05, AUC = 0.81, CBF BIC = –947.7, AUC = 0.81 Tmax BIC = –946.6, AUC = 0.81, NCCT BIC = –871.3, AUC = 0.81. Analysis within time epoch 1 (≤70 min) showed: CBV BIC = –172.02, AUC = 0.89, CBF BIC = –170.7, AUC = 0.89, Tmax BIC = –170.7, AUC = 0.89, NCCT BIC = –166, AUC = 0.88.

For the unadjusted model within time epoch 1 (≤70 min), ASPECTS >7 on all CTP modalities were stronger models than on NCCT: CBV BIC = –150.1, AUC = 0.52, CBF BIC = –151.2, AUC = 0.58, Tmax BIC = –152.8, AUC = 0.62, NCCT BIC = –146.3, AUC = 0.55. For the unadjusted model across all time epochs, the prognostic accuracy of ASPECTS >7 for 3 month mRS was as follows: CBV BIC = –896.1, AUC = 0.58, CBF BIC = –907.2, AUC = 0.60, Tmax BIC = –898.9, AUC = 0.56, NCCT BIC = –910.2, AUC = 0.58.

Discussion

This study has demonstrated that ASPECTS assessed on CTP parameters has greater inter-rater agreement and reduced magnitude of differences between raters than NCCT ASPECTS. This particularly applied to patients imaged within 70 min of stroke onset. We showed that for NCCT ASPECTS, the inter-rater agreement and the prognostic accuracy of ASPECTS >7 for 3-month mRS significantly increased as time from onset to imaging increased, which was not seen using any CTP parameter.

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Prognosis after stroke is critically dependent on age, baseline clinical severity, the extent of ischemic core and the speed and extent of reperfusion. NCCT changes represent irreversibly injured ischemic core [20]. CTP CBV reduction also represents ischemic core but, unlike NCCT, visual conspicuity of the abnormality is not dependent on the development of ionic edema, which takes time to evolve [9]. Extensive involvement on either NCCT or CBV is associated with poor prognosis regardless of reperfusion status [21, 22]. In this dataset, we have shown that after adjustment for both age and baseline stroke severity, ASPECTS >7 on CBV is a stronger predictor of good prognosis than ASPECTS >7 on NCCT, particularly in the early period after stroke onset. CBF is reduced in both ischemic core and salvageable penumbra and Tmax is prolonged in both tissue states. Therefore, extensive CBF or Tmax abnormality in the absence of extensive NCCT or CBV abnormality indicates tissue at risk and prognosis is critically dependent on reperfusion. The lack of reperfusion data in our series is therefore a limitation for analysis of the prognostic significance of CBF and Tmax, which would be expected to be greater in non-reperfused patients. This lack of reperfusion status is, however, the norm in clinical practice and similar studies. In contrast, studies of the prognostic value of automated processing of CTP with thresholded volumetric analysis have shown a strong relationship with outcome [23].

CTP has been shown to improve diagnostic accuracy and confidence. Our data on the reduced reliability of NCCT for early ischemic changes, particularly in the early epoch after stroke onset, and the lack of time-dependence when using CTP parameters illustrates some of the reasons for improved accuracy with CTP. Improved diagnostic sensitivity is important in allowing access to thrombolysis for certain scenarios like stroke with seizure at onset and reducing the risk of treating mimics [12]. CTP may also increase access to thrombolysis for patients who may be deemed clinically suboptimal candidates but have favourable imaging profiles [24], including CTP mismatch, which has previously been investigated [25–27]. Favourable CTP imaging profiles have also been shown to indicate an improved clinical response to reperfusion when thresholds for salvageable tissue and volumetric criteria are used [3]. Conversely, early ischemic change on NCCT is strongly time dependent. The earlier the scan is performed, the more difficult it is to detect early signs of ischemia and many studies report low inter-rater agreement for the presence and extent of early ischemic change (κ = 0.20–0.88) [28]. Hypoattenuation on CT reflects ionic edema with net tissue water increase, resulting in a decrease in X-ray attenuation and loss of grey-white matter differentiation indicating early ischemic change [29]. Within 4.5 h, the therapeutic window for IV-tPA the rate of detection is as low as <67% for images within 3 h of stroke onset, increasing to >80% at 6 h, outside the therapeutic window for thrombolysis [28, 30, 31]. Our study has demonstrated that the ASPECTS-based approach applied to CTP parameters as a prognostic tool performs similarly to ASPECTS using NCCT, with the added benefit of significantly greater reliability in assessing extent of ischemic changes, particularly in the ultra-early time period after stroke onset.

Unlike previous studies, we have specifically compared the influence of earlier time on inter-rater agreement and prognostic accuracy of ASPECTS on CTP and NCCT. Our choice of quartiles represents the earliest times stroke centres worldwide are imaging and treating their patients. They are comparable to a meta-analysis of stroke workflow times, which reported a median onset to imaging time in 2 primary centres of 100 min (IQR 67–175) and 108 min (IQR 75–155), respectively [32]. More recently, the combined workflow times of 4 randomized controlled trials demonstrating the superiority of endovascular thrombectomy over standard care for acute ischemic stroke patients with large artery occlusion highlighted the rapid processing times the intervention groups received, median (IQR) = 63 (46–85) in the HERMES meta-analysis [33]. Of the 5 randomized controlled trials in that meta-analysis, 3 used ASPECTS to grade the degree of EIC on NCCT as part of the eligibility criteria [34–37]. These fast onset to imaging times will be increasingly important as emergency systems evolve to more efficient workflow, especially if CT-equipped mobile stroke units continue to proliferate.

Our study has limitations. This was a retrospective analysis examining a pure IV-tPA population reflecting the processing times of a single centre. Our patient cohort did not exclusively comprise patients with large vessel occlusion, which is the main context in which ASPECTS-based treatment selection has been proposed. However, this avoids selection bias, which would likely occur if only endovascular patients were included. We were limited to 2 raters and reliability may vary with differing experience, although other many studies have reported a range of inter-rater agreements for the presence of early ischemic change (κ = 0.20–0.88) [28]. The ASPECTS itself has limitations. It considers only the

middle cerebral artery territory and can be compromised by motion and beam hardening artefacts. As discussed, reperfusion status was unknown in the patients who received only thrombolysis without thrombectomy and strongly modulates clinical outcome. However, the prognostic capacity of ASPECTS was initially developed on patients in whom reperfusion status was also unknown.

Conclusions

We conclude that the application of ASPECTS to CTP parameters significantly improves reliability and reproducibility in assessing the extent of ischemic changes, particularly in the ultra-early time period after stroke onset. The prognostic value of both NCCT and CTP ASPECTS was modest in this dataset but is dependent on reperfusion status and the particular CTP parameter used.

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Chapter 6

Objective 1 – Radiological Markers

Chapter 6 examines a comparison of the association between haemorrhagic transformation and post ischaemic stroke seizure development in patients receiving reperfusion therapies

Given that the ASPECTS is a marker for ischaemic lesion size which is consistently reported as a risk factor for post stroke seizure development, the Post Stroke Epilepsy lab group at the Royal Melbourne Hospital investigated the association of post stroke seizures with the extent of ischaemia assessed by ASPECTS in a population of patients who received IV-tPA. In univariate logistic regression, both ASPECTS on admission (OR 0.69 per 1-point increase; 95% CI 0.55–0.86; p = 0.001) and at 24 h (OR 0.80 per 1-point increase; 95% CI 0.70–0.92; p = 0.002) were significantly associated with decreased odds of post stroke seizures, highlighting that greater ischemic burden as adjudicated by lower ASPECTS was associated with increased likelihood of post stroke seizure development (see appendix for publication).

A further study conducted by the Post Stroke Epilepsy lab group at the Royal Melbourne Hospital aimed to examine whether cortical involvement, as detected on CT perfusion imaging, could be used to identify patients at higher risk of developing post ischaemic stroke seizures. It was found that cortical involvement was significantly associated with post stroke seizures across all CTP modalities. CBV had the highest hazard ratio (11.3, 95 % confidence interval (CI) 1.1–41.2), followed by NCCT (5.3, 95 % CI 1.5–18.0) and CBF (4.2, 95 % CI 1.1–15.2). Sensitivity was highest for Tmax (100 %), followed by CBV and CBF (both 76.9 %) and NCCT (63.6 %). Specificity was highest for CBV (77.8 %), then NCCT (75.6 %), CBF (54.0 %), and Tmax (29.1 %). Receiver-operating characteristic area under the curve was significantly different between imaging modalities ($p < 0.001$), CBV 0.77, NCCT 0.70, CBF 0.65, and Tmax 0.65. It was concluded that CTP may improve sensitivity and specificity for identifying cortical involvement

as a risk for post stroke seizures compared to NCCT (see appendix for publication).

The final study conducted by the Post Stroke Epilepsy lab group at the Royal Melbourne Hospital cemented the groundwork for Chapter 6 presented in this thesis. This was the first study that reported on post stroke seizures in patients treated with modern cerebrovascular stent devices and revascularization techniques, with the specific aim of assessing the association between haemorrhagic transformation (HT) and the development of post stroke seizures in this setting. It was found that patients with anterior circulation strokes who developed HT after treatment with endovascular therapy were nearly 5 times more likely to experience post stroke seizures compared to those without HT. It was concluded that HT has the potential to be used as an imaging marker when evaluating compounds with antiepileptogenic effects in patients after ischemic stroke (see appendix for publication).

Chapter 6 represents the follow-on study, which aimed to compare the association between haemorrhagic transformation and post stroke seizure development in patients receiving reperfusion therapies (including intra-venous tissue-plasminogen activator and intra-arterial therapies). The degree of haemorrhagic transformation was classified according to the European Cooperative Acute Stroke Study (ECASS), with further sub-classification based on size of the haemorrhage.

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Title: A comparison of the association between haemorrhagic transformation and post ischaemic stroke seizure development in patients receiving reperfusion therapies

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Abstract

Background: Epilepsy is a major complication of stroke. We aimed to compare the association between haemorrhagic transformation (HT) and seizure development in patients with acute ischaemic stroke receiving either intravenous thrombolysis (IV tPA) and/or intra-arterial thrombolysis (IAT). Improved understanding of this relationship may improve post-stroke monitoring and follow-up.

Methods: This was a retrospective cohort study conducted at the Royal Melbourne Hospital. We included patients with anterior circulation ischaemic stroke admitted 2008-2015. Patients were divided into two treatment groups 1. IV-tPA only versus 2. IAT (with/without IV-tPA). To test the hypothesis of an interaction between HT (ECASS classification) and treatment group for PSS occurrence, a logistic regression model with HT and treatment group together with an HT-by-treatment group interaction term was used, adjusted for age, stroke severity, stroke outcome and cortical involvement. Adjusted receiver operating characteristic curves were generated based on the adjusted logistic regression model.

Results: There were 363 patients who received IV-tPA only and 205 patients receiving IAT (with/without IV-tPA). Haemorrhagic transformation was associated with post stroke seizure development in the IAT (with/without IV-tPA) treated patients but not the IV-tPA treated patients: IAT adjusted OR 2.03 (1.06-4.36) p=0.03 AUC=0.80, IV-tPA only adjusted OR 0.6 (0.23-1.5) p=0.3 AUC=0.66. The

association between HT and seizures was affected by treatment group, p-value for interaction=0.04.

Conclusions: Patients receiving IAT who have subsequent HT are at the highest risk of post stroke seizures within two years and may benefit from longer follow-up. Novel strategies to reduce the risk of HT may also be beneficial.

Introduction

Epilepsy is one of the major complications of stroke. It has well been established that post-stroke epilepsy poses a considerable burden to stroke survivors and, even when well-controlled with medications, negatively impacts their quality of life [1]. Numerous randomised controlled trials have demonstrated the superiority of reperfusion therapies, including endovascular thrombectomy and IV-tPA, over standard treatment for acute ischaemic stroke patients with large artery occlusion [2-6]. Given the improved early prognosis, there is now a need to understand whether these interventions also affect long-term complications of stroke.

The incidence of seizures post primary intra-cerebral haemorrhage (ICH) is as high as 18% after 1 year and 25% after 5 years [7], whereas after ischaemic stroke it has been reported between 2-14% between 1 and 5 years post stroke [8-10]. Reasons for the higher rate of seizures following ICH are not well established. Similarly, pathophysiological mechanisms are not well understood. It has been suggested that products of blood metabolism, such as hemosiderin, may cause focal cerebral irritation leading to seizures [11]. In ischaemic stroke, haemorrhagic lesions have long been recognized as a risk factor for seizure development [8, 9, 11, 12]. However, only one of our previous studies has compared the influence of HT on seizure development in patients receiving intra-arterial therapies [13], but not in intra-venous reperfusion therapies. Given that HT is increased by reperfusion [14, 15], understanding the

potential influence of therapies on seizure outcome is of importance for stroke management and detection of these late onset sequelae.

This study aims to compare the association between haemorrhagic transformation and post ischaemic stroke seizure development in patients receiving reperfusion therapies (intra-venous thrombolysis (IV-tPA) and intra-arterial therapy (IAT) (with/without IV-tPA). Currently, minor degrees of haemorrhagic transformation are regarded as clinically inconsequential [16]. However, a better understanding of the relationship between haemorrhagic transformation from acute stroke therapies and the development of seizures may indicate a need to develop new approaches to prevent haemorrhagic transformation.

Methods

Setting

This was a retrospective, single centre cohort study conducted at the Royal Melbourne Hospital. Patients with an ischaemic stroke were identified from the prospectively maintained clinical stroke database. The Royal Melbourne Hospital, located in Victoria, Australia, provides IV-tPA therapy to acute ischaemic stroke patients who arrive to the hospital within 4.5 hours of stroke onset. It also serves as the statewide referral centre for intra-arterial therapies, including endovascular thrombectomy and intra-arterial urokinase. Ethical approval of the study was granted by the Melbourne Health Research Ethics Committee (project number QA2010089).

Patient groups

We included patients with anterior circulation ischaemic stroke admitted between 2008 and 2015. Patients with a history of epilepsy or seizures prior to their stroke were excluded. Patients included were divided into 2 groups based on the type of acute reperfusion treatment received: 1. IV-tPA only and 2. IAT (with/without IV-tPA).

Clinical Data Collection

Clinical data were collected and entered into the database when patients were admitted to the emergency department, transferred to the stroke unit and returned to stroke follow-up clinics. Data included patient demographics, age, sex, pre-morbid modified Rankin score (mRS), admission National Institute of Health Stroke Scale (NIHSS), stroke risk factors such as hypertension, atrial fibrillation, diabetes and dyslipidaemia. Clinical follow-up information included the modified Rankin Scale (mRS) at 3-months post onset with good outcome defined as mRS 0-2. Haemorrhagic transformation on follow-up 24hr CT brain imaging was assessed blinded to the patient's seizure status. The degree of HT was classified into HI (petechial haemorrhage without space-occupying effect) or PH (haemorrhage with mass effect) in accordance with The European Cooperative Acute Stroke Study (ECASS) [17], with further sub-classification based on size of the haemorrhage. We did not consider symptomatic HT as the clinical impact of bleeding does not necessarily correlate with HT extent, which is more likely to influence seizure risk [17].

Seizure Follow-Up

Patients with post stroke seizures up to two years from stroke onset were identified. This time window was chosen based on previous findings that suggest the highest risk

of seizure development is within the first year [9]. Patients and/or their families were contacted via telephone using a standardized questionnaire modified from our previous studies and a validated seizure screening questionnaire [18-21]. Information obtained from the telephone interview was corroborated with clinical records, EEG evidence, hospital correspondence or via the patient's primary care physician. Events were recorded as seizures if the symptoms included motor or autonomic components, with or without impairment of consciousness, as defined by the International League Against Epilepsy [22]. Final determination of occurrence of seizure was made by an epileptologist blinded to the imaging data. Early onset seizures were recorded if events occurred within 7 days of stroke onset and late onset seizures were recorded if events occurred after 7 days.

Statistical Analyses

The demographic, clinical and outcome characteristics of patients treated with either IV-tPA only versus IAT (with/without IV-tPA) were summarised as median (IQR) for continuous characteristics and as counts (proportions) for categorical characteristics, and compared using either Kruskall-Wallis test or Fisher's Exact test depending on the nature of the distribution.

Due to the very low incidence of early onset seizures (3 in the IV-tPA treated group ad 0 in the IAT with/without IV-tPA), early and late onset groups were combined for subsequent analyses.

Logistic regression was used to assess the association between haemorrhagic transformation and seizure occurrence within two years, adjusted for a priori chosen covariates: age, baseline NIHSS, mRS 0-2 and cortical involvement, as factors known to be associated with post stroke seizures [9, 23]. Corresponding effect sizes were

summarised as adjusted ORs with 95% confidence intervals. Receiver operating characteristic curves were generated from the adjusted logistic regression model. To test the hypothesis of a potential interaction between haemorrhagic transformation and treatment group for post stroke seizure occurrence, a logistic regression model with haemorrhagic transformation and treatment group together with a haemorrhagic transformation-by-treatment group interaction term was used. This analysis was conducted using the ECASS classification trichotomised as no HT, HI (H1 or H2) or PH (PH1 or PH2). As a sensitivity analysis, this was then repeated with further sub-classification based on size of the haemorrhage i.e. no ICH, H1, H2, PH1, PH2.

Statistical analyses were performed using STATA IC (v13.1, StataCorp, College Station, TX, USA), p-value <0.05 was treated as indicative of statistical significance.

Results

Patient characteristics

Of the patients treated with IV-tPA only, 21/363 (5.8%) patients developed post-stroke seizures compared to 16/205 (7.8%) of the patients who received IAT (with/without IV-tPA). Of the patients treated with IV-tPA 68/363 (18.7%) developed HT compared to 56/205 (27.3%) patients treated with IAT with/without IV-tPA (p<0.0001). Baseline characteristics are provided in Table 1.

The association between haemorrhagic transformation and seizure development within treatment groups

There was a significant association between haemorrhagic transformation and seizure development in patients treated with IAT with/without IV-tPA, OR 2.0, 95%CI 1.06-

4.4, p=0.03 after adjustment for age, NIHSS, mRS 0-2 and cortical involvement. There was no significant association found between HT and seizures in patients treated with IV-tPA, OR 0.6, 95%CI 0.23-1.5, p=0.3. Sensitivity analysis using 5 ECASS subcategories was similar; IAT with/without IV-tPA: OR 1.6, 95%CI 1.05-2.5, p=0.03 and IV-tPA-only: OR 0.8, 95%CI 0.5-1.3, p=0.3.

In receiver operating characteristic analyses based on these adjusted logistic regression models, the area under the curve for the IAT treated group was 0.80 and for the IV-tPA group was 0.66 (p=0.03) and in sensitivity analysis with 5-category ECASS 0.84 versus 0.66 (p=0.02). Using an adjusted multivariable logistic regression model with an appropriate HT-treatment interaction term, the association between HT and seizures was affected by treatment group (IAT with/without IV-tPA verses IV-tPA only), p=0.04 (p=0.05 in sensitivity analysis with 5-category ECASS)

Discussion

This study has demonstrated that patients undergoing intra-arterial therapy (with/without IV-tPA) who subsequently develop haemorrhagic transformation are at substantially greater risk of developing post stroke seizures than patients treated with IV-tPA only, independent of age, baseline stroke severity and functional outcome. We have also shown a significant increase in seizure occurrence between patients with haemorrhagic transformation receiving IAT compared to patients receiving IV-tPA, suggesting that patients receiving IAT therapy have additional underlying mechanisms for increased seizure development.

Numerous randomised controlled trials have demonstrated the superiority of IAT plus IV-tPA treatment over medical therapy (including IV-tPA) for acute ischaemic stroke patients with large artery occlusion [2-6]. Patients receiving combined therapies have the greatest chance of recanalisation and successful reperfusion. However, reperfusion is also associated with increased risk of haemorrhagic transformation. In this study, we showed a significant difference in the risk of haemorrhagic transformation between the treatment groups, with the highest HT rate in patients receiving IAT with/without IV-tPA. This was also suggested in a review of rates of HT in major clinical trials of ischaemic stroke interventions that found trends towards increased HT in patients with intra-arterial therapies [15].

There have also been suggestions that sudden changes in cerebral perfusion can cause a clinical syndrome that includes seizures [24] and seizures in the acute setting are a marker of good reperfusion [25]. When perfusion is improved by revascularization procedures, a cascade of inflammation causes changes to the blood brain barrier that may permit HT [25]. However, in this study we were unable to show an association between successful reperfusion, in patients receiving IAT therapy and increased risk of post stroke seizures. Additionally, the substantially higher risk of seizures post ICH supports a role for the blood products themselves in increasing the risk of seizures.

It has been demonstrated in both animal models and in patients that blood products, such as iron and hemosiderin, are epileptogenic, particularly in cortical brain metastases [26, 27]. For example, intra-tumoral haemorrhage is a common consequence of brain tumours, with seizures the presenting symptom in up to 50% of patients [28]. Other previous studies have reported that cortical ICH represents highly

epileptogenic lesions, again with hemosiderin deposits as the potential epileptogenic trigger in these patients [29]. In patients undergoing resection of cavernous malformations for refractory epilepsy, excision of the entire surrounding hemosiderin-stained region is recognized as necessary to maximise the probability of seizure control [30]. Intra-arterial therapy (with/without IV-tPA) is the standard treatment for large artery occlusions, which predominantly affect cortical locations, whereas IV-tPA is also used to treat lacunar and small vessel strokes. Although patients treated with IAT (with or without IV-tPA) had more cortical infarcts, this did not fully explain the increased seizure risk in this group. IAT-treated patients also tend to have larger infarcts than tPA-only patients, which may be a confounding factor. Assessment of HT extent between treatment groups would also be valuable.

A limitation of this study is the retrospective design with the potential of recall bias towards identifying seizures using the phone questionnaire. However, this limitation was minimized with corroboration of patient recall with reviewing follow-up medical records and contacting the patients' primary care physician. Additionally, despite a large, single centre cohort of endovascular treated acute stroke patients, the number of patients with post stroke seizures was relatively small. Thus, it was not feasible to perform separate sub-group analyses for early and late seizures or to perform interaction assessments on large vessel occlusion patients only. A larger sample size would allow for these sub-group analyses. Determining seizure diagnosis by phone interview may have been affected by recall bias and overestimation of seizure incidence.

Conclusion

Acute ischaemic stroke patients undergoing IAT with/without IV-tPA are at high risk of haemorrhagic transformation, which likely predisposes to subsequent seizures. Longer stroke follow-up for these late complications of treatment may be beneficial. Novel strategies to reduce the risk of haemorrhagic transformation may also be beneficial.

List of abbreviations

HT – Haemorrhagic Transformation

IAT – Intra-arterial therapy

IV-tPA – Intravenous-tissue plasminogen activator

OR – Odds ratio

95%CI – 95% confidence interval

mRS - modified Rankin scale

ECASS – European cooperative acute stroke study

NIHSS - National Institute of Health Stroke Scale

Declarations

Ethics approval and consent to participate:

This was a retrospective analysis of de-identified data collected as part of routine clinical care. Ethical approval for this study was granted by the Melbourne Health Human Research Ethics Committee and patient consent was waived.

Consent for publication: Not applicable

Availability of data and materials:

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request

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The authors declare that they have no competing interests

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Author Contributions:

JN – Conception and study design, drafting of manuscript, data acquisition, analysis and interpretation of data, revising manuscript critically for important intellectual content, given final approval for work to be published and will take accountability for all aspects of work

AT – Conception and study design, data acquisition, drafting of manuscript, analysis and interpretation of data, revising manuscript critically for important intellectual content, given final approval for work to be published and will take accountability for all aspects of work

LC – Conception and study design, statistical analysis, interpretation of results, drafting of manuscript, revising manuscript critically for important intellectual content, given final approval for work to be published and will take accountability for all aspects of work

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Table 1: Baseline demographics and stroke outcomes across treatment groups

	IV-tPA only, n=363	IAT (with/without IV-tPA), n=205	p-value
Age (median, IQR)	74 (65-82)	69 (57-78)	<0.001 [a]
Female Sex (n,%)	175 (45.1)	88 (42.9)	0.66 [b]
NIHSS baseline (median, IQR)	10 (6-17)	17 (13-21)	<0.001 [a]
Hypertension (n,%)	264 (68.0)	108 (52.7)	<0.001 [b]
Diabetes (n,%)	106 (27.3)	38 (18.5)	0.02 [b]
Dyslipidaemia (n,%)	191 (49.2)	61 (29.8)	<0.001 [b]
Atrial Fibrillation (n,%)	115 (29.6)	74 (36.1)	0.1[b]
Haemorrhagic Transformation (n,%) H1 H2 PH1 PH2	 21 (5.8) 23 (6.3) 11 (3) 13 (3.6)	 12 (5.9) 26 (12.7) 10 (2.5) 8 (3.9)	0.03 [b]
3-month mRS (0-2) (n,%)	191 (50)	95 (46.3)	0.14 [b]
Cortical Involvement	122 (46)	148 (72.2)	<0.0001[b]
Post Stroke Seizures (n,%)	21 (5.8)	16 (7.8)	0.2[b]

[a] Kruskall-Wallis test
[b] Fisher's Exact test

Table 2: European Cooperative Acute Stroke Study (ECASS) classification of haemorrhagic transformation in patients with and without post stroke seizures, by treatment group

	IV-tPA only		IAT with/without IV-tPA	
ECASS Classification	With post stroke seizures	Without post stroke seizures	With post stroke seizures	Without post stroke seizures
HI: petechial haemorrhage without space-occupying effect				
HI/H2	2	42	8	30
PH: haemorrhage (coagulum) with mass effect				
PH1/PH2	1	23	2	16
Without HT	18	277	6	143
OR, 95% CI, p-value	0.6, 0.23-1.5, 0.3		2.0, 1.06-4.4, 0.03	
*p-value for interaction= 0.04				

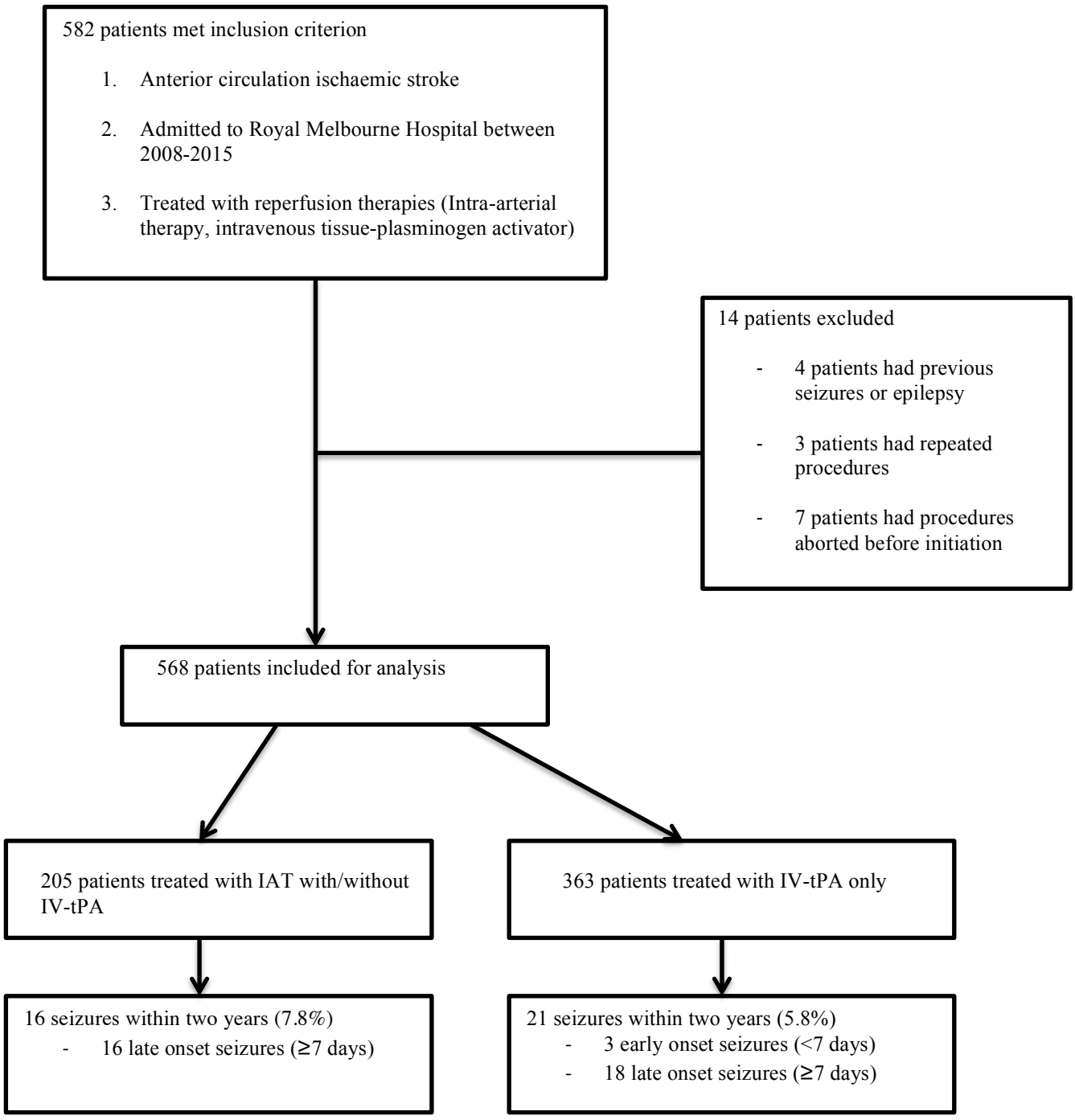
*adjusted for age, baseline NIHSS, cortical involvement and good functional outcome at 90 days (mRS 0-2)

Table 3: European Cooperative Acute Stroke Study (ECASS) 5 group sub-classification of haemorrhagic transformation in patients with and without post stroke seizures, by treatment group

	IV-tPA only		IAT with/without IV-tPA	
ECASS Classification	With post stroke seizures	Without post stroke seizures	With post stroke seizures	Without post stroke seizures
With HT	3	65	10	46
HI: petechial haemorrhage without space-occupying effect				
HI1	1	20	1	11
HI2	1	22	7	19
PH: haemorrhage (coagulum) with mass effect				
PH1	0	11	0	10
PH2	1	12	2	6
Without HT	18	277	6	143
OR, 95% CI, p-value	0.8, 0.5-1.3, 0.3.		1.6,1.05-2.5, 0.03	
*p-value for interaction= 0.05				

*****Adjusted for age, baseline NIHSS, cortical involvement and good functional outcome at 90 days (mRS 0-2)

Figure 1: Patient flow diagram



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Chapter 7

Objective 2 – Clinical Markers

Chapter 7 examines the association between different acute stroke therapies and the development of post stroke seizures

Reperfusion therapies with thrombolysis and, more recently, endovascular thrombectomy have transformed outcomes for patients. This chapter targeted groups of patients treated with modern cerebrovascular stent devices and revascularization techniques in order to assess the implications of these novel stroke interventions focusing on development of post stroke seizures, a potential late complication that has not previously been examined in relation to endovascular reperfusion. This was a retrospective, multicenter cohort study at the Royal Melbourne Hospital and the Jinling Hospital Nanjing that examined the association of difference acute stroke therapies (IV-tPA, IAT) and the development of post stroke seizures.

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RESEARCH ARTICLE Open Access

Association between different acute stroke therapies and development of post stroke seizures

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Abstract

Background: Epilepsy is a major complication of stroke. We aimed to establish whether there is an association between intravenous thrombolysis, intra-arterial thrombolysis and post stroke seizure (PSS) development. Improved understanding of the relationship between reperfusion therapies and seizure development may improve post-stroke monitoring and follow-up.

Methods: This was a retrospective, multicentre cohort study conducted at the Royal Melbourne Hospital and Jingling Hospital Nanjing. We included patients with anterior circulation ischemic stroke admitted 2008–2015. Patients were divided into four treatment groups 1. IV-tPA only, 2. Intra-arterial therapies (IAT) only, 3. IAT + IV-tPA and 4. stroke unit care only (i.e. no IV-tPA or IAT). To assess the association between type of reperfusion treatment and seizure incidence we used multivariable logistic regression models adjusted for age, stroke severity, 3-month functional outcome and prognostic factors.

Results: There were 1375 stroke unit care-only patients, of whom 28 (2%) developed PSS. There were 363 patients who received only IV-tPA, of whom 21 (5.8%) developed PSS. There were 93 patients who received IAT only, of whom 12 (12.9%) developed PSS and 112 that received both IV-tPA + IAT, of which 5 (4.5%) developed PSS. All reperfusion treatments were associated with seizure development compared to stroke unit care-only patients: IV-tPA only adjusted odds ratio (aOR) 3.7, 95%CI 1.8–7.4, $p < 0.0001$; IAT aOR 5.5, 95%CI 2.1–14.3, $p < 0.0001$, IAT + IV-tPA aOR 3.4, 95% CI 0.98–11.8, $p = 0.05$. These aORs did not differ significantly between treatment groups (IV-tPA + IAT versus IV-tPA $p = 0.89$, IV-tPA + IAT versus IAT, $p = 0.44$).

Conclusions: Patients receiving thrombolytic or intra-arterial reperfusion therapies for acute ischemic stroke are at higher risk of epilepsy and may benefit from longer follow-up. No evidence for an additive or synergistic effect of treatment modality on seizure development was found.

Keywords: Ischemic stroke, Post stroke seizures, Intravenous tissue plasminogen activator, Intra-arterial thrombectomy

Background

With greater public awareness of the importance of early stroke recognition and more efficient treatment delivery (e.g. code stroke, telemedicine), there has been a welcome increase in the proportion of patients arriving at hospital within the timeframe to be eligible for reperfusion therapies [1]. Numerous randomized controlled trials have demonstrated the superiority of reperfusion therapies, including endovascular thrombectomy and IV-tPA, over standard treatment for acute ischemic stroke patients with large artery occlusion [2–6]. These advances have contributed to a 68% increase in the number of stroke survivors between 1990 and 2010 [7]. Given the improved immediate outlook of acute stroke, there is a need to understand whether these interventions also affect long term complications.

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Epilepsy is one of the major complications of stroke. Post-stroke epilepsy poses a considerable burden to stroke survivors and, even when well-controlled with medications, negatively impacts their quality of life [8]. Seizures develop in 2–14% of patients who have had an ischemic stroke [9]. Such wide variation in the reported incidence and prognostic factors [10] has been attributed to differences in follow-up duration, definition and classification of seizures, and characteristics of the study population [11].

Whether the recent advances in reperfusion therapies for acute ischemic stroke have influenced the incidence of seizure development has not been well studied. Two studies have found that thrombolysis increases the likelihood of acute symptomatic seizures, within 7 days, post ischemic stroke, [12, 13] whilst others have shown no association [14]. Few have examined post-stroke seizures following intra-arterial therapies (IAT).

This study aimed to investigate whether there is an association between different acute stroke treatments and post stroke seizure development. A better understanding of the relationship between acute stroke therapies and the development of seizures may lead to improved post stroke monitoring and follow-up.

Methods

Setting

This was a retrospective, multicentre cohort study conducted at the Royal Melbourne Hospital and Jingling Hospital, Nanjing. Subjects at both centres were identified from prospectively maintained clinical databases of patients admitted with an ischemic stroke. The Royal Melbourne Hospital, located in Victoria, Australia, provides IV-tPA therapy to acute ischemic stroke patients who arrive to the hospital within 4.5 h of stroke onset. It also serves as the state-wide referral centre for intra-arterial therapies, including endovascular thrombectomy and intra-arterial urokinase. Jingling Hospital is located in Jiangsu province, China, where most patients received stroke unit care only. Ethical approval of the study was granted by the Melbourne Health Human Research Ethics Committee (project number QA2010089) and patient consent was waived.

Patient groups

We included patients with anterior circulation ischemic stroke admitted to the two hospitals between 2008 and 2015. Patients with a history of epilepsy or seizures prior to their stroke were excluded. Patients included were divided into 4 groups based on the type of acute reperfusion treatment received: 1. IV-tPA only, 2. IAT only, 3. IAT + IV-tPA and 4. stroke unit care only (i.e. no IV-tPA or IAT). For the purpose of analyses, patients receiving 'stroke unit care only' were regarded as controls.

Clinical data collection

Clinical data was collected and entered into the databases at both centres when the patients were admitted to the emergency department, transferred to stroke wards and returned to stroke follow-up clinics. Data included patient demographics, age, sex, pre-morbid modified Rankin score (mRS), admission National Institutes of Health Stroke Scale (NIHSS) score, admission blood pressure and stroke risk factors such as hypertension, atrial fibrillation, diabetes, dyslipidemia, previous stroke or transient ischemic attack (TIA) and smoking. Clinical follow-up information included the modified Rankin Scale (mRS) at 3-months post onset with good outcome defined as mRS 0–2. Hemorrhagic transformation was assessed using the ECASS classification [15] on follow-up 24 h CT brain imaging, blinded to seizure data collection.

Seizure follow-up

Patients with post stroke seizures were identified if they experienced seizures up to 2 years from stroke onset. This cut-off was chosen based on previous findings that suggested that the highest risk of seizure development was within the first year [16]. Occurrence of post stroke seizures was ascertained by reviewing follow-up medical records and via telephone interview using a questionnaire from previous studies [14, 17–19], modified from a validated screening questionnaire [20]. This questionnaire was translated into Chinese for use at the Jingling Hospital Nanjing. Events were recorded as seizures if the symptoms included motor or autonomic components, with or without impairment of consciousness, as defined by the International League Against Epilepsy [21].

Statistical analyses

To assess homogeneity between the two sites, the seizure occurrence within 2 years post-stroke between the control groups across the two sites was compared using Fisher's Exact test with corresponding effect estimated as Odds Ratios (OR) with 95% confidence interval (95%CI). When no significant difference in seizure incidence between the two sites was found, the control groups were combined.

The demographic, clinical and risk factor characteristics for the control group, IAT only, IV-tPA only and IAT + IV-tPA combined groups were summarised as median (IQR) for continuous characteristics and as counts (proportions) for categorical characteristics, and compared using either Kruskall-Wallis test or Fisher's Exact test depending on the nature of the distribution.

To assess the association between the types of treatment (reperfusion: IAT only, IV-tPA only, IAT + IV-tPA; with controls) and seizure occurrence within 2 years, logistic regression modelling with seizure occurrence as an

output and treatment groups as inputs was used. The analysis was adjusted for the following a priori chosen covariates known to be associated with post stroke seizures: age, baseline NIHSS and 3 month mRS [16, 22–26]. Corresponding effect sizes were summarised as adjusted ORs with 95%CI. For robustness analysis, extra covariates that demonstrated statistically significant association with seizure occurrence on univariate analyses were subsequently included in the model.

To further investigate the robustness of the modelling outcomes, we performed a sensitivity analysis to assess the potential association between thrombectomy and seizures with a control group more closely matching the patients who receive thrombectomy. We used a selection criterion of NIHSS≥6 as per AHA/ASA guidelines for thrombectomy eligibility [27]. We additionally performed a sensitivity analysis including only patients with an NIHSS> 8 to further increase specificity for large vessel occlusion [28].

Statistical analyses were performed using STATA IC (v13.1, StataCorp, College Station, TX, USA), p-value < 0.05 was treated as indicative of statistical significance.

Results

Patient characteristics

A total of 1943 patients with anterior circulation ischemic stroke were included in the analysis (757 from Melbourne and 1186 from Nanjing). The overall incidence of post-stroke seizures within 2 years was 3.3% (65/1943). No significant difference in seizure occurrence was identified between the Melbourne (1/189, 0.53%) and the Nanjing (27/1186, 2.3%) stroke unit care-only patients (OR 0.24, 95%CI 0.0–1.4, $p = 0.1$). Consequently, these patients were combined into a single control group for the multivariable regression models to assess the influence of treatment on seizure development. In the combined cohort, 1375 patients were controls, of which 27 (2, 95%CI 1.3–2.8%) developed post stroke seizures. There were 363 patients treated with IV-tPA only. Of these, 21/363 (5.8, 95%CI 3.6–8.7%) patients developed post stroke seizures. There were 93 patients who received IAT only, with 12 (12.9, 95%CI 6.8–21.4%) developing post stroke seizures. There were 112 patients treated with IAT and IV-tPA, of whom 5 (4.5, 95%CI 1.5–10%) developed post stroke seizures.

There was a significant difference in baseline NIHSS across treatment groups median (IQR): IAT + IV-tPA = 17 (13–21), IAT = 18 (13–21), IV-tPA only = 10 (6–17), control = 2 (0–7), $p = 0.0001$. There was a significant difference in age at stroke across the treatment groups median (IQR): IAT + IV-tPA = 70 (59–78), IAT = 67 (54–77), IV-tPA only = 74 (65–77) control = 62 (52–70), $p = 0.0001$. There was a significant difference in mRS 0–2 at 3 months across the treatment groups: IAT + IV-tPA = 59/112 (52.7%), IAT = 36/93 (38.7%), IV-tPA only = 191/363 (52.6%) control = 839/1375 (61.0%), $p < 0.0001$. Baseline characteristics are detailed in Table 1.

The association of IAT, IV-tPA and IAT + IV-tPA with post stroke seizure development

The univariate analysis for baseline variables age, NIHSS at baseline and the 3-month mRS of 0-2 and their association with post stroke seizures are provided in Table 2. In multivariable logistic regression adjusted for baseline NIHSS, age, and mRS at 3 months, administration of IV-tPA (without IAT) was significantly associated with increased odds of seizure development: adjusted OR 3.7,

Table 1 Baseline demographics and stroke risk factors across treatment group

	IAT + IV-tPA, $n = 112$	IAT only $n = 93$	IV-tPA only, $n = 363$	Control, $n = 1375$	p-value
Age (median, IQR)	70 (59–78)	67 (54–77)	74 (65–82)	62 (52–70)	0.0001[a]
Female Sex (n,%)	45 (40.2)	50 (54)	175 (48.2)	477 (34.7)	< 0.001[b]
Systolic Blood Pressure (median, IQR)	-[x]	-[x]	150 (133–167)	136 (128–150)	0.0001[a]
Diastolic Blood Pressure (median, IQR)	-[x]	-[x]	80 (70–90)	80 (72–88)	0.50[a]
NIHSS baseline (median, IQR)	17 (13–21)	18 (13–21)	10 (6–17)	2 (0–7)	0.0001[a]
Hypertension (n,%)	66 (58.9)	42 (45)	264 (73.0)	823 (59.8)	< 0.001[b]
Diabetes (n,%)	19 (17.0)	19 (20.4)	106 (29.2)	299 (21.7)	0.05[b]
Dyslipidaemia (n,%)	33 (29.5)	28 (30)	191 (52.6)	53 (4)	< 0.001[b]
Smoking (n,%)	-[x]	-[x]	88 (24)	438 (32)	0.001[b]
Atrial Fibrillation (n,%)	43 (38.4)	31 (33.3)	115 (31.7)	114 (8.3)	< 0.001[b]
Hemorrhagic Transformation (n,%)	28 (25)	28 (30)	63 (17)	-[x]	0.001[b]
3-month mRS (0–2) (n,%)	59 (53)	36 (39)	191 (53)	839 (61)	< 0.001[b]
Post Stroke Seizures (n,%)	5 (4.5)	12 (12.9)	21 (5.8)	28 (2)	< 0.001[b]

[a]Kruskall-Wallis test
[b]Fisher's Exact test
[x]unavailable

Table 2 Univariate Analysis for baseline variables and the association with post stroke seizures

	IAT + IV-tPA, $n = 112$ OR, 95% CI, p-value	IAT only $n = 93$ OR, 95% CI, p-value	IV-tPA only, $n = 363$ OR, 95% CI, p-value	Control, $n = 1375$ OR, 95% CI, p-value
Age	1.0, 0.94–1.1, $p = 0.9$	0.94, 0.9–0.98, $p = 0.003$	1.0, 0.96–1.0, $p = 0.95$	0.99, 0.96–1.0, $p = 0.33$
NIHSS baseline	1.1, 0.9–1.3, $p = 0.36$	1.0, 0.92–1.1, $p = 0.98$	1.1, 1.0–1.1, $p = 0.05$	1.1,1.0–1.1, $p < 0.0001$
3-month mRS (0–2)	0.13, 0.01–1.3, $p = 0.08$	0.52, 0.12–2.2, $p = 0.37$	0.37, 0.14–0.97, $p = 0.04$	0.27, 0.12–0.62, $p = 0.002$

95%CI 1.8–7.4, $p < 0.0001$. Similarly, there was an independent association between treatment with IAT only and increased odds of seizure development compared to controls: adjusted OR 5.5, 95%CI 2.1–14.3, $p < 0.0001$. There was also a trend towards increased seizure risk in patients treated with combined IAT + IV-tPA compared to controls: OR 3.4, 95%CI 0.98–11.8, $p = 0.05$ (Fig. 1). For the purpose of robustness analysis, we included extra baseline adjustment covariates and risk factors that were significantly associated with seizure development in univariate analysis and were recorded for all treatments: sex, hypertension, atrial fibrillation and dyslipidemia (Table 1). Including these variables as extra covariates increased the collinearity in the regression model and rendered some standard error estimates less stable, but the adjusted estimates for the effects for individual treatment groups remain quantitatively similar: IAT only OR 6.6, 95%CI 2.5–17.8, $p < 0.001$, IV-tPA only OR 2.9, 95%CI 2.3–10.7, $p < 0.001$, IV-tPA+ IAT OR 4.3, 95%CI 1.2–15.3, $p = 0.03$. Consistently with largely overlapping confidence intervals, the odds of post stroke seizures in the three treatment groups did not differ (IV-tPA + IAT versus IV-tPA $p = 0.89$, IV-tPA + IAT versus IAT, $p = 0.44$, Fig. 1).

For the sensitivity analysis including only patients with an NIHSS ≥6 (to better match the controls with patients eligible for thrombectomy [27]), the results of the logistic regression were similar to the previous results: IV-tPA verses controls OR 3.2, 95%CI 1.4–7.4, $p = 0.005$, IAT verses controls OR 4.6, 95%CI 1.6–13.0, $p = 0.004$, IV-tPA + IAT versus controls OR 3.2, 95%CI 0.84–12.1, $p = 0.09$.

For the sensitivity analysis including only patients with an NIHSS> 8 (to further increase specificity for large vessel occlusion [28]) the results of the logistic regression were similar to the previous results: IV-tPA versus controls OR 3.9, 95%CI 1.5–9.7, $p = 0.004$, IAT versus controls OR 5.3, 95%CI 1.9–15.3, $p = 0.002$, IV-tPA + IAT versus controls OR 4.0, 95%CI 1.03–16.2, $p = 0.045$. Additional file 1 for the univariate and multivariable logistic regression has been presented.

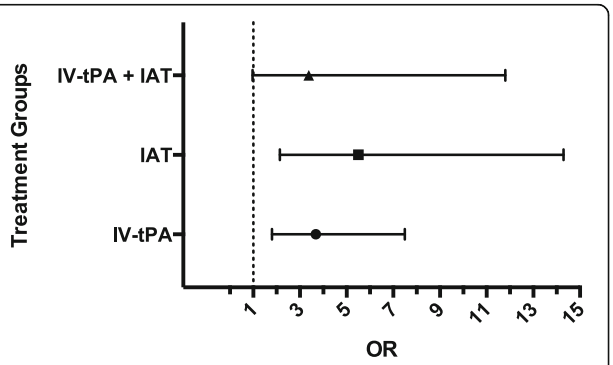

Fig. 1 Forest plot of treatment effects on seizure development post stroke. The effects shown are ORs with 95% CIs (adjusted for age, baseline NIHSS and 3 month mRS) using stroke unit care only group as a reference. No significant difference in seizure development post stroke between IAT, IV-tPA and IV-tPA + IAT treatment groups (IV-tPA + IAT versus IV-tPA $p = 0.89$, IV-tPA + IAT versus IAT, $p = 0.44$)

Discussion

This study has demonstrated that acute stroke reperfusion therapies are significantly associated with seizure development. Specifically, we showed that in patients treated with IV-tPA only, independent of age, baseline stroke severity, stroke outcome and other baseline variables, there was a greater than threefold increase in the likelihood of developing seizures in comparison to controls. This was a similar effect to those patients treated with IV-PA+ IAT. We also found that in patients treated with IAT only, there was a greater than fivefold increase in the likelihood of developing seizures in comparison to controls. However, there was no evidence for an additive or synergistic effect of treatment modality.

There have been two studies that have reported an association between thrombolysis with IV-tPA and increased likelihood of acute symptomatic seizures, within 7 days, after an ischemic stroke [12, 13]. We have shown that the risk of seizures extends further than the acute symptomatic period. In this study, we found the development of seizure occurred over a 24-month period, and recommend longer-term follow-up of these higher risk patients. This could significantly improve the detection of late onset complications of stroke.

Our analysis was adjusted for prognostic factors for seizure development as well as factors that were significantly different between treatment groups, suggesting that the increased risk of post stroke seizure development may be inherent to the treatment itself. A number of potential mechanisms may account for this. Firstly, sudden changes

in cerebral perfusion have been described to cause a clinical syndrome that includes seizures [29–31]. When perfusion is improved by revascularization procedures, it is suggested a cascade of inflammatory responses causes the reestablishment of brain circulation, contributing to the development of the reperfusion syndrome and subsequent seizures, with seizures a sign of good reperfusion [32]. Given that early onset seizures are a risk factor for later onset seizures and epilepsy development, potentially the benefit of reperfusion from acute stroke therapies is concomitantly increasing the risk of seizure development in these populations.

Although IV-tPA has been reported to have some neurotoxicity [33], the greater odds of developing seizures from any IAT treatment compared to any IV-tPA treatment would argue against a specific effect of IV-tPA and perhaps reperfusion itself is the issue. In our study, we showed that patients who underwent any intra-arterial therapy were significantly more likely to develop seizures than those with any IV-tPA. Of interest, there was a significant difference in rates of hemorrhagic transformation between the treatment groups (IAT, IV-tPA and IAT + IV-tPA). This was also suggested in a review of rates of HT in major clinical trials of ischemic stroke interventions where they found observable trends in increased HT in patients with intra-arterial therapies [34]. Potentially, the greater likelihood of developing post stroke seizures in patients undergoing IAT rather than IV-tPA may be due to increased rates of HT within this treatment group. An analysis of post stroke seizures due to HT across different treatment groups is a planned follow up analysis.

A limitation of this study is the retrospective design with the potential of bias towards identifying seizures in those patients receiving reperfusion therapies given that they may have received more monitoring in the stroke unit than those patients without reperfusion therapies. However, given that all patients were contacted via a phone call questionnaire we believe this would have minimised any bias towards treatment groups. Another limitation is that we do not understand the potential ethnicity-treatment interaction without a treated group from Nanjing. We cannot discount the potential effect of the treatment itself differing due to ethnicity, although we do not expect this. Future studies should incorporate a treatment group from separate ethnicities to examine a possible treatment-ethnicity interaction. Additionally, due to the low incidence of post stroke seizure development, future larger studies are required to improve the precision of incidence estimates. Finally, we were unable to perform a subanalysis on patients with large vessel occlusion as vessel occlusion status was not available in our control population. Even with adjustment for baseline NIHSS, initial stroke severity may still be contributing to the higher odds of seizures in the treated patients. It would be of interest to perform a future analysis specifically in large vessel occlusion patients.

Conclusion

Patients undergoing reperfusion therapies with IV-tPA and IAT were at considerably higher risk of post-stroke epilepsy than control patients. However, no additional effect from combined treatment was evident in this dataset. This association persisted despite adjustment for differences in stroke severity and other prognostic variables. We conclude that patients undergoing IAT and/or IV-tPA in acute ischemic stroke may benefit from longer stroke follow-up for late complications of treatment such as epilepsy.

Additional file

Additional file 1: Table S1. Univariate logistic regression with baseline risk factors (age, NIHSS and mRS02 at 90 days) and treatment for seizure outcome. **Table S2.** Logistic regression model with treatment groups plus age for seizure outcome. **Table S3.** Logistic regression model with treatment groups plus NIHSS for seizure outcome. **Table S4.** Logistic regression model with treatment groups plus mRS02 for seizure outcome. **Table S5.** Logistic regression model with treatment groups unadjusted. **Table S6.** Logistic regression model with treatment groups adjusted for age, NIHSS and mRS02. **Table S7.** Median (IQR) of the baseline NIHSS and number (percentage) of mRS (0–2) in the sensitivity analysis NIHSS ≥ 6. **Table S8.** Median (IQR) of the baseline NIHSS and number (percentage) of mRS (0–2) in the sensitivity analysis NIHSS > 8. (DOCX 63 kb)

Abbreviations

95%CI: 95% confidence interval; ECASS: European cooperative acute stroke study; HT: Hemorrhagic Transformation; IAT: Intra-arterial therapy; IV-tPA: Intravenous-tissue plasminogen activator; mRS: modified Rankin scale; OR: Odds ratio

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JN – Conception and study design, drafting of manuscript, data acquisition, analysis and interpretation of data, revising manuscript critically for important intellectual content, given final approval for work to be published and will take accountability for all aspects of work. AT – Conception and study design, data acquisition, drafting of manuscript, analysis and interpretation of data, revising manuscript critically for important intellectual content, given final approval for work to be published and will take accountability for all aspects of work. LC – Conception and study design, statistical analysis, interpretation of results, drafting of manuscript, revising manuscript critically for important intellectual content, given final approval for work to be published and will take accountability for all aspects of work.
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take accountability for all aspects of work. ZC2 – Conception and study design, data acquisition, analysis and interpretation of data, revising manuscript critically for important intellectual content, given final approval for work to be published and will take accountability for all aspects of work. XL – Conception and study design, data acquisition, analysis and interpretation of data, revising manuscript critically for important intellectual content, given final approval for work to be published and will take accountability for all aspects of work. PK - Conception and study design, data acquisition, analysis and interpretation of data, revising manuscript critically for important intellectual content, given final approval for work to be published and will take accountability for all aspects of work. BC - Conception and study design, drafting of manuscript, analysis and interpretation of data, revising manuscript critically for important intellectual content, given final approval for work to be published and will take accountability for all aspects of work.

Ethics approval and consent to participate

This was a retrospective analysis of de-identified data collected as part of routine clinical care. Ethical approval for this study was granted by the Melbourne Health Human Research Ethics Committee and patient consent was waived. This study was submitted to the local Medical Ethics Committee at Jinling Hospital, Nanjing University School of Medicine and received ethical approval.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

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Chapter 8

Objective 2 – Clinical Markers

Chapter 8 examines the association between atrial fibrillation and post ischaemic stroke seizure development.

Patients with atrial fibrillation (AF) are at a six-fold increased risk of developing ischaemic stroke, with AF the commonest cause of cardioembolic stroke. Cardioembolic stroke is increasingly more frequent; contributing to 32% of stroke cases in some studies and is associated with worse 90-day stroke outcome in comparison to other stroke subtypes. Additionally, there have been suggestions that patients with cardioembolic stroke are at a greater risk of developing seizures than other stroke subtypes. However, the incidence of atrial fibrillation and cardioembolic strokes varies considerably across countries, generally higher in Western populations than in Asian populations, with numerous genes associated with the development of AF in certain populations. This may explain the inconsistencies in incidence and prognostic factors for post stroke seizure development. Chapter 8 aimed to assess whether ethnicity affects the association between atrial fibrillation and post stroke seizures. This study included patients from the Royal Melbourne Hospital and Nanjing Jinling Hospital.

This chapter is presented as it appears in publication in the Journal of Stroke and Cerebrovascular Disease.

The Association Between Atrial Fibrillation and Poststroke Seizures is Influenced by Ethnicity and Environmental Factors

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Goal: Epilepsy is a major complication of stroke. There have been suggestions that patients with cardioembolic stroke are at a greater risk of developing seizures than other stroke subtypes. However, the incidence of atrial fibrillation (AF) and cardioembolic stroke varies considerably across countries, generally higher in Western populations than in Asian populations. This study assessed whether ethnicity affects the association between AF and poststroke seizure (PSS) development. We hypothesized that Royal Melbourne Hospital ([RMH] Melbourne) patients will have significantly higher incidence of AF-related PSS than in the Jinling Hospital (Nanjing) population. *Materials and Methods:* This was a retrospective, multicenter cohort study including patients with anterior circulation ischemic stroke admitted between 2008 and 2015. Occurrences of PSS were ascertained by reviewing medical records or telephone follow-up. To test the hypothesis of an interaction between ethnicity and AF for PSS occurrence, a logistic regression model with AF and ethnicity together with an ethnicity-by-AF interaction term was used. *Findings:* Of 782 patients followed-up for seizure development at RMH, 247 (31.6%) patients had AF, of whom 10 (4%) developed PSS. Of 1185 patients followed-up and included at JH, 54 (4.8%) patients with AF, of whom 4 (7.4%) developed PSS. At RMH, no significant association was found between AF and PSS; odds ratio .75, 95% confidence interval .4-1.6, ($P = .4$). At JH, there was a significant association between AF and increased PSS: OR 4.0, 95% CI 1.3-12.1, ($P = .01$), P for interaction = .03. *Conclusion:* Further understanding of genetic risks and environmental differences across ethnic populations and the role in PSS is required.

Key Words: Atrial fibrillation—poststroke seizure—ethnicity—ischemic stroke

Introduction

Epilepsy is one of the major complications of stroke. Poststroke epilepsy poses a considerable burden to stroke survivors and, even when well-controlled with medications, negatively impacts their quality of life.[1] Seizures develop in 2%-14% of patients who have had an ischemic stroke.[2] The reported incidence and prognostic factors varied widely between studies.[3-5] This has been attributed to differences in follow-up times, epilepsy and seizure definitions, heterogeneity in study designs, methodology for seizure ascertainment and classification and the

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potential underlying genetic and environmental differences between populations.[6]

Patients with atrial fibrillation (AF) are at a 6-fold increased risk of developing ischemic stroke, with AF the commonest cause of cardioembolic stroke.[7] Cardioembolic stroke is increasingly more frequent; contributing to 32% of stroke cases in some studies[8] and is associated with worse 90-day stroke outcome in comparison to other stroke subtypes.[9] Additionally, there have been suggestions that patients with cardioembolic stroke are at a greater risk of developing seizures than other stroke subtypes.[10-12] This is potentially due to cardioembolic strokes being generally larger and more severe strokes, associated with worse patient outcomes. It also could be attributed to greater cortical involvement with cardioembolic strokes,[13] which is a significant risk factor for seizure development.[14-16] In patients treated with intravenous tissue plasminogen activator, poststroke seizures have been associated with atrial fibrillation and early mortality.[17] Atrial fibrillation has also been shown to be significantly more prevalent in late onset seizures, a risk factor for the development of poststroke epilepsy.[18] However, the incidence of atrial fibrillation and cardioembolic strokes varies considerably across countries, generally higher in Western populations than in Asian populations, with numerous genes associated with the development of AF in certain populations.[19] This may explain the inconsistencies in incidence and prognostic factors for poststroke seizure development.

The hereditary component of seizures after AF-related stroke is unknown. Whilst genome-wide association studies (GWAS) have enabled the discovery of genetic loci associated with AF,[20] it is unknown whether these have any involvement in the development of poststroke seizures. Given the suggestion of an underlying genetic predisposition to developing atrial fibrillation, understanding the ethnicity-specific interaction may provide insight into the role AF has in poststroke seizure development.

Aim and Hypothesis

This study aimed to assess whether ethnicity affects the association between atrial fibrillation and poststroke seizure development. We hypothesized that Royal Melbourne Hospital ([RMH] Melbourne) patients will have significantly higher incidence of AF-related poststroke seizures than in the Jinling Hospital (Nanjing) population.

Methods

Subjects

This was a retrospective, multicentre cohort study conducted at the RMH and Nanjing Jinling Hospital. Subjects at both centers were identified from prospectively maintained clinical databases of patients admitted to their respective hospitals with an anterior circulation ischemic stroke. The RMH, located in Victoria, Australia, provides intravenous thrombolysis (IV-tPA) therapy to acute ischemic stroke patients who arrive to the hospital within 4.5 hours of stroke onset. It also serves as the statewide referral center for intra-arterial therapies (IAT), including endovascular clot retrieval and intra-arterial urokinase. Nanjing Jinling Hospital is located in Jiangsu province, China, where most patients received stroke unit care only. Ethical approval was granted by the Melbourne Health Human Research Ethics Committee (project number QA2010089).

Clinical Stroke Data Collection

Clinical data were collected and entered into the databases at both centers when the patients were admitted to the emergency department, transferred to stroke wards and returned to stroke follow-up clinics. Data included patient demographics, age, sex, premorbid modified Rankin score (mRS), admission National Institutes of Health Stroke Scale (NIHSS), admission blood pressure, and stroke risk factors such as hypertension, atrial fibrillation (detected at the RMH on patient admission via a 12-lead ECG or Holter monitor and at Jingling hospital through a 12-lead ECG), diabetes, and dyslipidemia. Clinical follow-up information includes the mRS at 3-months postonset.

Seizure Follow-Up

Patients with poststroke seizures were identified if they experienced seizures up to 2 years from stroke onset. This cut-off was chosen based on previous findings that suggest the highest risk of seizure development is within the first year.[21] Occurrences of poststroke seizures were ascertained by reviewing follow-up medical records, contacting the patients' primary care physicians, or via telephone interview using a questionnaire validated in a previous study.[22-25] This questionnaire was translated into Chinese for use at the Nanjing Jinling Hospital. Excluded patients were those with a history of epilepsy or seizures previous to their stroke.

Ethnicity

The ability to statistically adjust for 'ethnicity' is compromised by the fact that there is no gold standard definition of ethnicity, with only fair agreement shown between self-reported and actual country of birth.[26,27] Thus, for the purposes of this study, we treated ethnicity as the country of residence at the time of stroke onset. For sensitivity analysis, we also removed those patients within the RMH who were born in Mainland China or Hong Kong (n = 9) and there were no patients in the Chinese cohort who were born in Australia.

Statistical Analyses

The demographic, clinical, and risk factor characteristics for the 2 populations were summarized as median (interquartile range [IQR]) for continuous characteristic and as counts (proportions) for categorical characteristics.

To assess the association between atrial fibrillation on seizure occurrence within 2 years within each population, the logistic regression modeling with seizure occurrence as an output and atrial fibrillation as the input was used. The analysis was adjusted for the following prespecified covariates: age and baseline NIHSS (due to previous knowledge of their association with poststroke seizures)[10,14,21,28,29] and any reperfusion therapies (IV-tPA, IAT, and IAT+IV-tPA).

Corresponding effect sizes were summarized as adjusted odds ratios (ORs) with corresponding 95% confidence intervals (CIs).

To test the hypothesis of a potential interaction between ethnicity and atrial fibrillation for poststroke seizure occurrence, a logistic regression model with atrial fibrillation and ethnicity together with an ethnicity-by-atrial fibrillation interaction term was used. We performed robust analysis of the model using Firth penalized maximum likelihood regression to investigate the effect of potential reduction in bias due to separation conditions in the model.

Statistical analyses were performed using STATA IC (v13.1, StataCorp, College Station, TX), P value $<.05$ was treated as indicative of statistical significance.

Results

Baseline Demographics by Atrial Fibrillation

RMH

A total of 782 patients were followed-up for seizure development and included at the Melbourne center. There were 247 (31.6%) patients with atrial fibrillation, of whom 10 (4%) developed poststroke seizures. The median age of the group with atrial fibrillation was 78 (IQR 71-84), median IQR NIHSS was 14 (6-20), female sex was 129 (52.2%), and 89 (36.0%) had independent functional outcome at 3 months (mRS 0-2). Of the 247 patients with atrial fibrillation, 115 (46.6%) received IV tPA treatment alone, 31 (12.6%) received IAT alone, and 43 (17.4%) received IV-tPA and IAT combined (Table 1).

Nanjing Hospital

A total of 1185 patients were followed-up and included at the Nanjing center. There were 54 (4.8%) patients with atrial fibrillation, of whom 4 (7.4%) developed poststroke seizures. The median (IQR) age of the group with atrial fibrillation was 73 (65-79), median IQR NIHSS was 6 (2-13), female sex was 34 (63%), and 17 (3.1%) had independent functional outcome at 3 months (mRS 0-2) (Table 1).

Ethnicity-by-AF Interaction

Using an adjusted multivariable logistic regression model with an appropriate ethnicity-AF interaction term, the association between AF and seizures was affected by ethnicity, $P = .03$. In the Melbourne population, there was no significant association found between atrial fibrillation and poststroke seizure development; OR .75, 95% CI .4-1.6, ($P = .4$). In the Nanjing population, there was a significant association between atrial fibrillation and increased poststroke seizure development: OR 4.0, 95% CI 1.3-12.1, ($P = .01$). (Table 2) The results remained qualitatively similar with the use of Firth penalized maximum likelihood model, P for interaction = .025. For the sensitivity analysis, 9 patients were removed from the RMH population as they were born in either Mainland China or Hong Kong and 0 patients were removed from the Nanjing population. The P value for interaction again remained qualitatively similar, $P = .026$.

Discussion

This study assessed whether ethnicity affects the association between atrial fibrillation and seizures. Atrial fibrillation (AF) was significantly associated with increased likelihood of seizure development in the Nanjing cohort but not in the Melbourne population. Furthermore, within our ethnicity-interaction regression models, we found that the association between atrial fibrillation and seizures was significantly affected by ethnicity. This may suggest an underlying environmental and/or genetic basis for seizure development in those patients with AF.

The incidence of AF in this RMH acute ischemic stroke patient cohort was 31.6%. The reported incidence of AF in other centers within Australia has been 25%[30] and in other Western centers as high as 33.4%.[31,32] The incidence of AF in ischemic stroke in our Nanjing cohort was 4.8%. This, again, is similar to the China National Stroke Registry, which out of 21,902 patients included, 1200 (5.5%) were confirmed with AF through at least 1 electrocardiogram, or the presence of the arrhythmia during hospitalization, or through a history of AF.[33] Similarly, in a larger study from Southwest China, the incidence of stroke in patients with AF was 6.4% across an 11-year period. Understanding the ethnic and regional variation in AF has not yet been identified, but most likely attributed to different study designs across populations, genetic influence, and environmental factors.

The hereditary component of seizures after AF-related stroke is unknown. Whilst GWAS have enabled the discovery of genetic loci associated with AF,[20] it is unknown whether these have any involvement in the development of poststroke seizures. A number of candidate gene approach studies and GWAS studies have identified genes, such as Pitx2, KCNN3, and ZFHX3, that are associated with atrial fibrillation in populations of European descent.[34] In particular, SNP rs2200733 on chromosome 4q25 has been strongly associated with AF in both European and Chinese cohorts,[35,36] but SNP rs10033464 on chromosome 4q25 has reached genome-wide significance

Table 1. *Baseline demographics and outcomes by atrial fibrillation across populations*

	Patients with AF, n = 301		Patients without AF, n = 1666	
	Royal Melbourne Hospital, n = 247	Nanjing Jinling Hospital, n = 54	Royal Melbourne Hospital, n = 535	Nanjing Jinling Hospital, n = 1131
Age (median, IQR)	78 (71-84)	73 (65-79)	69 (58-79)	60 (51-67)
Female sex (n, %)	129 (52.2)	34 (63.0)	209 (39.0)	790 (69.8)
Systolic blood pressure (median, IQR)	150 (130-165)	135 (126-150)	150 (135-167)	135 (128-149)
Diastolic blood pressure (median, IQR)	80 (70-90)	80 (75-85)	80 (70-90)	80 (72-87)
NIHSS baseline (median, IQR)	14 (6-20)	6 (2-13)	10 (5-18)	2 (0-6)
Hypertension (n, %)	174 (70.4)	31 (57.4)	209 (39.0)	686 (60.7)
Diabetes (n, %)	64 (25.9)	9 (1.7)	124 (23.2)	245 (21.7)
Dyslipidaemia (n, %)	80 (32.4)	1 (1.9)	182 (34.0)	42 (3.7)
IV-tPA treatment (n, %)	115 (46.6)	- *	273 (51.0)	- *
IAT treatment (n, %)	31 (12.6)	- *	62 (11.6)	- *
IV-tPA + IAT treatment (n, %)	43 (17.4)	- *	69 (12.9)	- *
Post stroke Seizures (n, %)	10 (4.0)	4 (7.4)	29 (5.4)	22 (1.9)
3-month mRS (0-2) (n, %)	89 (36.0)	17 (3.1)	256 (47.9)	761 (67.3)

AF, atrial fibrillation; IQR, interquartile range; IV-tPA, intravenous thrombolysis; mRS, modified Rankin score.
* Indicates data not applicable to population.

in 4 European studies, but failed to be replicated in Chinese cohorts.[37] These findings prompt further investigation of genetic risks across ethnic populations and its role in stroke outcomes, particularly poststroke seizures [34,38].

In addition to the genetic contribution to AF, AF therapy may influence development of poststroke seizures. Patients with AF are often administered anticoagulant therapy to prevent cardioembolic stroke. A number of studies have investigated the effects of anticoagulant therapy on increased seizure risk. One study assessing risk factors for epileptic seizures in patients following surgical treatment of acute subdural hematoma concluded that existing anticoagulant therapy in patients was an independent risk factor for seizure development.[39] Several other studies have reported frequent re-bleeding rates in these patients on anticoagulation.[40,41] It is suggested that the larger distribution and contact area of blood with

Table 2. *The prevalence of PSS in patients with and without atrial fibrillation*

	Royal Melbourne Hospital, n = 718	Nanjing General Hospital, n = 1159
Patients with AF	10/227 (4.2%)	4/50 (7.4%)
Patients without AF	29/491 (5.6%)	22/1109 (1.95%)
*OR (95%CI) of patients in AF group versus non-AF group	.75 (.4-1.6)	4.0 (1.3-12.1)

AF, atrial fibrillation; CI, confidence interval; IV-tPA, intravenous thrombolysis; OR, odds ratio; PSS, poststroke seizure.
P value for interaction = .03.
* adjusted for age, baseline NIHSS and reperfusion treatment with either IAT or IV-tPA or IV-tPA+IAT.

cortical surface might be the underlying pathophysiological mechanism for increased seizure risk.[42] Similarly, it is well known that acute ischemic stroke patients with atrial fibrillation and on anticoagulant therapy, particularly in those receiving thrombolysis or thrombectomy, have an increased bleeding risk.[43] Given that the risks of major bleeding in patients with AF and on anticoagulation is higher in Asian populations than non-Asian populations, regardless of any reperfusion treatment,[44] this may contribute to why patients with atrial fibrillation in China were 4 times more likely to develop seizures than patients with atrial fibrillation in Melbourne. However, the follow-up bleeding rates were unknown in the Chinese cohort. Future studies could assess the effects of oral anticoagulant therapies and bleeding rates on development of postischemic stroke seizures in patients with atrial fibrillation.

A limitation of this study is the difference in clinical care between centers. The routine use of 24-hour Holter ECG monitoring in Melbourne but not in Nanjing may have contributed to the increased proportion of patients with AF identified in Melbourne. However, the use of Holter monitoring in a larger study at this Melbourne center was recorded in 20.2% of patients, with the increasing prevalence of atrial fibrillation across a 10-year period remaining significant after adjustment for both age and the use of Holter monitoring.[45] Secondly, the rate of hemorrhagic transformation is unknown in the Chinese cohort and therefore was unable to be included into the adjustment. Similarly, we do not know if patients were on anticoagulation and in what form at the time of and after their stroke. Understanding if anticoagulation has an influence on poststroke seizure development in cardioembolic stroke is an area of further interest. A further limitation is the small sample size of patients with poststroke seizures and atrial fibrillation and further investigation using larger sample sizes are warranted. However as discussed, the rates of AF within the 2 populations are consistent with previous literature. Similarly, the rates of poststroke seizure patients with AF-related stroke are consistent with previous Western literature.[46,47]

Conclusions

The association between atrial fibrillation and poststroke seizures may be influenced by ethnicity and environmental factors, prompting further understanding of genetic risks and environmental differences across ethnic populations and its role in stroke outcomes, particularly poststroke seizures.

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Chapter 9

Objective 2 – Clinical Markers

Chapter 9 is a protocol chapter entitled A registry of clinical and MRI outcomes following early versus late administration of novel oral anticoagulation after ischaemic stroke or transient ischaemic attack in patients with atrial fibrillation.

This represents an ongoing observational clinical trial conducted at multiple centres.

In Chapter 8, it was concluded that the association between atrial fibrillation and post stroke seizures may be influenced by ethnicity and environmental factors, prompting further understanding of genetic risks and environmental differences across ethnic populations and its role in stroke outcomes, particularly post stroke seizures. This protocol represents a much larger study aiming at investigating the timing of novel oral anticoagulation after an acute ischaemic stroke in patients with atrial fibrillation on the rate of early haemorrhagic transformation and early embolic ischaemic stroke. A key secondary outcome is to assess the incidence of post stroke epilepsy at 1-year post stroke in these patients treated with novel oral anticoagulant therapy. Patient recruitment is due to close in early 2019.

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A registry of clinical and MRI outcomes following early versus late initiation of anticoagulation after ischaemic stroke or transient ischaemic attack in patients with atrial fibrillation

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Abstract

Background: Three clinical trials compared the efficacy and safety of oral anticoagulants (NOACs) versus warfarin, demonstrating favourable risk-benefit profiles. We aimed to investigate the timing of initiation of NOACs after recent stroke/TIA in patients with atrial fibrillation on the rate of early haemorrhagic transformation and early embolic ischaemic stroke. Our primary hypothesis is that patients initiated on oral anticoagulation within 7 days of stroke/TIA will have less recurrent infarction on MRI than patients initiated on an NOAC after 7 days.

Methods: This is prospective, multicentre, 3-month observational cohort study using a registry to examine 1-month MRI and 3-month clinical outcomes of patients initiated on NOAC or warfarin within 1-month after acute stroke. The primary study outcome is new ischaemic lesions on follow up MRI at 1-month. Secondary outcomes include new clinical ischaemic stroke by 90-day visit, and intracerebral haemorrhage on follow up MRI at 1-month. Based on the literature, the predicted new ischemic lesion rate on DWI if NOAC commenced within 7 days is 28%, and commenced after 7 days is 48%. Thus, a sample size of 248 patients was calculated, assuming 62 patients receive oral anticoagulation within 7 days and 186 patients beyond 7 days. Allowing a 10% dropout, we expect to recruit 70 patients within 7 days and 210 patients after 7 days.

Discussion: The optimal timing of commencement of anticoagulation after ischaemic stroke is an ongoing clinical dilemma, which this study will attempt to address.

Trial Registration: ID ISRCTN44617730

Introduction and Rationale

Atrial Fibrillation (AF) accounts for 18-28% of all strokes [1]. The risk of recurrent ischaemic stroke or transient ischaemic attack (TIA) following cardioembolism to the brain is high (between 10% and 20%) during the 90 days post initial event, with 50% of that risk present in the first week [2]. The recommendations for initiation of an anticoagulant after embolic stroke/TIA are unclear due to the potential risk of haemorrhagic transformation, with expert consensus suggesting deferral of anticoagulation from anywhere between 2-4 weeks post stroke/TIA [3].

Three randomised controlled trials compared the efficacy of oral anticoagulants (NOACs), dabigatran, rivaroxaban and apixaban, with a vitamin K antagonist (VKA), warfarin [4-6]. A meta-analysis of these trials demonstrated a favourable risk profile when compared to warfarin, with a 19% reduction in stroke or systemic embolic event (RR 0.81, 95% CI 0.73-0.91; $p<0.0001$, mainly driven by a reduction in haemorrhagic stroke (RR 0.49, 95% CI 0.38-0.64; $p<0.0001$) [7].

The key clinical trials of the NOACs excluded patients from 7 days to 6 months after stroke/TIA. The advantageous safety profile of an NOAC, however, makes it an attractive agent to investigate the balance between risk of haemorrhage versus benefit of

METHODS

Study Design

This is a prospective, 3-month observational cohort study using an established clinico-radiological stroke registry to examine clinical and MR imaging outcomes of patients initiated on NOAC (Apixaban, Rivaroxiban and Dabigatran, note that Edoxaban is currently unavailable in Australia) or VKA (Warfarin) within one month after acute stroke or TIA. Subjects will be analysed according to whether anticoagulant initiation was within 7 days, or after 7 days of stroke symptom onset. As this is an observational cohort study, collecting patients who are undergoing "usual care", the decision of when and what type of oral anticoagulant is used is at the discretion of the treating clinician.

Patient Population

Inclusion Criteria

1. Patients who present with an acute ischaemic stroke or TIA of cardioembolic (AF-related) origin and who have an MRI following their primary ischaemic event, and are deemed suitable for initiation of NOAC or VKA therapy.
2. Subjects must be enrolled within 30 days of symptom onset.

Exclusion Criteria

1. Evidence of primary intracranial haemorrhage.

prevention of early embolic ischaemic stroke if the anticoagulant was initiated earlier than consensus guidelines recommend (within 7 days of ictus onset). We aim to collect baseline and follow-up clinical, process of care, and MRI data from patients who are initiated on NOACs or VKAs within 1 month of acute ischaemic stroke/TIA, and examine the potential benefit of early (<7 days) versus later (>7 days) initiation.

We hypothesise that:

(i) Patients initiated on NOACs within 7 days of the stroke/TIA will have less recurrent infarction than patients initiated more than 7 days after their stroke/TIA;

(ii) There will be no difference in haemorrhagic transformation (HT) or new intra-cerebral haemorrhage (ICH) in patients initiated on NOAC within 7 days of the stroke/TIA compared to initiation after 7 days;

(iii) Patients initiated on NOAC within 7 days of the stroke/TIA will have less recurrent ischaemic events than patients initiated after 7 days.

2. Inability to have baseline and follow up MRI

Imaging and clinical data

The data collection will utilise current infrastructure from the INSPIRE registry (INternational Stroke Perfusion Imaging REgistry), which is a web-based registry for clinical and imaging data collection for patients after stroke thrombolysis. The registry (www.inspire.apollomit.com) allows participating sites to upload imaging data as well as enter clinical data to a secure server. Patients will be eligible if they have an MRI prior to initiation of anticoagulation. A repeat 30 day MRI will assess asymptomatic new lesions (infarction or ICH). Sequences required for baseline and follow up include: diffusion-weighted imaging (DWI), T1 sequence, FLAIR and gradient echo (GRE) or susceptibility weighted imaging (SWI).

Clinical data will include: patient demographics, pre-stroke history, previous medication history, in-hospital data (baseline and 24 hour National Institute of Health Stroke Score), reperfusion treatment, antiplatelet and anticoagulant treatment post stroke. Follow up information includes clinical evidence of recurrent ischaemic stroke, TIA, ICH, and the 3 month modified Rankin scale (mRS). Three-month outcomes will be recorded centrally by phone call from the coordinating centre. This includes a scripted, validated mRS assessment [8].

Central Review of Imaging

All imaging will be reviewed by principal investigators of the study. Investigators will be blinded to both anticoagulation type (NOAC versus warfarin) and time frame for initiation. Imaging review will follow a proforma that assesses ischaemic change as well as ICH.

Primary Outcome

- New ischaemic lesions on MRI at one month

Secondary Outcomes

- New clinical stroke within 90 days determined by clinic review or telephone follow up
- Intracerebral haemorrhage on MRI at one month
- mRS at 90 days determined by clinic review or telephone follow up
- Non-intracranial bleeding within 90 days
- Post stroke seizure occurrence at 1 year post stroke

Data Monitoring Body

This study received Australian national multicentre ethics approval by the Hunter New England Human Research Ethics Committee, HREC reference number: 16/02/17/4.01.

Management and governance will be overseen by the project Steering Committee, chaired by the principal investigators.

Sample Size Estimates

The expected event rate (new ischaemic lesions on MRI) for patients starting anticoagulants within 7 days is 28%. This estimate was based on two studies that reported a 28% ischaemic lesion recurrence rate on DWI at 2 weeks post stroke in patients administered with either Warfarin or NOAC [9, 10]. For the delayed initiation group (after 7 days post stroke), the expected ischaemic lesion recurrence rate on DWI was estimated at 48%, an average of multiple studies assessing new ischaemic lesions on DWI from 1 week to 3 months post acute stroke onset [11-13]. Using a two-tailed Chi-squared test comparing two independent proportions, a sample size of 62 patients in the earlier group (within 7 days) and 186 patients beyond 7 days was calculated, which yields 80% power. Assuming a 10% dropout rate, we plan to recruit 70 patients in the earlier group and 210 patients in the later group. Significance was set at an alpha 0.05. Analysis was performed using STATA IC (v13.1, StataCorp, College Station, TX, USA).

Statistical Analyses

The primary outcome, new ischaemic lesions on follow-up 30 day MRI, will be tested using multivariate regression techniques.

All continuous variables will be described as mean with standard deviation or median with interquartile range and analyses using either the paired or unpaired t test, the Mann Whitney U test or the Wilcoxon signed-rank test depending on the underlying data distribution and the relationship between sample populations.

Study Organisation and Funding:

The funding body (Bayer Pharmaceuticals) has provided financial support towards the establishment of the study database, central co-ordination and site recruitment costs including follow-up MR imaging. The funding body has/will have no role in the conduct of the study, analysis of data or publication/presentation of study results.

Discussion

The optimal timing of anticoagulation therapy initiation following acute ischaemic stroke should balance the risk of both early recurrent embolic stroke and HT of infarction. Currently, however, the optimal timing remains an important and

unanswered clinical question. There is limited consensus and no randomized data to guide clinicians on the timing of oral anticoagulation after stroke. The American Stroke Guidelines suggest that it is reasonable to initiate oral anticoagulation within 14 days after the onset of neurological symptoms, and in the presence of high risk for haemorrhagic conversion (larger infarcts, more severe stroke, HT on initial imaging or uncontrolled hypertension), to initiate oral anticoagulation after 14 days [3]. Other guidelines suggest the *1-3-6-12 Day Rule [14]*. Although consideration has been given to the conduct of a randomised trial comparing clinical and radiological outcomes in patients where anticoagulation is commenced earlier versus later, an observational registry is an alternative means of addressing the question. Additionaly, using MRI as a means to detect both new ischaemia and ICH allows us a much smaller sample size than a clinical registry. We acknowledge certain limitations in the study design. Due to the observational nature, patients with TIA or smaller non-disabling strokes will have less confounders and probably more likely to be in the earlier <7 days group. This potential bias, along with any confounding factors, may be accounted for using propensity score matching analyses.

Summary and Conclusion

This will be the only NOAC and VKA registry worldwide that collects baseline and follow-up imaging data. Results from this trial will provide critical insight into the optimal timing for initiation of anticoagulation after an ischaemic stroke/TIA

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Chapter 10

Objective 3 – Genetic Markers

This objective is of exploratory nature aiming to understand if genetics contribute to the development of seizures and epilepsy post ischaemic stroke.

In most epilepsies there is no known environmental cause (i.e.; stroke, tumour, CNS infections, head trauma) of the epilepsy (i.e. non-acquired epilepsies). Most of the genetic findings thus far have been identified in this patient group. However, even in patients with a known risk factor for epilepsy such as ischaemic stroke, only a subpopulation of patients develop epilepsy. Studies of epilepsy patients with ischaemic strokes suggest that, in addition to the primary ischaemic insult, there may be other underlying epileptogenic pathologies present. Factors that contribute to this risk for epileptogenesis in a given individual generally remain unknown. No antiepileptic treatments are available at present for patients at risk of developing epilepsy after brain insult, emphasising the need to understand cause-specific mechanisms that can be targeted towards epileptogenesis in individual patients.

Chapter 10 is an exploratory pilot study aiming at understanding whether in a population of acute ischaemic stroke patients, is there an association between genetic variants and the development of post stroke seizures.

This chapter has been prepared as a traditional thesis chapter.

10.1 Introduction

Even in patients with a known risk factor for epilepsy such as those who have suffered an ischaemic stroke, only a subpopulation of patients will develop epilepsy (1). Studies involving patients who have developed epilepsy post ischaemic stroke suggest that, in addition to the primary ischaemic insult, there may be other underlying epileptogenic pathologies that may have contributed to the development of epilepsy. However, the factors that contribute to this increased risk for epileptogenesis generally remain unknown. Further, currently there are no antiepileptic treatments available to treat patients at risk of developing epilepsy post stroke, emphasising the need to understand causative mechanisms that can be targeted to prevent the development of epileptogenesis in individual patients (1).

It has been estimated that approximately 30% of all epilepsies are of genetic origin, with more than 500 loci linked to epilepsy in human beings and mice (1). However it has been speculated that the genetic contribution to epilepsy is likely a lot higher than those reported, with only a small number of epilepsies (10-30% depending on epilepsy type) are Mendelian (single gene) disease, and the most common epilepsies having a complex pattern of inheritance, making them less likely to be identified in a clinical setting (2). Despite this, even when a cause for epilepsy is identified (e.g. a stroke), there is clear evidence that epilepsies develop only in some susceptible individuals, suggesting the presence of genetic modifiers and susceptibility alleles. According to some studies assessing post-traumatic epilepsies, the incidence of family history of epilepsy in patients with closed head trauma (i.e., stroke) who develop late seizures is 6-17%, compared with only 3-4% for individuals with no family history

(3). These findings strongly suggest that certain genetic factors may predispose these patients to the development of post-traumatic epilepsies (3). Accordingly, for patients who have had an ischaemic stroke and developed epilepsy, genetic factors may similarly contribute to the subsequent epileptogenesis.

Although only a few studies have investigated the hypothesised genetic link to post stroke epilepsy, the ALDH2 and CD40-1C/T polymorphisms have been identified and proposed to be involved. Specifically, Allele A of the rs671 polymorphism in a gene encoding mitochondrial aldehyde dehydrogenase 2 (ALDH2) has been associated with post stroke epilepsy in a Han Chinese population (4). This study showed a significant difference in the distribution of this allele and genotype frequencies of the rs671 polymorphism (A allele) on the ALDH2 gene, between patients who develop seizures post stroke and patients who do not (4). Additionally, a CD40-1C/T polymorphism has also been associated with post stroke epilepsy susceptibility in a Chinese group (5), with carriers of the T allele showing increased plasma sCD40L levels (i.e., oxidative stress) and a pro-thrombotic state (5). Currently these genes have not been investigated and validated in other populations. Additionally, there are known genetic determinants (PCDH2 and CAMSAP1L1) (6, 7) of common epilepsies. Understanding whether these variants are similarly associated with epilepsy development in a stroke population, or if the development of post stroke seizures is a result of the stroke injury alone is of additional interest to this study.

In contrast, the genetic link to stroke outcome, and the development of stroke comorbidities, has been widely investigated. For example, the APOE4 gene is associated with impairment of neuronal repair processes, and has been associated with

significantly poorer recovery over the first month post-stroke, as well as with a lower proportion of patients with minimal or no disability at 3 months post stroke (8). Similarly, the IGF1 locus affects brain plasticity after brain injury and a polymorphism at the IGF1 locus (rs7136446) has been associated with good functional outcome at 3 months post stroke (1). As stroke severity and the persistence of stroke disability are risk factors for the development of post stroke seizures, variants such as IGF1 and APOE4 could be potential candidates for further exploration in regards to the development of seizures post stroke. So far, no literature has been published on the influence of genetics on stroke progression and the development of subsequent seizures.

By identifying genetic markers in patients at a higher risk of developing post stroke seizures, there is potential to better design and develop trials that explore the anti-epileptogenic properties of specific agents, including AEDs, in a higher risk population. This chapter is a pilot study that will explore the associations between genetic biomarkers and the development of post stroke seizures.

10.2 Exploratory Aim and Hypotheses

10.2.1 Exploratory Aim

We aimed to explore if, in a population of acute ischaemic stroke patients, there was an association between genetic variants and the development of post stroke seizures.

10.2.2 Exploratory Hypotheses

1. In an ischaemic stroke population, are known epilepsy variants associated with the development of post stroke seizures (ie- variants directly associated with epilepsy)?
2. There have been two variants (ALDH2 and CD40) that have been associated with reduced likelihood of post stroke seizure development in a Han Chinese cohort. Are these variants associated with reduced likelihood of post stroke epilepsy in a non-Chinese cohort and can we replicate this in our Chinese cohort?
3. There are variants that predispose a patient to poorer stroke outcomes/ greater stroke severity that are also associated with post stroke seizure development

10.3 Methods

10.3.1 Study Design and Participants

This was an exploratory, multi-centre, case-control pilot study. Five centres were included in this study including two in China, two in Australia and one in Brazil. The centres were as follows: Prince of Wales Hospital in Hong Kong, Jinling Hospital in Nanjing China, the University of Campinas, São Paul, Brazil, the John Hunter Hospital in Australia and the Royal Melbourne Hospital in Australia.

Controls were patients who were admitted for an ischaemic stroke at the respective hospital who did not develop post stroke seizures within two years. Cases were ischaemic stroke patients admitted at the respective hospitals who did develop post stroke seizures within two years. This cut-off was chosen based on previous findings that suggested that the highest risk of seizure development was within the first year (9). Occurrence of ischaemic stroke was ascertained by imaging and subtyped by specialised stroke neurologists before being entered into the respective centres databanks. Occurrence of post stroke seizures was ascertained by reviewing a seizure questionnaire modified from that used in our center previously, and from a validated screening questionnaire (Keezer MR, et al. Epilepsia 2014, 55:1763-1771). Responses were then corroborated with the patient's hospital medical records and records from their primary physicians. Events were recorded as seizures if the symptoms included motor or autonomic components, with or without impairment of consciousness, as defined by the International League Against Epilepsy (10). If discrepancies were found between patient recall and medical records/notes, final determination was made

by an epileptologist. This questionnaire was translated into Chinese for use at the centres in China and also into Portuguese for centres in Brazil.

10.3.2 Sample Size Estimation

This was an exploratory, pilot study and thus sample size was not estimated a priori. Due to the multiplicity of our comparisons, multiplicity corrections at the level of the hypothesis was applied to control for type 1 error. Despite that, our emphasis is on effect size and thus results are not to be considered confirmatory.

10.3.3 Genetic data collection, processing and testing

Please refer to the *General Methods* (Chapter 3) section.

10.3.4 Statistical Analyses

For the purpose of analyses, patients were divided into 3 ethnic groups. The first group were those recruited from two centres in China, the second from two Australian centres and the third from one centre in Brazil. Ethnicity was defined as country of birth and patients were removed if not in concordance with the centre they presented at. Baseline and follow-up stroke data are presented as count (proportion) for categorical variables, and as median (interquartile range) for continuous variables. Statistical analysis plans are provided for each hypothesis. For each hypothesis, proportions of genotypes within each ethnicity (case/control) are provided in table form at the beginning of each section. These analyses were conducted on PLINK

version 1.09 and data were converted to plink format (.ped and .map) files. A p-value of less than 0.05 was considered significant at the level of the hypothesis, however Bonferroni thresholds were additionally applied and reported. From here, within ethnic group association tests were conducted using the **–model fisher** command, which includes the Fisher's test, 1df dominant gene action, 1df recessive gene action, 2df genotypic and Cochrane-Armitage trend tests. Consequently, only the Fisher's test and the Cochrane-Armitage results have been reported. Across group tests were conducted using the **-mh** command, which provides a Cochrane-Mantel-Haenszel test based on an average odds ratio to control for potential confounders due to ethnicity.

For each hypothesis, a logistic regression model testing the association between variant and outcome was performed, with adjustment for covariates as described below. These analyses were conducted using STATA IC (v13.1, StataCorp, College Station, TX, USA). Due to the retrospective nature of data collection, only age of patient at stroke onset was available for the Prince of Wales, Hong Kong patients. Because of this, analyses were conducted with adjustment for age only for China (Hong Kong and Nanjing), and then a sensitivity analysis within Nanjing patients alone was performed to allow adjustment for multiple covariates when required.

Hypothesis One:

To test the association between known epilepsy variants (CAMPSAP1L1, PCDH7) and post stroke seizure development, a Fisher's Exact test (and Cochrane-Armitage trend) and a logistic regression model with adjustment for multiple covariates was performed. The covariates included baseline stroke severity score (NIHSS), presence of atrial fibrillation (AF), stroke outcome mRS0-2, haemorrhagic transformation

(HT), treatment with intra-venous tissue plasminogen activator (IV-tPA) and age at stroke onset. These covariates were chosen due to their known association with post stroke seizures *(Refer to Literature Review Chapter 2)*.

Hypothesis Two:

To validate the association between known post stroke epilepsy variants in a Han Chinese population (ALDH2, CD40) and post stroke seizures and then to test this association in another population, a Fisher's Exact test (and Cochrane-Armitage trend) and a logistic regression model with adjustment for multiple covariates was performed. The covariates include NIHSS, AF, mRS0-2, HT, IV-tPA and age.

Hypothesis three:

For part 1, both a Fisher's Exact test (and Cochrane-Armitage trend) and a logistic regression model with adjustment for age were used to test the association between genotype (APOE, IGF1, 4q25) and either stroke severity (as measured by the NIHSS), good stroke outcome (as measured by mRS 0-2) or atrial fibrillation. These models were adjusted for covariates known to be associated with each outcome (refer to Chapter 1).

Outcome AF: adjusted for age

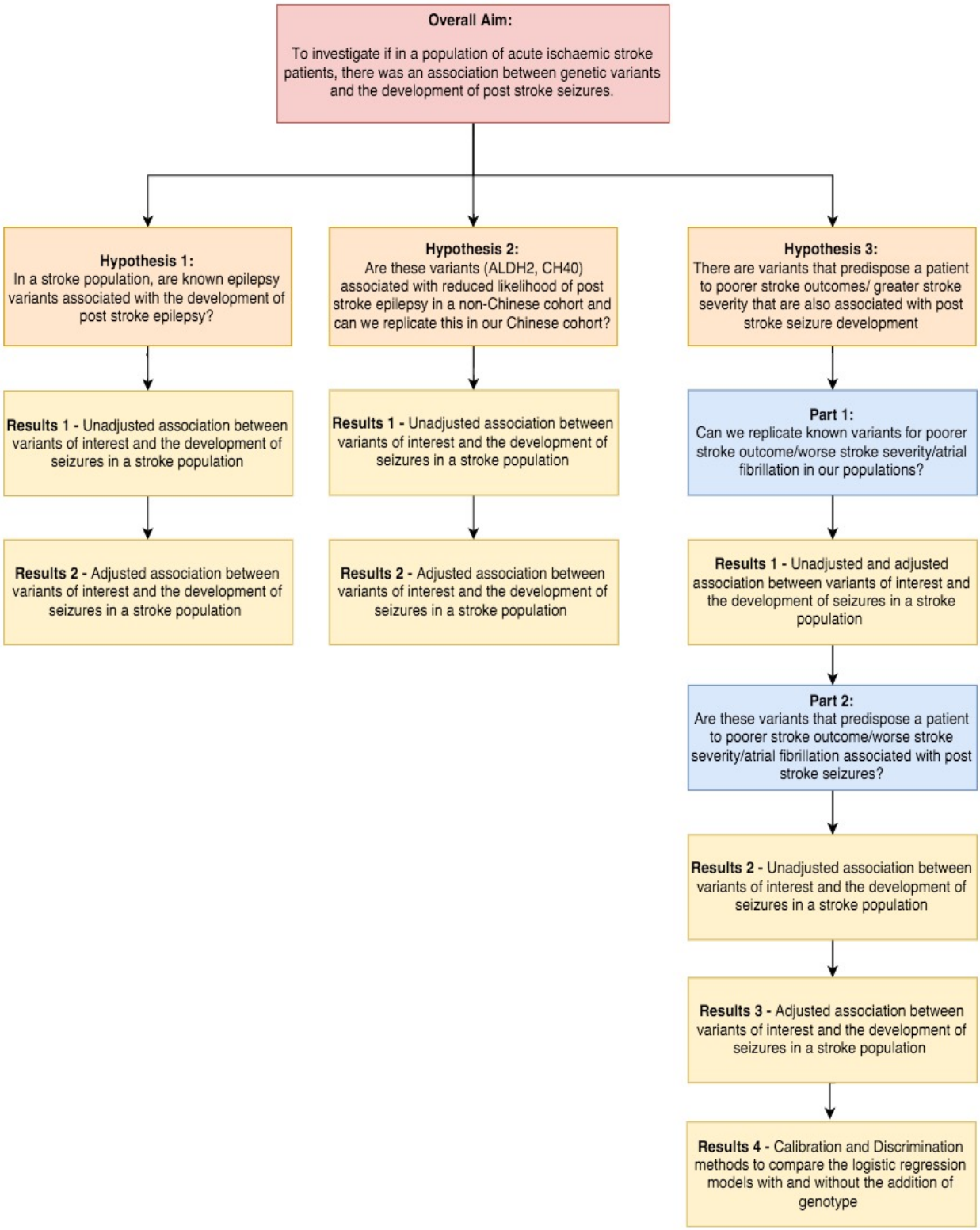
Outcome NIHSS: adjusted for age, AF, IV-tPA treatment

Outcome mRs0-2: adjusted for age, AF, NIHSS, IV-tPA treatment, HT

For part 2, to test the association between genotype and post stroke seizures, both a Fisher's Exact test (and Cochrane-Armitage trend) and a logistic regression model

was used with adjustment for age and stroke phenotype. The covariates include NIHSS, AF, mRS0-2, HT, IV-tPA treatment and age at stroke onset. We then compared the prognostic accuracy of adding genetic information into the logistic regression models with models not containing genetic information using calibration methods (receiver operating characteristic (ROC) analyses with area under curve (AUC)). We also used discrimination methods to calculate the Bayesian Information Criterion for these logistic regression models testing the association of genetic variants on post stroke seizure development, adjusting for age. The comparisons of the logistic regression models (**logit)**, receiver-operating characteristic curves **(lroc)** and **(roccomp)** and Bayesian information criterion models **(estat ic)** were conducted using STATA IC (v13.1, StataCorp, College Station, TX, USA).

10.3.5 Diagrammatic Outline of Analyses



10.6 Results

10.6.1 Patient Characteristics

A total of 171 cases and 384 controls were identified and included in the genetic testing and subsequent analyses. In the Australian sample, there were a total of 164 patients included, of which 59 were cases and 105 were controls; median (IQR) age of cases were 62 (48-72) and controls were 63 (51-71). In the Chinese sample, there were a total of 318 patients, of which 90 were cases and 228 were controls; median (IQR) age of cases were 62 (48-76) and controls were 59 (46-72). In the Brazilian sample, there were a total of 73 patients included, of which 22 were cases and 51 were controls (Table 1); median (IQR) age of cases were 63 (50-70) and controls were 62 (54-71).

The percentage females for each sample were as follows: Australian cases, 68%, controls, 67%; Chinese cases, 61%, controls, 59% cases; Brazilian cases, 77% females, controls 48%.

The median (IQR) NIHSS for Australia cases were 11 (4-19) and controls 4 (2-9), Chinese cases were 8 (2-12) and controls 2 (0-2) and Brazilian cases were 5 (3-8) and controls 8 (4-16) (Table 3).

The variants available at each source are provided in Table 2. The gnomAD for each variant are provided in Table 4 and the allele frequencies across the three populations are provided in Table 5.

Table 1: A breakdown of case/control patients from each group

	Case Ischaemic stroke patients who developed epilepsy within 2 years	**Control** Ischaemic stroke patients who did not develop epilepsy within 2 years	**Total**
Group 1 *(Royal Melbourne Hospital and John Hunter Hospital)*	59	105	164
Group 2 *(Nanjing Hospital and Prince of Wales Hospital)*	90	228	318
Group 3 *(University of Campinos- Brazil)*	22	51	73
Total	171	384	555

Table 2: Total contributing centres per variant

Variant	Number of contributing centres	Missing centre
rs2292096	5	-
rs6660197	5	-
rs671	4	John Hunter Hospital (Group 1)
rs2200733	4	University of Campinos (Group 3)
rs28498976	5	-
rs7136446	5	-
rs429358	5	-
rs7412	5	-
rs1883832	5	-

Table 3: Baseline and follow up data across groups for cases and controls

	Australian		Chinese		Brazilian	
	Case, n=59	Control n=105	Case n=90 Nanjing and HK n=27 Nanjing only	Control n=228 Nanjing only	Case n=22	Control n=58
Age Median (IQR)	62 (48-72)	63 (51-71)	62 (48-76) Nanjing and HK	59 (46-72) Nanjing and HK	63 (50-70)	62 (54-71)
Sex (n, % female)	40 (68)	70 (67)	53 (59) Nanjing and HK	140 (61) Nanjing and HK	17 (77)	28 (48)
NIHSS Median (IQR)	11 (4-19)	4 (2-9)	8 (2-12) Nanjing only	2 (0-4) Nanjing only	5 (3-8)	8 (4-16)
Atrial Fibrillation (n, %)	8 (14)	5 (5)	4 (14.8) Nanjing only	11 (5) Nanjing only	3 (14)	9 (16)
IV-tPA treatment (n, %)	14 (24)	13 (12)	0 (0) Nanjing and HK	0 (0) Nanjing and HK	2 (9)	17 (29)
Haemorrhagic transformation (n, %)	5 (8)	3 (3)	-* Nanjing and HK	-* Nanjing and HK	1 (5)	8 (14)
mRS (02) (n, %)	24 (41)	65 (62)	10 (37.0) Nanjing only	157 (69) Nanjing only	14 (64)	29 (50)

**data unavailable*

Table 4: gnomAD information for each variant

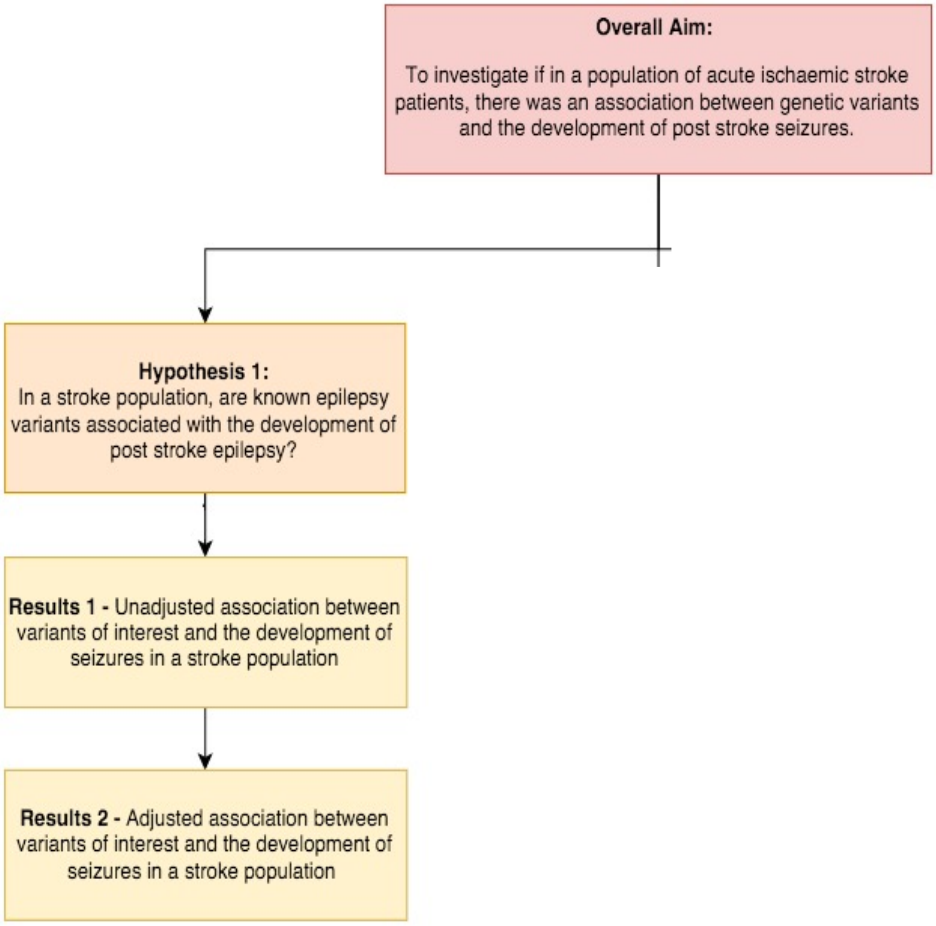
rs number	Ref_Alt	gnomAD
rs1883832	C/T	20-44746982-T-C
rs2200733	C/T	4-111710169-C-T
rs2292096	A/G	1-200826769-A-G
rs28498976	G/A	4-31151357-G-A
rs429358	T/C	19-45411941-T-C
rs6660197	C/T	1-200703541-C-T
rs671	A/G	12-112241766-G-A
rs7136446	T/C	12-102838515-C-T
rs7412	C/T	19-45412079-C-T

Table 5: Allele frequencies across populations

Allele Frequencies- GnomAD			
Population	Non Finnish European	East Asian	Latino
rs1883832	0.742	0.5678	0.8038
rs2200733	0.1578	0.532	0.2392
rs2292096	0.1145	0.1787	0.1663
rs28498976	0.3796	0.4814	0.3096
rs429358	0.1489	0.08854	0.1041
rs6660197	0.1141	0.1995	0.1619
rs671	0.00002444	0.2565	0.0003903
rs7136446	0.6006	0.829	0.756
rs7412	0.07669	0.07669	0.03175

10.7 Hypothesis 1 Results:

In a stroke population, are known epilepsy variants associated with the development of post stroke?



Variants of interest:

- PCDH7 – rs28498976
- CAMPSAP1L1 – rs6660197
- CAMPSAP1L1 – rs2292096

10.7.1 Unadjusted association between variants of interest and post stroke seizures in the different populations

Table 6- Australia

rs28498976	AA (Dose 0)	GA (Dose 1)	GG (Dose 2)
Case	6	31	22
Control	13	50	42
			p-value <0.99
rs6660197	AA (Dose 0)	AG (Dose 1)	GG (Dose 2)
Case	51	7	1
Control	80	23	2
			p-value = 0.20
rs2292096	CC (Dose 0)	CT (Dose 1)	TT (Dose 2)
Case	51	7	4
Control	80	23	2
			p-value = 0.20

**p-value represents Fisher's Exact test for genotype dose 0 versus dose 1+2*
**Bonferroni corrected significance threshold for this analysis (alpha=0.017)*

Table 7- China

rs28498976	CC (Dose 0)	TC (Dose 1)	TT (Dose 2)
Case	21	44	21
Control	52	122	54
			p-value = 0.93
rs2292096	AA (Dose 0)	AG (Dose 1)	GG (Dose 2)
Case	63	26	1
Control	153	63	12
			p-value = 0.31
rs6660197	CC (Dose 0)	CT (Dose 1)	TT (Dose 2)
Case	61	26	3
Control	151	65	12
			p-value = 0.65

**p-value represents Fisher's Exact test for genotype dose 0 versus dose 1+2*
**Bonferroni corrected significance threshold for this analysis (alpha=0.017)*

Table 8- Brazil

rs28498976	AA (Dose 0)	GA (Dose 1)	GG (Dose 2)
Case	8	12	2
Control	7	25	19
			p-value =0.006
rs2292096	AA (Dose 0)	AG (Dose 1)	GG (Dose 2)
Case	15	7	1
Control	35	14	2
			p-value = 0.82
rs6660197	CC (Dose 0)	CT (Dose 1)	TT (Dose 2)
Case	17	5	1
Control	35	15	1
			p-value <0.99

**p-value represents Fisher's Exact test for genotype dose 0 versus dose 1+2*
**Bonferroni corrected significance threshold for this analysis (alpha=0.017)*

10.7.2 Adjusted association between variants of interest and post stroke seizures

PCDH7 and post stroke seizures

In Australia, no significant association between ***PCDH7*** rs28498976 and post stroke seizures was found; adjusted Odd's Ratio (aOR) 0.89, p=0.68, 95% CI (0.51-1.55), after adjustment for (age, intra-IV-tPA, NIHSS, mRS0-2, haemorrhagic transformation (HT))

In China, no significant association between ***PCDH7*** rs28498976 and post stroke seizures was found; aOR 0.89, p=0.60, 95% CI (0.46-1.6), after adjustment for age. When only patients from Nanjing were analysed, with adjustment for age, NIHSS, mRS0-2, no association between ***PCDH7*** rs28498976 and post stroke seizures was found: aOR 0.91, p=0.76, 95% CI (0.49-1.7).

In Brazil, a significant association between ***PCDH7*** rs28498976 and post stroke seizures was found; aOR 5.3, p=0.005, 95% CI (1.7-16.8), after adjustment for age,

IV-tPA, NIHSS, mRS0-2, HT and AF. CMH test for 2 groups: OR 1.16, p=0.23, 95% CI (0.9-1.5).

CAMPSAP1L1 and post stroke seizures

In Australia, no significant association between ***CAMPSAPILI*** rs6660197 and post stroke seizures was found; aOR 0.74, p=0.5, 95% CI (0.32-1.7), after adjustment for age, IV-tPA, NIHSS, mRS0-2, HT.

In China, no significant association between ***CAMPSAPILI*** rs6660197 and post stroke seizures was found; aOR 1.05, p=0.89, 95% CI (0.52-2.1), after adjustment for age. When only patients in Nanjing were analysed, with adjustment for age, NIHSS, mRS0-2, no association between ***CAMPSAPILI*** rs6660197 and post stroke seizures was found: aOR 0.89, p=0.76, 95% CI (0.43-1.9).

In Brazil, no significant association between ***CAMPSAPILI*** rs6660197 and post stroke seizures was found; aOR 1.2, p=0.74, 95% CI (0.37-4.0), after adjustment for age, NIHSS, mRS0-2, HT, IV-tPA.

CAMPSAP1L1 and post stroke seizures

In Australia, no significant association between ***CAMPSAPILI*** rs2292096 and post stroke seizures was found; aOR 0.74, p=0.5, 95% CI (0.32-1.7), after adjustment for (age, IV-tPA, NIHSS, mRS0-2, HT.

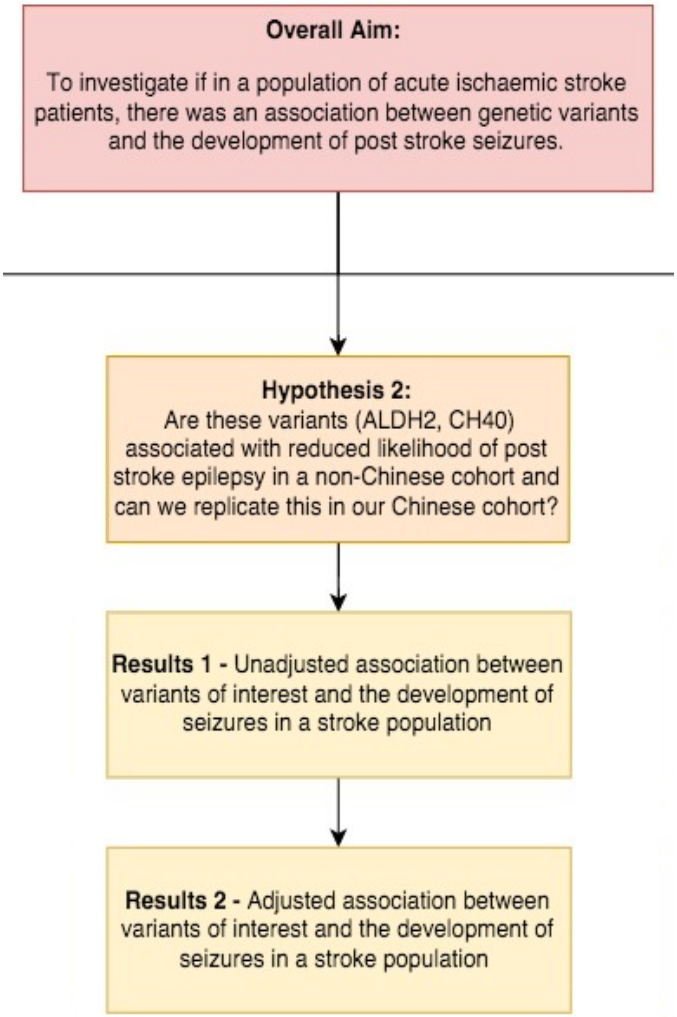
In China, no significant association between ***CAMPSAPILI*** rs2292096 and post stroke seizures was found; aOR 1.0, p=0.9, after adjustment for age. When only patients from Nanjing were analysed, with adjustment for age, NIHSS, mRS0-2, no

association between ***CAMPSAPILI*** rs2292096 and post stroke seizures was found: aOR 0.91, p=0.79, 95% CI (0.43-1.9).

In Brazil, no significant association between ***CAMPSAPILI*** rs2292096 and post stroke seizures was found; aOR 1.7, p=0.36, 95% CI (0.56-5.0), after adjustment for age, IV-tPA, NIHSS, mRS0-2, HT, IV-tPA.

10.8 Hypothesis 2 Results:

There have been two variants that have been associated with post stroke seizure development in a Han Chinese cohort. Are these variants associated with post stroke epilepsy in a non-Chinese cohort and can we replicate this in our Chinese cohort?



Variants of interest:

- ALDH2 – rs671
- CD40 – rs1883832

10.8.1 Unadjusted association between variants of interest and post stroke seizures in the different populations

Table 9- Australia

rs1883832	CC (Dose 0)	TC (Dose 1)	TT (Dose 2)
Case	29	27	3
Control	55	41	9
			p-value <0.99

**p-value represents Fisher's Exact test for genotype dose 0 versus dose 1+2*
** ALDH2 rs671 unavailable in Australia*
**Bonferroni corrected significance threshold for this analysis (alpha=0.025)*

Table 10- China

rs671	GG (Dose 0)	AG	AA
Case	12	50	28
Control	7	69	152
			p-value = 0.03
rs1883832	CC (Dose 0)	TC (Dose 1)	TT (Dose 2)
Case	35	44	11
Control	93	101	34
			p-value = 0.93

**p-value represents Fisher's Exact test for genotype dose 0 versus dose 1+2*
**Bonferroni corrected significance threshold for this analysis (alpha=0.025)*

Table 11- Brazil

rs671	GG (Dose 0)	AG	AA
Case	23	0	0
Control	51	0	0
			p-value <0.99
rs1883832	CC (Dose 0)	TC (Dose 1)	TT (Dose 2)
Case	35	44	11
Control	93	101	34
			p-value = 0.84

**p-value represents Fisher's Exact test for genotype dose 0 versus dose 1+2*
**Bonferroni corrected significance threshold for this analysis (alpha=0.025)*

10.8.2 Adjusted association between variants of interest and post stroke seizures

ALDH2 and post stroke seizures

Variant unavailable in Australian sample.

In China, a significant association between ***ALDH2*** rs671 and reduced likelihood of post stroke seizures was found; Cochrane-Armitage trend p=0.02, aOR 0.587, p=0.036, 95% CI (1.032-2.44), adjusted for age. In the Nanjing only population, no significant association was found between ***ALDH2*** rs671 and post stroke seizures when additionally adjusted for age, NIHSS and mRS0-2: aOR 0.62, p=0.3, 95% CI (0.25-1.6).

In Brazil, no significant association between ***ALDH2*** rs671 and post stroke seizures was found; Fisher's Exact (FE) p<0.99.

CMH test for 2 groups: OR 1.02, p=0.93, 95% CI (0.67-1.6).

CD40 and post stroke seizures

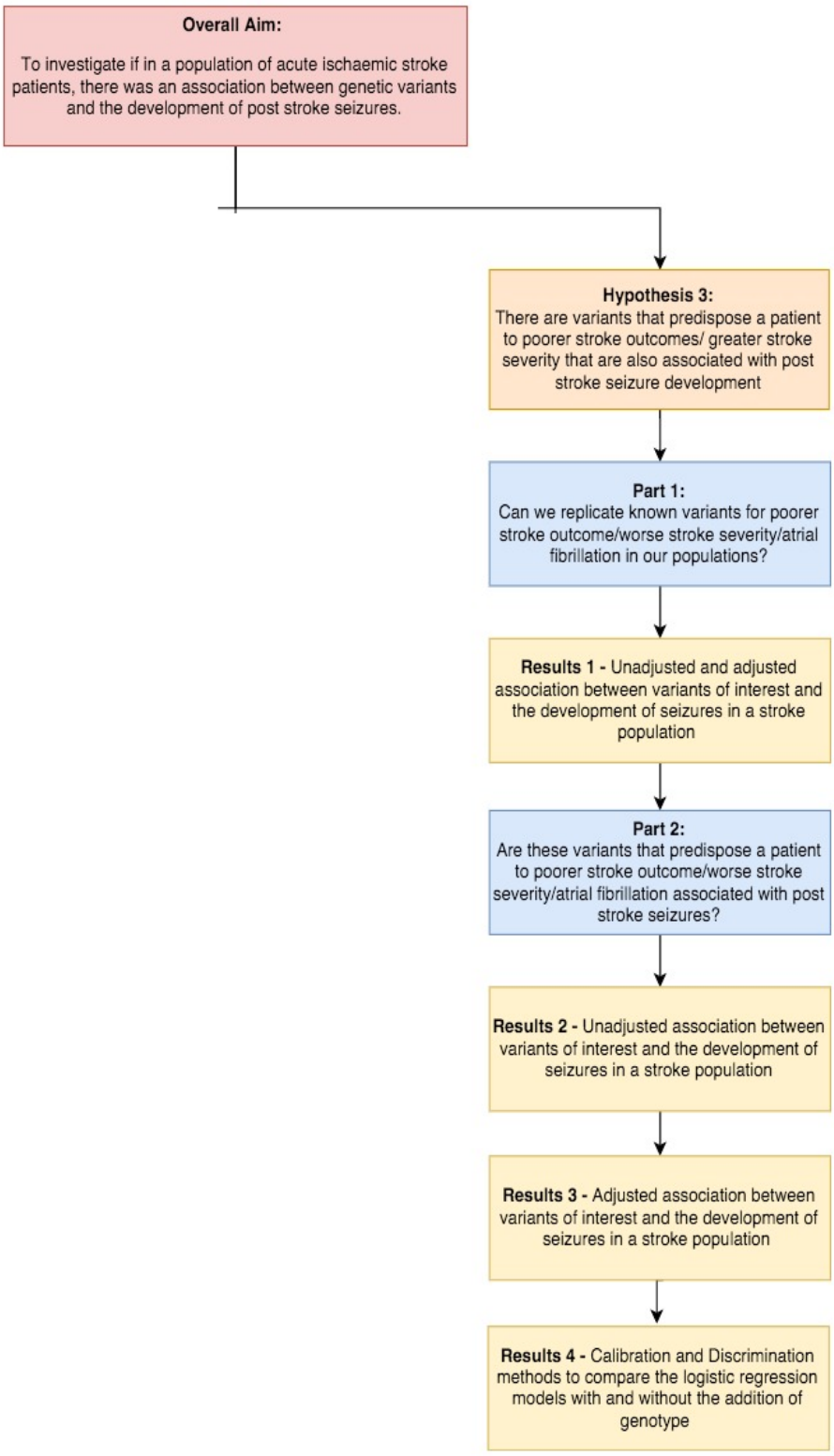
In Australia, no significant association between ***CD40*** rs1883832 and post stroke seizures was found; FE p<0.99.

In China, no significant association between ***CD40*** rs1883832 and post stroke seizures was found; aOR 1.0, p<0.99, 95% CI (0.18-0.69), adjusted for age. In the Nanjing only population, approaching significance was found between ***CD40*** rs1883832 and post stroke seizures when additionally adjusted for age, NIHSS and mRS0-2: aOR 0.5, p=0.06, 95% CI (0.25-1.03).

In Brazil, no significant association between ***CD40*** rs1883832 and post stroke seizures was found; FE p= 0.8.

10.9 Hypothesis 3 Results:

Are variants that predispose a patient to poorer stroke outcomes/ greater stroke severity associated with post stroke seizure development?



Variants of interest:

- APOE4 – rs429358
- APOE4 – rs7412
- IGF1- rs7136446
- 4q25 – rs2200733

Part 1:

Can we replicate known variants for poorer stroke outcome/worse stroke severity/atrial fibrillation?

10.9.1 Unadjusted and adjusted association between genotype and stroke phenotypes (poorer stroke outcome/greater stroke severity/atrial fibrillation)

**Bonferroni corrected thresholds for significance*
-For mRS0-2 outcome (alpha=0.025)
-For NIHSS outcome (alpha=0.05)
-For AF outcome (alpha=0.025)

APOE4 and good stroke outcome (mRS0-2):

In Australia, there was a significant association between APOE rs429358 and reduced likelihood of good stroke outcome (mRS0-2); Fisher's Exact (FE) p=0.03, logistic regression with adjustment for age: aOR 0.40, p= 0.03, 95% CI (0.17-0.92). When additionally adjusted for age, IV-tPA, NIHSS and HT, this association remained significant: OR: 0.23, p=0.016, 95% CI (0.068-0.76).

In Nanjing, no significant association was found between APOE rs429358 and reduced likelihood of good stroke outcome (mRS0-2), with adjustment for age and NIHSS aOR 0.91, p=0.92, 95% CI (0.146-5.69).

In Brazil, no significant association between APOE rs429358 and reduced likelihood of good stroke outcome (mRS0-2) was found; FE p=0.82, aOR 1.2, p=0.70, 95% CI

(0.47-3.1), when adjusted for age. When additionally adjusted for age, IV-tPA, NIHSS and HT, no significance was found: aOR 0.74, p=0.61, 95% CI (0.23-2.4).

**Bonferroni corrected threshold for significance (alpha=0.025)*

APOE4 and atrial fibrillation:

In Australia, no significant association between APOE rs7412 and presence of atrial fibrillation was found; FE p=0.79, aOR 3.23, p=0.19, 95% CI (0.56-18.7), with adjusted for age.

In Nanjing, a significant association between APOE rs7412 and presence of atrial fibrillation was found; FE p=0.005, aOR 5.2, p=0.004, 95% CI (1.04-1.2), with adjustment for age.

In Brazil, no significant association between APOE rs7412 and presence of atrial fibrillation was found; FE p=0.30 aOR 1.9, p=0.32, 95% CI (0.5-7.0), with adjustment for age.

**Bonferroni corrected threshold for significance (alpha=0.025)*

IGF1 and good stroke outcome:

In Australia, there was a significant association between IGF1 rs7136446 and increased likelihood of good stroke outcome (mRS 0-2); FE p=0.03, aOR 1.433, p=0.01, 95% CI (0.23-0.84), with adjustment for age. When additionally adjusted for age, IV-tPA, NIHSS and HT: aOR 0.26, p=0.01, 95% CI (0.094-0.74).

In Nanjing, no association between IGF1 rs7136446 and post stroke seizures with adjustment for age, NIHSS; aOR 2.56, p=0.068, 95% CI (0.92-7.01) was found.

In Brazil, no significant association between IGF1 rs7136446 and reduced likelihood of good stroke outcome (mRS 0-2) was found; FE p=0.71, aOR 1.2, p=0.7, 95% CI

(0.54-2.6), with adjustment for age. When additionally adjusted for age, IV-tPA, NIHSS and HT: aOR 1.1, p=0.83, 95% CI (0.43-2.85).

**Bonferroni corrected threshold for significance (alpha=0.025)*

IGF1 and stroke severity:

In Australia, no significant association between IGF1 rs7136446 and decreased stroke severity was found; FE p= 0.5, aOR 1.3, p= 0.62. 95% CI (-2.2-1.3), with adjustment for age and atrial fibrillation.

In Nanjing, no significant association between IGF1 rs7136446 and decreased stroke severity was found; FE p=0.1, aOR 1.3, p= 0.1 95% CI (-2.7-0.216), with adjustment for age.

In Brazil, no significant association between IGF1 rs7136446 and decreased stroke severity was found; FE p=0.5, aOR 0.55, p= 0.66, 95% CI (-1.91-3.007), with adjustment for age and AF.

**Bonferroni corrected threshold for significance (alpha=0.05)*

4q25 and presence of atrial fibrillation:

In Australia, a non-significant trend was found between 4q25 rs2200733 and presence of atrial fibrillation; FE p=0.05, aOR 1.7, p= 0.06, 95% CI (0.33-9.1), with adjustment for age.

In Nanjing, there was a significant association between 4q25 rs2200733 and presence of atrial fibrillation; FE p=0.003, aOR 4.1, p=0.005, 95% CI (1.6-10.9), with adjustment for age.

This variant was unavailable in Brazil.

**Bonferroni corrected threshold for significance (alpha=0.025)*

Part 2:

Are these variants that predispose a patient to poorer stroke outcome/worse stroke severity/atrial fibrillation associated with post stroke seizures?

10.9.2 Unadjusted association between variants of interest and the development of seizures in a stroke population in the different populations

Table 12: Australia

rs429358	CC (Dose 0)	TC (Dose 1)	TT (Dose 2)
Case	1	19	39
Control	2	57	46
			p-value =0.025
rs7412	CC (Dose 0)	CT (Dose 1)	TT (Dose 2)
Case	32	17	0
Control	64	41	0
			p-value = 0.29
rs7136446	CC (Dose 0)	CT (Dose 1)	TT (Dose 2)
Case	10	28	20
Control	15	56	34
			p-value <0.99
rs2200733	CC (Dose 0)	CT (Dose 1)	TT (Dose 2)
Case	49	5	4
Control	80	24	1
			p-value =0.86

**p-value represents Fisher's Exact test for genotype dose 0 versus dose 1+2*
**Bonferroni corrected threshold for significance (alpha=0.0125)*

Table 13: China

rs429358	CC (Dose 0)	TC (Dose 1)	TT (Dose 2)
Case	3	18	69
Control	2	35	191
			p-value =0.077
rs7412	CC (Dose 0)	CT (Dose 1)	TT (Dose 2)
Case	80	9	1
Control	203	23	2
			p-value <0.99
rs7136446	CC (Dose 0)	CT (Dose 1)	TT (Dose 2)
Case	4	25	60
Control	6	56	166
			p-value = 0.28
rs2200733	CC (Dose 0)	CT (Dose 1)	TT (Dose 2)

Case	25	44	18
Control	57	108	63
			p-value =0.25

**p-value represents Fisher's Exact test for genotype dose 0 versus dose 1+2*
**Bonferroni corrected threshold for significance (alpha=0.0125)*

Table 14: Brazil

rs429358	CC (Dose 0)	TC (Dose 1)	TT (Dose 2)
Case	1	5	16
Control	1	18	32
			p-value=0.65
rs7412	CC (Dose 0)	CT (Dose 1)	TT (Dose 2)
Case	17	4	1
Control	41	10	0
			p-value = 0.57
rs7136446	CC (Dose 0)	CT (Dose 1)	TT (Dose 2)
Case	4	10	8
Control	6	25	20
			p-value= 0.71

**p-value represents Fisher's Exact test for genotype dose 0 versus dose 1+2*
**4q25 rs2200733 unavailable in Brazil*
**Bonferroni corrected threshold for significance (alpha=0.0125)*

10.9 Adjusted association between variants of interest and the development of seizures in a stroke population

APOE4 rs429358 and post stroke seizures:

In Australia, a significant association between *APOE4 rs429358* and post stroke seizures was found; Cochrane-Armitage trend p=0.009, aOR 1.4, p=0.005, 95% CI (1.7-4.4) with adjustment for age and IV-tPA. However, when other covariates were added to the model (age, IV-tPA, NIHSS, mRS0-2, HT and AF), no significance was found; aOR 0.9, p=0.76, 95% CI (0.34-2.17).

In China, no significant association between *APOE4 rs429358* and post stroke seizures was found; aOR 1.59, p=0.08, 95% CI (0.94-2.97), with adjustment for age.

In patients only from the Nanjing centre, no significant association between *APOE4 rs429358* and post stroke seizures was found, with adjustment for age, NIHSS and mRS0-2; aOR 0.42, p=0.41, 95% CI (0.05-3.2).

In Brazil, no significant association between *APOE4 rs429358* and post stroke seizures was found; aOR 0.72, p= 0.52, 95% CI (0.32-02.1), with adjustment for age. aOR 0.7, p=0.55, 95% CI (0.215-2.26), with adjustment for age, IV-tPA, NIHSS, mRS0-2, HT, AF.

The differences in the genotype and allele frequencies between the groups were not significant, OR: 0.9, p= 0.6.

Cochrane-Mantel-Haenszel (CMH) test for 2 groups: OR 0.92, p=0.69, 95% CI (0.60-1.38). CMH test for 3 groups: OR 0.891, p=0.58, 95% CI (0.61-1.29).

APOE4 rs7412 and post stroke seizures:

In Australia, no significant association between *APOE4 rs7412* and post stroke seizures was found; aOR 0.6, p=0.24, with adjustment for age and IV-tPA. aOR 1.2, p=0.75, 95% CI (0.457-2.97), with adjustment for age, IV-tPA, NIHSS, mRS0-2, HT and AF.

In China, no significant association between *APOE4 rs7412* and post stroke seizures was found; aOR 0.97, p=0.93, with adjustment for age. In patients only from the Nanjing centre, no significant association between *APOE4 rs7412* and post stroke seizures was found, with adjustment for age, NIHSS and mRS0-2; aOR 1.5, p=0.5, 95% CI (0.46-5.12).

In Brazil, no significant association between *APOE4 rs7412* and post stroke seizures was found; aOR 1.6, p=0.41. aOR 1.3, p=0.75, 95% CI (0.289-5), with adjustment for age, IV-tPA, NIHSS, mRS0-2, HT, AF.

IGF1 and post stroke seizures

In Australia, no significant association between ***IGF1*** rs7136446 and post stroke seizures was found; aOR 1.1, p=0.8, adjusted for age and IV-tPA. When other covariates were added to the model (age, IV-tPA, NIHSS, mRS0-2, HT and AF), no significance was found; aOR 1.2, p=0.71, 95% CI (0.56-2.325).

In China, no significant association between ***IGF1*** rs7136446 and post stroke seizures was found; aOR 1.2, p=0.37, with adjustment for age. In patients only from the Nanjing centre, no significant association between ***IGF1*** rs7136446 and post stroke seizures was found, with adjustment for age, NIHSS and mRS0-2; aOR 0.80, p=0.73, 95% CI (0.23-2.75).

In Brazil, no significant association between ***IGF1*** rs7136446 and post stroke seizures was found; aOR 1.3, p=0.5. aOR 0.97, p=0.9, 95% CI (0.38-2.5), with adjustment for age, IV-tPA, NIHSS, mRS0-2, HT, AF)

4q25 and post stroke seizures

In Australia, no significant association between ***4q25*** rs2200733 and post stroke seizures was found; aOR 0.8, p=0.55, 95% CI (0.41-1.6), with adjustment for age and IV-tPA. However, when other covariates were added to the model (age, IV-tPA, NIHSS, mRS0-2, HT and AF), the model approached significance; aOR 0.29, p=0.06, 95% CI (0.08-1.06).

In China, no significant association between ***4q25*** rs2200733 and post stroke seizures was found; aOR 0.81, p=0.2, adjusted for age. In patients only from the Nanjing centre, no significant association between ***4q25*** rs2200733 and post stroke seizures

was found, with adjustment for age, NIHSS and mRS0-2; aOR 1.5, p=0.5, 95% CI (0.46-5.12).

Variant unavailable in Brazil.

10.9.3 Calibration and Discrimination – comparing the logistic regression models with and without the addition of genotype

*A BIC >10 points lower is regarded as very strong evidence of model superiority(11)
*A p-value will be reported if the area under the logistic regression receiver operating characteristic curves (AUC LROC) is significantly different between models

Table 15: APOE rs429358 in Australia

Logistic Regression model (adjusted age)	OR, p-value	LROC	BIC
rs429358→PSS	1.4, p=0.006	0.62	221.7
mRS0-2→PSS	2.4, p=0.05	0.58	167.4
mRS0-2+rs429358→PSS	-	0.63	171

Table 16: APOE rs7412 in Australia

Logistic Regression model (adjusted age)	OR, p-value	LROC	BIC
rs7412→PSS	0.63, p=0.2	0.57	228
AF→PSS	3.3, p=0.048	0.56	223.5
AF+rs7412→PSS	-	0.58	227

Table 17: IGF1 rs7136446 in Australia

Logistic Regression model (adjusted age)	OR, p-value	LROC	BIC
rs7136446→PSS	1.1, p=0.81	0.53	229.3
mRS0-2→PSS	2.4, p=0.05	0.58	167.4
mRS0-2+rs7136446→PSS	-	0.62	170.8

Table 18: IGF1 rs7136446 in Australia

Logistic Regression model (adjusted age)	OR, p-value	LROC	BIC
rs7136446→PSS	1.1, p=0.81	0.53	229.3
NIHSS→PSS	1.1, p<0.001	0.75	145
NIHSS+rs7136446→PSS	-	0.75	147.3

Table 19: 4q25 rs220073 in Australia

Logistic Regression model (adjusted age)	OR, p-value	LROC	BIC
rs220073→PSS	0.8, p=0.6	0.53	228
AF→PSS	1.1, p<0.001	0.75	145
AF+rs220073→PSS	-	0.58	227

*p<0.05 for differences in AUC between models

Table 20: APOE rs429358 in China (Nanjing only)

Logistic Regression model (adjusted age)	OR, p-value	LROC	BIC
rs429358→PSS	1.03, p=0.96	0.53	174.7
mRS0-2→PSS	3.1, p=0.01	0.64	167.9
mRS0-2+rs429358→PSS	-	0.64	173.5

Table 21: APOE rs7412 in China (Nanjing only)

Logistic Regression model (adjusted age)	OR, p-value	LROC	BIC
rs7412→PSS	1.2, p=0.82	0.54	174.7
AF→PSS	2.65, p=0.18	0.56	172.7
AF+rs7412→PSS	-	0.57	178.3

Table 22: IGF1 rs7136446 in China (Nanjing only)

Logistic Regression model (adjusted age)	OR, p-value	LROC	BIC
rs7136446→PSS	1.4, p=0.5	0.55	167.9
mRS0-2→PSS	3.1, p=0.011	0.64	167.9
mRS0-2+rs7136446→PSS	-	0.65	166.4

Table 23: IGF1 rs7136446 in China (Nanjing only)

Logistic Regression model (adjusted age)	OR, p-value	LROC	BIC
rs7136446→PSS	1.4, p=0.5	0.55	167.9
NIHSS→PSS	1.1, p=0.005	0.71	166
NIHSS+rs7136446→PSS	-	0.71	164.9

Table 24: 4q25 rs220073 in China (Nanjing only)

Logistic Regression model (adjusted age)	OR, p-value	LROC	BIC
rs2200733→PSS	0.56, p=0.2	0.58	173
AF→PSS	2.7, p=0.18	0.56	172.7
AF+rs2200733→PSS	-	0.60	176.2

Table 25: APOE rs429358 in Brazil

Logistic Regression model (adjusted age)	OR, p-value	LROC	BIC
rs429358→PSS	0.72, p=0.5	0.58	103.8
mRS0-2→PSS	1.4, p=0.54	0.53	97.2
mRS0-2+rs429358→PSS	-	0.58	101

Table 26: APOE rs7412 in Brazil

Logistic Regression model (adjusted age)	OR, p-value	LROC	BIC
rs7412→PSS	1.6, p=0.4	0.53	104
AF→PSS	0.75, p=0.7	0.53	104
AF+rs7412→PSS	-	0.56	107.8

Table 27: IGF1 rs7136446 in Brazil

Logistic Regression model (adjusted age)	OR, p-value	LROC	BIC
rs7136446→PSS	1.3, p=0.5	0.53	103.8
mRS0-2→PSS	1.4, p=0.5	0.53	97.2
mRS0-2+rs7136446→PSS	-	0.57	100.6

Table 28: IGF1 rs7136446 in Brazil

Logistic Regression model (adjusted age)	OR, p-value	LROC	BIC
rs7136446→PSS	1.3, p=0.5	0.53	103.8
NIHSS→PSS	0.92, p=0.11	0.66	86.8
NIHSS+rs7136446→PSS	-	0.65	90.9

Table 29– Summary of Results

		Adjusted Association Found			
		Australia	**China**	**China (Nanjing only)**	**Brazil**
Hypothesis 1					
	PCDH7 rs28498976	×	×	×	✔
	CAMPSAP1L1 rs6660197	×	×	×	×
	CAMPSAP1L1 rs2292096	×	×	×	×
Hypothesis 2					
	ALDH2 rs671	–	✔	×	×
	CD40 rs1883832	×	×	×	×
Hypothesis 3					
Part 1	APOE rs429358 (for mRS0-2)	✔	-	×	×
	APOE4 rs7412 (for AF)	×	-	✔	×
	IGF1 rs7136446 (for mRS0-2)	✔	-	×	×
	IGF1 rs7136446 (for NIHSS)	×	-	×	×
	4q25 rs2200733 (for AF)	×	-	✔	-
Part 2	APOE rs429358	✔	×	×	×
	APOE4 rs7412	×	×	×	×
	IGF1 rs7136446	×	×	×	×
	4q25 rs2200733	×	×	×	×

- Association unable to be performed ✔ Association found ×No association found

10.10 Discussion

We aimed to understand if, in a population of acute ischaemic stroke patients, there was an association between genetic variants and the development of post stroke seizures. To do this, we aimed to assess whether known epilepsy variants are associated with the development of post stroke epilepsy in a stroke population (hypothesis 1). Secondly, we aimed to replicate the association between known genetic variants of post stroke epilepsy development in a Han Chinese population, and to test whether this association is present in other populations (hypothesis 2). Finally, we assessed whether known genetic variants of ischaemic stroke outcome were also associated with the development of post stroke epilepsy (hypothesis 3). The findings will be discussed in turn.

Hypothesis 1

CAMSAP1L1 has been reported in multiple studies to be associated with the development of common epilepsies (6, 12), although this has been largely restricted to Chinese populations. In contrast, studies using patients of European ancestry have not detected GWAS significance for the CAMSAP1L1 SNPS for focal epilepsies (13). The CAMSAP1L1 gene encodes a cytoskeletal protein of little-known function. It has previously been reported in the mammalian nervous system (expressed in neurons and astrocytes) (14), and other work has reported that it is able to inhibit neurite extension by blocking microtubule function (15). It is now thought to potentially play a role in neurotransmission, neuronal networking and connectivity (12). In our study, we did not find a significant association or increased odds between variant CAMPSAP1L1

(rs2292096 or rs6660197) and the development of post stroke seizures in any of the acute ischaemic stroke populations investigated.

PCDH7 encodes a calcium-dependent adhesion protein, is member of the cadherin gene family. The gene is expressed in the CNS, in particular the thalamocortical circuit and the hippocampus. In one recent genome-wide association study, the 4p.15.1 locus of the PCDH7 reached genome wide significance for the development of epilepsy (6). It was suggested that the protocadherin gene is a plausible candidate for common forms of epilepsy, as mutations in the PCDH19 gene causes epilepsy and mental retardation in female patients (Rett syndrome) (16) (6) (17). In our study, we showed a significant association between the PCDH7 rs28498976 and post stroke seizure development in our Brazilian acute stroke population, with adjustment for age, stroke severity, stroke outcome, haemorrhagic transformation and atrial fibrillation (OR=5.1). This association remains significant with the Bonferroni threshold applied. It is currently unclear as to why this association would only be present in the Brazilian sample, with further exploration of the role of PCDH7 in epilepsy and post stroke epilepsy warranted.

Hypothesis 2

The ALDH2 (mitochondrial aldehyde dehydrogenase 2) rs671 SNP leads to a genetic codon change in which glutamate is replaced with lysine at position 504 (18). This results in adverse effects on the dehydrogenase activity of ALDH2, decreasing its activity by 90% (4). ALDH2 is a key enzyme that metabolizes reactive aldehydes, such as 4-HNE, which is increased in rats with middle cerebral artery occlusions and associated with worse brain damage (4). One study has assessed the role of ALDH2 rs671 on the influence of post stroke epilepsy in a Han Chinese population and found

a significant association between the variant and decreased risk of post stroke epilepsy development (4). In our study, we confirmed a significant association between *ALDH2* rs671 and reduced likelihood of post stroke seizures in the Han Chinese population, but were unable to replicate this result in the Australian or Brazilian population. This is most likely due to the much smaller allele frequency in these populations (0.00002444 and 0.0003903 versus 0.2565 in Chinese).

The CD40/CD40L system is reported to be involved in the progression of multiple disease states (5). It serves as a link between inflammation, immunity and tumourigenesis and when bound to its ligand CD40L, CD40 is activated and leads to the up-regulation of proinflammatory and proatherogenic genes (5). It has also been shown to produce reactive oxygen species resulting in oxidative stress, which is involved in the pathogenesis and progression of epilepsy (19, 20). CD40L is now considered to be a predictor for the progression and prognosis of patients with cardiovascular diseases (21, 22). One study assessed the relationship between post stroke epilepsy and the CD40/CD40L system and showed the T allele was significantly associated with post stroke epilepsy in a Han Chinese population (5). Our study aimed to replicate this association in a Han Chinese population and validate it in another population. We found no association between CD40 rs1883832 and the development of seizures after stroke in any population studied.

Hypothesis 3

Performed primarily in animal studies, a number of molecular events have been theorised to contribute to epileptogenesis after traumatic brain injuries (23, 24). These include release of oxygen free radicals, production of cytokines, release of excitatory

amino acids such as glutamate, activation of proteinases and eventual apoptosis (25). Genes that regulate and influence these processes have been the focus of previous studies as they are potential targets for therapeutic interventions. In patients with an ischaemic stroke, it is known that greater ischaemic stroke burden (for example, worse stroke severity, poorer collateral vessel recruitment, and thus poorer stroke outcome) is a major risk factor for the development of post stroke seizures. Thus, we aimed to confirm known genetic variants for ischaemic stroke outcome and assess whether these are additionally associated with the development of post stroke epilepsy in patients with an acute ischaemic stroke. In previous literature, the APOE4 gene variants have been associated with increased stroke risk and increased stroke recovery (26), reduced likelihood of good stroke outcome, increased early death from stroke and worse stroke severity (27, 28), results that have also not been replicated in other studies (29, 30). In our study, we were able to confirm a significant association between APOE rs429358 and reduced likelihood of good stroke outcome with adjustment for age in the Han Chinese population. Work with transgenic mice has indicated that APOE4 has a role in structural plasticity during development and aging, and cell death after ischaemic (31, 32) or convulsive brain injury (33). In humans, the APOE polymorphisms have also been associated with increased risk of late post-traumatic seizures in patients with traumatic brain injuries (25). In our study, we showed that the APOE rs429358 was significantly associated with post ischaemic stroke seizures, after controlling for stroke outcome, age and intra-venous thrombolysis treatment within the Han Chinese population. APOE4 has a major role in lipid redistribution, which is important for membrane maintenance and repair in the brain, including synaptic remodelling, during or after brain injuries (34-37). However, some published studies have demonstrated the ethnic differences between APOE

genotypes and the patient's lipid profile (38, 39), which may account for the lack of association in the Australian and Brazilian populations. The result of our exploratory study suggests that in Han Chinese patients with an acute ischaemic stroke, patients with the APOE rs429358 variant are at an increased likelihood of developing seizures. However when we compared the BIC models, we found that across all populations, the lowest BIC value was for the model testing the association between poor stroke outcome (mRS0-2) and the development of post stroke seizures, with the addition of genotypic information in the model adding no prognostic value.

We also showed a significant association between APOE rs7412 and presence of atrial fibrillation, a common arrhythmia, in the Australian group. Atrial fibrillation is not just a risk factor for stroke, but it is a risk factor for worse stroke severity and poorer stroke outcome (40, 41). Additionally, some literature has suggested that cardioembolic stroke is a risk factor for post stroke seizure development (42). Of particular interest is the literature on the interaction between AF and the APOE genotype in promoting cognitive decline and increasing atherosclerotic burden in patients with Alzheimer's Disease, as well as the synergistic role played by the APOE4 genotype in the presence of atherosclerotic diseases (43). We showed a significant association between APOE4 rs7412 and the development of atrial fibrillation in the Australian population, controlling for age OR=3.2. However, we found no significant association between this variant and the development of post stroke seizures in any population. When we compared the BIC models for seizure outcome, we found that in all populations the lowest BIC was for association between atrial fibrillation and post stroke seizure outcome, with the addition of genetic information reducing the strength of the model. This suggests that it may be the

presence the APOE rs7412 variant that increases the likelihood of developing atrial fibrillation, which in turn is driving the association with increased likelihood of post stroke seizures.

As discussed, atrial fibrillation is a common heart arrhythmia with evidence of genetic susceptibility. The rs2200733 single-nucleotide polymorphism (SNP) in a non-coding region on chromosome 4q25 has previously been strongly and independently associated with an increased risk of AF in multiple populations, including Greek, Italian, Chinese and Taiwanese (44-48). In our study, we showed that in an Australian acute stroke population there was a significant association between 4q25 rs2200733 and presence of atrial fibrillation, however we were unable to replicate this in our Han Chinese population (p=0.05). When we assessed post stroke seizure outcome, there was a non-significant increased odds of developing post stroke seizures across all populations. Similar to APOE rs7412, when we compared the BIC models for seizure outcome, we found that in all populations the lowest BIC was for association between atrial fibrillation and post stroke seizure outcome, with the addition of genetic information reducing the strength of the model. This again suggests that it is the presence the 4q25 rs2200733 variant that increases the likelihood of developing atrial fibrillation, which in turn is driving the association with increased likelihood of post stroke seizures.

Insulin-like growth factor-1 (IGF1) in the literature has been associated with neurogenesis and survival, atherosclerosis, and lower degree of disability 2 years post stroke (1, 49-51). IGF1 has also been studied in cancers, Alzheimer's disease, diabetes and endocrine disorders (52, 53). It has neuroprotective effects in both white

and gray matter under different detrimental conditions and is a key regulator of cell proliferation and an inhibitor of cell apoptosis and necrosis (54) (55). Several studies have found a relationship between low plasma IGF1 levels and increased risk of ischaemic stroke (56). Additionally, some recent studies have suggested an association between IGF1 and increased hippocampal excitatory and seizure activity in epileptic brain (51). In our study, we found no association between IGF1 rs7136446 and stroke severity across any groups, but we were able to show a significant association between IGF1 rs7136446 and reduced likelihood of poor stroke outcome in the Australian population. Additionally, we showed no association between IGF1 rs7136446 and the development of post stroke seizures across any population. In the Han Chinese population, the addition of genetic information to the logistic regression models reduced the BIC value indicating a greater strength of the model, however this was not seen in the other populations where it remained relatively similar.

Overview

This study was limited by a small number of cases, so results are not considered confirmatory. Additionally, where no associations were found we cannot be certain that this represents a real finding or if it's a consequence of the small sample size. However, as an exploratory study we have identified some potential variants that warrant further investigation in a larger sample. In this study, our control population were the acute stroke patients who did not develop seizures within 2 years of stroke. A future study should consider incorporating a group of epilepsy patients without a previous history of stroke. This would allow for greater insight and comparison into whether the onset of epilepsy after stroke is largely genetic based or a result of the stroke itself. Additionally, due to the small sample size we were unable to perform a

genome-wide association study, which is preferred to a candidate gene approach study. However, a GWAS study requires great numbers which given the relatively low incidence of post stroke epilepsy is a time consuming and labour intensive endeavour. It is recommended that further studies should not just focus on genetic variants for the development of post stroke epilepsy, but should include patients with seizures and epilepsy from traumatic brain injuries, which has a much higher incidence and is often more poorly controlled with antiepileptic drugs. Due to the retrospective nature of data collection across some of the centres, we were limited by the baseline and follow up stroke data originally collected at each site. For example, the Hong Kong cohort was limited to age at stroke onset and sex of patient, and because of this they were often removed from logistic regression models, further reducing the sample size.

10.11 Conclusions

We have identified a number of variants such as PCDH7 rs28498976, ALDH2 rs429358 and APOE4 rs429358 that have shown association with the development of post stroke seizures. Additionally, we have confirmed a number of variants (APOE rs429358, rs7412, IGF1 rs7136446, 4q25 rs2200733) that have shown association for stroke comorbidities (atrial fibrillation, worse stroke severity and poor stroke outcome). As this was an exploratory, pilot study these results warrant further investigations with a larger sample size.

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General Discussion and Conclusion

Summary of key findings

Acute stroke outcome is not entirely represented by mortality rates and level of disability. There are a range of neurological sequelae that contribute an important additional burden to patients. Over the past 20 years, major advances in acute stroke treatment and management has led to a reduction in stroke-related mortality, however an unavoidable side effect has been the concomitant rise in survivors living with life-changing disability. As discovered, post stroke seizures can affect up to 13% of the ischaemic stroke population, are difficult to predict, and are associated with a poorer health related quality of life (206). This multidisciplinary thesis examined the role of clinical, imaging and genetics factors in determining stroke outcome with a focus on post stroke epilepsy.

The objectives were three-fold 1) to optimise the role of imaging in hyperacute stroke and assess the potential use of acute stroke imaging in detecting post stroke seizures, 2) to assess whether stroke reperfusion therapies affect post stroke seizure occurrence 3) to assess whether the occurrence of post stroke seizures has a genetic influence. The key findings from each chapter as well as limitations and future directions for each section have been summarised below.

Objective 1- Radiological markers: How can we optimise the role of imaging in hyperacute stroke and assess the potential use of acute stroke imaging in longer-term complications of stroke, in particular epilepsy?

Study 1: Reliability and utility of the Alberta Stroke Program Early CT Score in hyperacute stroke

Study Overview: The aim of this chapter was to evaluate whether time from ischaemic stroke onset to initial non-contrast CT influences inter-rater variability and prognostic accuracy of the Alberta Stroke Program Early CT Score for 3-month functional outcome, as determined by the modified Rankin scale (mRS).

Key Findings: This study confirmed that ASPECTS in earlier times (within 100 minutes) have greater inter-rater variability, but additionally demonstrated that the prognostic accuracy of ASPECTS is weaker in the earlier time window. We concluded that extra care should be exercised in adopting the ASPECTS on NCCT to ultra-early times as a tool for selection patients for revascularization.

Limitations: The ASPECTS itself has limitations and is only applicable to the middle cerebral artery territory. ASPECTS assessment can be compromised by streak artifacts at the base of the skull, watershed infarcts, subcortical and age-related periventricular white matter changes, and poor-quality scans due to patient motion. In this study, only scans that were severely movement degraded were

removed. Moreover, the anatomical regions covered by ASPECTS are not equally distributed across the MCA territory with 3 of the 10 regions allotted to subcortical areas that are within close proximity to each other and the remaining 7 distributed across the entire MCA territory (280). This has led to concern that ASPECTS may unjustifiably exclude patients from receiving IV-tPA treatment in striatocapsular stroke and thus is not a reliable marker for lesion volume in such stroke subtypes (281). Thus, lesion location should also be factored into the consideration for excluding or including patients for treatments (280, 281).

The success of reperfusion therapies has a substantial impact on prognosis and was not assessed in the routine clinical care of these patients who received predominantly thrombolysis without thrombectomy. However, the literature on the prognostic significance of ASPECTS >7 was developed in similar patient cohorts where reperfusion status was unknown. Our patient cohort was not exclusively composed of patients with LVO, which is the main context in which ASPECTS-based treatment selection has been proposed. However, this avoids selection bias, which would likely occur if only endovascular patients were included. The conspicuity of ASPECTS changes on NCCT over time is likely to be similar in the LVO and non-LVO patients, although this is something that can be examined further.

Future Directions: We proposed further investigation and validation of the ASPECTS as well as the role of alternative imaging modalities in hyperacute stroke.

Study 2: Reliability and utility of the Alberta Stroke Program Early CT Score on CT perfusion and non-contrast CT in hyperacute stroke

Study Overview: Given the results of study 1, this chapter aimed to assess and compare the practical evaluation (including reliability, prognostic accuracy and reproducibility) of ASPECTS on CT perfusion and NCCT in early versus later times after stroke onset.

Key Findings: This study demonstrated that ASPECTS assessed on CTP parameters has greater inter-rater agreement and reduced magnitude of differences between raters than NCCT ASPECTS. This particularly applied to patients imaged within 70 min of stroke onset. We showed that for NCCT ASPECTS, the inter-rater agreement and the prognostic accuracy of ASPECTS >7 for 3-month mRS significantly increased as time from onset to imaging increased, which was not seen using any CTP parameter. Finally, we showed that after adjustment for both age and baseline stroke severity, ASPECTS >7 on CBV is a stronger predictor of good prognosis than ASPECTS >7 on NCCT, particularly in the early period after stroke onset.

Limitations: This was a retrospective analysis examining a pure IV-tPA population reflecting the processing times of a single centre. Our patient cohort did not exclusively comprise patients with large vessel occlusion. However, this avoids selection bias, which would likely occur if only endovascular patients were

included. We were limited to 2 raters and reliability may vary with differing experience.

Future Direction: The prognostic value of both NCCT and CTP ASPECTS was modest in this dataset but is dependent on reperfusion status and the particular CTP parameter used. This should be considered in the future.

Given that the ASPECTS is a marker for ischaemic lesion size which is consistently reported as a risk factor for post stroke seizure development, the Post Stroke Epilepsy lab group at the Royal Melbourne Hospital investigated the association of post stroke seizures with the extent of ischaemia assessed by ASPECTS (282) in a population of patients who received IV-tPA. In univariate logistic regression, both ASPECTS on admission (OR 0.69 per 1-point increase; 95% CI 0.55–0.86; p = 0.001) and at 24 h (OR 0.80 per 1-point increase; 95% CI 0.70–0.92; p = 0.002) were significantly associated with decreased odds of post stroke seizures, highlighting that greater ischemic burden as adjudicated by lower ASPECTS was associated with increased likelihood of post stroke seizure development.

A further study conducted by the Post Stroke Epilepsy lab group at the Royal Melbourne Hospital aimed to examine whether cortical involvement, as detected on CT perfusion imaging, could be used to identify patients at higher risk of developing post ischaemic stroke seizures (283). It was found that cortical

involvement was significantly associated with post stroke seizures across all CTP modalities. CBV had the highest hazard ratio (11.3, 95% confidence interval (CI) 1.1–41.2), followed by NCCT (5.3, 95% CI 1.5–18.0) and CBF (4.2, 95% CI 1.1–15.2). Sensitivity was highest for Tmax (100%), followed by CBV and CBF (both 76.9%) and NCCT (63.6%). Specificity was highest for CBV (77.8%), then NCCT (75.6%), CBF (54.0%), and Tmax (29.1%). Receiver-operating characteristic area under the curve was significantly different between imaging modalities ($p < 0.001$), CBV 0.77, NCCT 0.70, CBF 0.65, and Tmax 0.65. It was concluded that CTP may improve sensitivity and specificity for identifying cortical involvement as a risk for post stroke seizures compared to NCCT.

The final study conducted by the Post Stroke Epilepsy lab group at the Royal Melbourne Hospital cemented the groundwork for Study 3 included in this thesis. This was the first study that reported on post stroke seizures in patients treated with modern cerebrovascular stent devices and revascularization techniques, with the specific aim of assessing the association between HT and the development of post stroke seizures in this setting (284). It was found that patients with anterior circulation strokes who developed HT after treatment with endovascular therapy were nearly 5 times more likely to experience post stroke seizures compared to those without HT. It was concluded that HT has the potential to be used as an imaging marker when evaluating compounds with antiepileptogenic effects in patients after ischemic stroke.

Study 3: A comparison of the influence of hemorrhagic transformation on post ischaemic stroke seizure development in patients receiving reperfusion therapies

Study Overview: This chapter aimed to compare the influence of hemorrhagic transformation on post ischaemic stroke seizure development in patients receiving reperfusion therapies (intra-venous thrombolysis (IV-tPA) and intra-arterial therapy (IAT) (with/without IV-tPA).

Key Findings: This study demonstrated that patients undergoing intra-arterial therapy (with/without IV-tPA) who subsequently develop hemorrhagic transformation are at substantially greater risk of developing post stroke seizures than patients treated with IV-tPA only, independent of age, baseline stroke severity and functional outcome. We showed a significant increase in seizure occurrence between patients with hemorrhagic transformation receiving IAT compared to patients receiving IV-tPA, suggesting that patients receiving IAT therapy have additional underlying mechanisms for increased seizure development.

Limitations: This study was retrospectively designed with the potential limitation of recall bias towards identifying seizures using the phone questionnaire. However, this limitation was minimized with corroboration of patient recall with reviewing follow-up medical records and contacting the patients' primary care

physician. Additionally, despite a large, single centre cohort of endovascular treated acute stroke patients, the number of patients with post stroke seizures was relatively small. Thus, it was not feasible to perform separate sub-group analyses for early and late seizures or to perform interaction assessments on large vessel occlusion patients only.

Future Directions: Given the increasing use of modern endovascular stroke therapies, larger controlled studies specifically comparing the seizure incidence following endovascular therapy and medical management are warranted.

Objective 2- Clinical markers: Given the improved immediate outlook of acute stroke, how do these novel stroke interventions affect longer-term complications of stroke, in particular epilepsy?

Study 4: The association between different acute stroke therapies and development of post stroke seizures

Study Overview: This was a retrospective, multi-centre cohort study conducted at the Royal Melbourne Hospital and Jinling General Hospital, Nanjing. This study aimed to establish whether there is an association between different acute stroke treatments and post stroke seizure development.

Key Findings: This study demonstrated that acute stroke reperfusion therapies

are significantly associated with seizure development. Specifically, we showed that in patients treated with IV-tPA only, independent of age, baseline stroke severity, stroke outcome and other baseline variables, there was a greater than threefold increase in the likelihood of developing seizures in comparison to controls. This was a similar effect to those patients treated with IV-PA+ IAT. We also found that in patients treated with IAT only, there was a greater than fivefold increase in the likelihood of developing seizures in comparison to controls. However, there was no evidence for an additive or synergistic effect of treatment modality.

Limitations: A limitation of this study is the retrospective design with the potential of bias towards identifying seizures in those patients receiving reperfusion therapies given that they may have received more monitoring in the stroke unit than those patients without reperfusion therapies. However, given that all patients were contacted via a phone call questionnaire we believe this would have minimised any bias towards treatment groups. Another limitation is that we do not understand the potential ethnicity-treatment interaction without a treated group from Nanjing. We cannot discount the potential effect of the treatment itself differing due to ethnicity, although we do not expect this.

Future Directions: Future studies should incorporate a treatment group from separate ethnicities to examine a possible treatment-ethnicity interaction. Additionally, due to the low incidence of post stroke seizure development, future larger studies are required to improve the precision of incidence estimates.

Finally, we were unable to perform a sub-analysis on patients with large vessel occlusion as vessel occlusion status was not available in our control population. Even with adjustment for baseline NIHSS, initial stroke severity may still be contributing to the higher odds of seizures in the treated patients. It would be of interest to perform a future analysis specifically in large vessel occlusion patients.

> **Study 5:** Does ethnicity affect the association between atrial fibrillation and post stroke seizure development?

Study Overview: This chapter assessed whether ethnicity affects the association between atrial fibrillation and post stroke seizure development.

Key Findings: Atrial fibrillation (AF) was significantly associated with increased likelihood of seizure development in the Nanjing cohort but not in the Melbourne population. Furthermore, within our ethnicity-interaction regression models, we found that the association between atrial fibrillation and seizures was significantly affected by ethnicity. This may suggest an underlying environmental and/or genetic basis for seizure development in those patients with AF.

Limitations: A limitation of this study is the difference in clinical care between centres. Secondly, the rate of haemorrhagic transformation was unknown in the Chinese cohort and therefore was unable to be included into the adjustment. Similarly, we do not know if patients were on anticoagulation and in what form at the time of and after their stroke. A further limitation is the small sample size of

patients with post stroke seizures and atrial fibrillation and further investigation using larger sample sizes are warranted.

Future Directions: Further understanding of genetic risks and environmental differences across ethnic populations and the role in PSS is required.

Study 6: A registry of clinical and MRI outcomes following early versus late initiation of anticoagulation after ischaemic stroke or transient ischaemic attack in patients with atrial fibrillation

Study Overview: This is a protocol/methods chapter, which follows on from the previous study. This is a prospective, observational clinical trial aiming at assessing clinical and MRI outcomes following early versus late initiation of anticoagulation after ischaemic stroke in patients with atrial fibrillation.

Future Directions: We anticipate the end date of recruitment to be 01/02/2019 and publication of results 01/02/2020.

Objective 3 – Genetic markers: Is there a potential genetic association to the development of seizures post stroke or is it a complication of the stroke itself?

Study 7: Is there a genetic association on the development of post stroke seizures?

Study Overview: This chapter aims to examine whether there is a potential genetic component to the development of seizures post stroke or whether it is a complication of the stroke itself.

Key Findings: In our study, we showed a significant association between the PCDH7 rs28498976 and post stroke seizure development in our Brazilian acute stroke population, with adjustment for age, stroke severity, stroke outcome, haemorrhagic transformation and atrial fibrillation. We also confirmed a significant association between *ALDH2* rs671 and reduced likelihood of post stroke seizures in the Hans Chinese population, but were unable to replicate this result in the Australian population. This is most likely due to the much smaller allele frequency in the Australian population.

Limitations: This study was limited by a small number of cases, so that only associations with relatively large effects could be detected. In this study, our control population were the acute stroke patients who did not develop seizures within 2 years of ictal onset. A future study should consider incorporating a group of epilepsy patients without a previous history of stroke. This would allow for greater insight and comparison into whether the onset of epilepsy after stroke is largely genetic based or a result of the stroke itself. Additionally, due to the small sample size we were unable to perform a genome-wide association study, which is preferred to a candidate gene approach study. Due to the retrospective nature of

data collection across some of the centres, we were limited by the baseline and follow up stroke data originally collected at each site. For example, the Hong Kong cohort was limited to age at stroke onset and sex of patient, and because of this they were often removed from logistic regression models, further reducing the sample size.

Future Directions: It is recommended that further studies should not just focus on genetic variants for the development of post stroke epilepsy, but should include patients with seizures and epilepsy from traumatic brain injuries, which has a much higher incidence and is often more poorly controlled with antiepileptic drugs. The results of our exploratory study warrant further investigations with a larger sample size.

Research Implications

Ischaemic stroke is the most common type of stroke and responsible for a substantial burden of death and disability worldwide. Reperfusion therapies with thrombolysis and, more recently, endovascular thrombectomy have transformed outcomes for patients. This body of research targeted patient groups treated with modern cerebrovascular stent devices and revascularization techniques, in order to assess the implications of these novel stroke interventions, focusing on development of post stroke seizures, a potential late complication that has not previously been examined in relation to endovascular reperfusion. Post stroke

epilepsy is relatively infrequent but constitutes the majority of adult-onset epilepsy and substantially degrades quality of life. The recently published SeLECT score was derived from a cohort of 1200 ischaemic stroke patients (12% received thrombolysis but none were treated with intra-arterial therapy). The reported post stroke seizure incidence was 7% at 5 years. It was concluded that, in addition to the SeLECT predictors (severity of stroke, large-artery atherosclerotic aetiology, early seizures, cortical involvement and middle cerebral artery territory involvement), future research should aim at refining the score to include lesion size, biomarkers, EEG findings, psychiatric comorbidities, genetic data and advanced neuroimaging. Data presented in this thesis may inform some of these factors.

Multiple advanced neuroimaging techniques capable of identifying patients at the highest risk of developing post stroke seizures have been examined. Specifically, these include the Alberta Stroke Program Early CT score on NCCT, cortical involvement on CTP parameters and extent of haemorrhagic transformation on NCCT. Our exploratory study assessing the genetic influence on the development of post stroke seizures has also laid important groundwork in developing genetic biomarkers for future studies. Additionally, unlike previous work, international sites were included along with Australian sites, allowing the interrogation of whether ethnicity and environment influences the development of post stroke seizures. Results from this investigation revealed that, not only does occurrence of seizures differ across populations from different countries, but certain clinical

markers, such as presence and treatment of atrial fibrillation, may influence seizure occurrence across populations.

Finally, not only has this thesis identified that patients undergoing novel reperfusion therapies (such as intra-venous thrombolysis and intra-arterial therapy) are at a higher risk of developing seizures in comparison to standard medical care, but results have also suggested that, within these patient groups, those with secondary haemorrhagic transformation are at the highest risk of developing seizures. This has potential implication for the ongoing care of these patients, with those identified at risk benefitting from longer-term stroke follow-up, and could represent an enriched population for targeted antiepileptogenic treatments. This finding also suggests that novel strategies to reduce the risk of haemorrhagic transformation after reperfusion therapies should be considered. For example, collateral status among patients with acute ischaemic stroke differs among patients and recent literature suggests that poor baseline collaterals and successful therapeutic recanalization may result in clinically significant haemorrhagic complications (285). Although recanalization to restore antegrade flow may be crucial to achieve a favourable outcome in patients undergoing revascularization therapy, research has suggested that therapeutic recanalization in the setting of poor collaterals resulted in a high frequency of HT with worsened clinical neurological status (285). As endovascular treated patients with subsequent HT were at a significantly increased risk of developing seizures, of

interest would be a study examining the effects of collateral status and post stroke seizure development in patients receiving such reperfusion therapies.

Several other strategies to prevent haemorrhagic transformation induced by reperfusion therapies have also been evaluated, including inhibiting metalloproteinases (MMPs), reducing oxygen radicals and modulating targets that affect the blood-brain barrier (BBB) permeability. Inhibition of MMP by therapeutics such as batimastat and minocycline were shown to reduce the BBB permeability and risk of HT and improve neurological outcomes in rat and rabbit models of stroke (286, 287). One mechanism of minocycline's protective effect may be its ability to suppress microglial activation and maintain the BBB via MMP inhibition (288). Minocycline has also been used in experimental models of drug-resistant epilepsies as a potent anticonvulsive (289). This is because minocycline has anti-inflammatory actions by inhibiting microglial activation and transmigration of T-lymphocytes (290). Patients taking minocycline shortly after an acute ischaemic stroke also showed improved functional outcome at 3 months compared to those receiving placebo (291). Additionally, in the setting of brain arteriovenous malformations, preliminary evidence suggests the minocycline may also reduce the risk for developing epilepsy (292).

Although several preclinical studies have demonstrated the potential effect of many drugs to reduce the risk of HT in IV-tPA experimental models, few are under investigation in clinical trials. Edaravone (a free radical scavenger) was

trialled in patients in the PROTECT 4.5 study, which suggested the frequency of HT is lowered (although non-significantly) with combined use of IV-tPA and edaravone (293). However, this was an observational study without a control group and it did not show any improved clinical outcomes (293).

Therapeutic hypothermia, which is thought to target multiple reperfusion injury mechanisms, has been tested in experimental models of MCA stroke, showing combination therapy of IV-tPA and therapeutic hypothermia reduces BBB disruption and risk of HT (294). The ReCCLAIM (Reperfusion and Cooling in Cerebral Acute Ischaemia) and the ICTuS-2 (Intravascular Cooling in the Treatment of Stroke) trials examined this combination in acute stroke patients, both showing the approach was safe and feasible (295, 296). However, more robust, randomized clinical trials are needed to establish its utility in the clinical setting. Of note, post-traumatic seizure susceptibility has been shown to be attenuated by hypothermia therapy in rats with traumatic brain injuries (297). In the clinical setting, the POLAR-RCT (Prophylactic Hypothermia Trial to Lessen Traumatic Brain Injury) study is currently recruiting traumatic brain injury patients to assess the effect of hypothermia on neurological outcomes (including post-traumatic epilepsy) (298).

However, at present no indications for antiepileptic drugs in preventing post stroke seizures and epilepsy exist and as such, only weak recommendations have been made against the use of AED prophylactic treatment in acute ischaemic

stroke patients for prevention of seizures (275). This is because undertaking clinical anti-epileptogenesis trials is difficult and trials with unselected populations require large sample sizes and are costly. This thesis has identified potential higher risk target populations as well as laid important groundwork for defining biomarkers of seizures after acute ischaemic stroke. This will better allow for designing and developing trials that explore the anti-epileptogenic properties of specific agents, including AEDs, as well as guide the clinical management of stroke survivors (259).

Final Conclusion

The results presented in this thesis have the potential to guide identification of individuals at a higher risk of developing post stroke seizures and represent a step towards personalised medicine. In the future, if antiepileptogenic treatments become available, these results may inform the selection of an enriched population for trials and guide recruitment for biomarker studies of epileptogenesis.

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Appendix 1 - Questionnaire for Patients with Post-stroke Epilepsy for Phone Interviews and Clinic Reviews

1. Patient Name：_____ Phone No 1：_____
2. Study Number：_____ 2：_____
3. Patient hospital specific number: _____ 3：_____
4. Date of Birth：_____/_____/_____(dd/mm/year) Year of Admission：_____
5. Did you ever have, or did anyone ever tell you that you had, a seizure disorder or epilepsy before the stroke[s]?

   No ☐ Yes ☐ (If yes, exclude; If no, move on)

6. Have you ever had or has anyone ever told you that you've had seizures, epilepsies or convulsions since the stroke[s]?

   No ☐ Yes ☐ Possible ☐ Not-Known ☐

   (If yes, go to Q7-12; If no, possible or not-known, go to q13)
7. What is your seizure type?

   Simple Partial Seizure ☐ Complex Partial Seizure ☐ Secondary GTCS ☐
8. How soon after the stroke did you experience your first seizure?_____
   Or the date of the first seizure: _____/_____/_____(dd/mm/year)
9. Do you have a family history of seizures, epilepsy or febrile convulsions?

   No ☐ Yes ☐ If yes, give details of 1st/2nd degree family members with positive hx.

Family Member: _____Diagnosis:_____AEDs:_____

10. Have you ever had any of the following:

    ☐ Head injury or head trauma ☐ Intracranial Infection ☐ Birth Trauma

    ☐ Excessive drug or alcohol use ☐ Cerebral Tumour
11. Have you ever taken medications for seizures?

    No ☐ Yes ☐ Possible ☐ Not-Known ☐
12. Details of the medications

	Name	Date of start dd/mm/year	Max. Dose mg/day	Date of cease dd/mm/year
1st AED				
2nd AED				

13. Date of last seizure:_____/_____/_____(dd/mm/year)⇨ End
    1) Have you ever had, or has anyone ever told you that you've had, the following
    2) A seizure, convulsion, fit or spell under any circumstances?

       No ☐ Yes ☐ Possible ☐ Not-Known ☐

    3) Uncontrolled movements of part of your body such as twitching, jerking, shaking or feelings without consciousness impairment?

       No ☐ Yes ☐ Possible ☐ Not-Known ☐

    4) An unexplained change in your mental state or level of awareness; or an episode of ''spacing out'' that you could not control?

       No ☐ Yes ☐ Possible ☐ Not-Known ☐

Name of Interviewer: _____Date:_____/_____/_____

Appendix 2 – Publication

Chen Z, Churilov L, Koome M, Chen Z, **Naylor J**, Kwan P, Yan B. Post stroke Seizures Is Associated with Low Alberta Stroke Program Early CT Score. *Cerebrovasc Dis* 2017;43:259-265

Original Paper

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Post-Stroke Seizures Is Associated with Low Alberta Stroke Program Early CT Score

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Keywords
Post-stroke seizures · Alberta Stroke Program Early CT Score · Non-contrast CT · Cortical involvement · Ischemic lesion size

Abstract
Background: Ischemic stroke is a leading cause of new-onset seizures. Cortical ischemia and large ischemic lesion size are among the most consistently reported risk factors for post-stroke seizures. Alberta Stroke Program Early CT Score (ASPECTS) is a simple and reliable tool for quantifying the extent of cerebral ischemia and may function as a screening tool for patients with high risk of seizure development. We investigated the association of post-stroke seizures with the extent of ischemia assessed by ASPECTS and with cortical involvement identified on non-contrast CT (NCCT). ***Methods:*** This cohort study was based on a prospectively maintained clinical database of acute ischemic stroke patients who were given intravenous tissue plasminogen activator treatment. We included patients with anterior circulation stroke admitted between January 2008 and October 2014. Patients with pre-stroke seizures were excluded. Clinical data and seizure follow-up data were collected. NCCT scans acquired both on stroke admission and at 24 h were analyzed. Logistic regression and cox regression were performed in statistical analysis. ***Results:*** A total of 348 patients (median age 73 years, interquartile range [IQR] 63–80, 55% male) were included. During follow-up (median duration 559 days, IQR 107.5–1188.5 days), 22 (6.3%) patients developed post-stroke seizures. Median time from stroke to seizure onset was 138 days (IQR 10–342 days). In univariate logistic regression, both ASPECTS on admission (OR 0.69 per 1-point increase; 95% CI 0.55–0.86; $p = 0.001$) and at 24 h (OR 0.80 per 1-point increase; 95% CI 0.70–0.92; $p = 0.002$) were significantly associated with post-stroke seizures. Cortical involvement at 24 h also correlated with seizure occurrence (OR 3.01; 95% CI 1.08–8.34; $p = 0.03$). Cox regression confirmed the higher risk of developing seizures at any time point in patients with lower ASPECTS value and cortical ischemia. Of note, ASPECTS was the only independent predictor for post-stroke seizures in multivariate logistic regression. ***Conclusion:*** The extent of

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Appendix 3– Publication

Koome M, Churilov L, Chen Z, Chen Z, **Naylor J**, Thevathasan A, Yan B, Kwan P. Computed tomography perfusion as a diagnostic tool for seizures after ischemic stroke. *Neuroradiology* 2016;58:577-584

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DIAGNOSTIC NEURORADIOLOGY

Computed tomography perfusion as a diagnostic tool for seizures after ischemic stroke

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Abstract
Introduction Cerebral cortical ischemia is a risk factor for post-stroke seizures. However, the optimal imaging method is unclear. We investigated CT perfusion (CTP) in detecting cortical ischemia and its correlation with post-stroke seizures compared with non-contrast CT (NCCT).
Methods We included patients with acute ischemic stroke admitted to the Royal Melbourne Hospital between 2009 and 2014. Post-stroke seizure information was collected. Cortical involvement was determined on acute NCCT and CTP (T_{max}, cerebral blood volume [CBV], and cerebral blood flow [CBF]). The association between cortical involvement detected by different imaging modalities and post-stroke seizures was examined.
Results Three-hundred fifty-two patients were included for analysis. Fifty-nine percent were male, and median age was 73 years (inter-quartile range 61–82). Follow-up was available for 96 %; median follow-up duration was 377 days (inter-quartile range 91–1018 days). Thirteen patients had post-stroke seizures (3.9 %). Cortical involvement was significantly associated with post-stroke seizures across all modalities. CBV had the highest hazard ratio (11.3, 95 % confidence interval (CI) 1.1–41.2), followed by NCCT (5.3, 95 % CI 1.5–18.0) and CBF (4.2, 95 % CI 1.1–15.2). Sensitivity was highest for T_{max} (100 %), followed by CBV and CBF (both 76.9 %) and NCCT (63.6 %). Specificity was highest for CBV (77.8 %), then NCCT (75.6 %), CBF (54.0 %), and T_{max} (29.1 %). Receiver-operating characteristic area under the curve was significantly different between imaging modalities ($p < 0.001$), CBV 0.77, NCCT 0.70, CBF 0.65, and T_{max} 0.65.
Conclusion CTP may improve sensitivity and specificity of cortical involvement for post-stroke seizures compared to NCCT.

Keywords Ischemic stroke · CT perfusion · Non-contrast CT · Post-stroke epilepsy · Seizures

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Introduction

Stroke is the most common risk factor identified in adults with new onset epilepsy in developed countries [1]. Up to 12 % of patients experience seizures after stroke [2–5]. Post-stroke seizures are associated with increased mortality, prolonged hospital stays, and loss of independence [6, 7]. Involvement of cerebral cortex, whether hemorrhagic or ischemic, is one of the most consistent risk factors for post-stroke seizures [2–5, 8, 9]. The association appears independent of infarct size [1].

The optimal method to detect cortical involvement has not been established. Studies have tended to combine findings from different modalities for analysis despite their differing sensitivities. Most studies have relied on non-contrast CT (NCCT) because of its widespread availability and its

Appendix 4 – Publication

Thevathasan A, **Naylor J**, Churilov L, Mitchell P.J, Dowling R.J, Yan B, Kwan P. Association between hemorrhagic transformation after endovascular therapy and poststroke seizures. *Epilepsia* 2018;59:403-409

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FULL-LENGTH ORIGINAL RESEARCH

Epilepsia®

Association between hemorrhagic transformation after endovascular therapy and poststroke seizures

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Summary

Objective: Endovascular therapy has recently become standard therapy for select patients with acute ischemic stroke. Infarcted brain tissue may undergo hemorrhagic transformation (HT) after endovascular therapy. We investigated the association between HT and occurrence of poststroke seizures in patients treated with endovascular therapy.

Methods: Consecutive patients treated with endovascular therapy for acute anterior circulation ischemic stroke were included. HT was assessed with computed tomography/magnetic resonance imaging (CT/MRI) at 24 h after stroke onset. Patients were followed for up to 2 years for seizure occurrence.

Results: A total of 205 (57.1% male) patients were analyzed. Median age was 69 years (interquartile range [IQR] 57-78). Among patients with HT, 17.9% (10/56) developed poststroke seizures compared with 4.0% (6/149) among those without HT (hazard ratio [HR] 5.52; 95% confidence interval [CI] 2.00-15.22; $P = .001$). The association remained significant after adjustment for cortical involvement, baseline National Institutes of Health Stroke Scale score, age and use of intravenous tissue plasminogen activator and clot retrieval (HR 4.85; 95% CI 1.60-14.76; $P = .005$). In patients who developed seizures within the follow-up period, median time to first seizure was 111 days (IQR 28-369) in patients with HT and 36 days (IQR 0.5-183) in patients without HT.

Significance: A patient who develops HT following endovascular therapy for acute ischemic stroke had a nearly 5 times higher rate of developing poststroke seizures within 2 years. HT may be used as an imaging biomarker for poststroke seizures.

KEYWORDS
hemorrhage, poststroke epilepsy, revascularization

1 | INTRODUCTION

The management of acute ischemic stroke is undergoing a paradigm shift. Based on the recent reports of several randomized controlled trials, including one performed at our center,[1] endovascular therapy, alone or in combination with intravenous thrombolysis, has become standard therapy for selected patients.[2]

It is well recognized that reperfusion techniques may be associated with hemorrhagic complications. Hemorrhagic transformation (HT) is a natural consequence of cerebral infarction.[3] HT occurs because of the disruption of the

Appendix 5- Publication

Chen Z, Churilov L, Chen Z, **Naylor J**, Koome M, Yan B, Kwan P. Association between implementation of a code stroke system and poststroke epilepsy. *Neurology*2018;90:e1126-e113

ARTICLE CLASS OF EVIDENCE

Association between implementation of a code stroke system and poststroke epilepsy

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Abstract

Objective
We aimed to investigate the effect of a code stroke system on the development of poststroke epilepsy.

Methods
We retrospectively analyzed consecutive patients treated with IV thrombolysis under or outside the code stroke system between 2003 and 2012. Patients were followed up for at least 2 years or until death. Factors with $p < 0.1$ in univariate comparisons were selected for multivariable logistic and Cox regression.

Results
A total of 409 patients met the eligibility criteria. Their median age at stroke onset was 75 years (interquartile range 64–83 years); 220 (53.8%) were male. The median follow-up duration was 1,074 days (interquartile range 119–1,671 days). Thirty-two patients (7.8%) had poststroke seizures during follow-up, comprising 7 (1.7%) with acute symptomatic seizures and 25 (6.1%) with late-onset seizures. Twenty-six patients (6.4%) fulfilled the definition of poststroke epilepsy. Three hundred eighteen patients (77.8%) were treated with the code stroke system while 91 (22.2%) were not. After adjustment for age and stroke etiology, use of the code stroke system was associated with decreased odds of poststroke epilepsy (odds ratio = 0.36, 95% confidence interval 0.14–0.87, $p = 0.024$). Cox regression showed lower adjusted hazard rates for poststroke epilepsy within 5 years for patients managed under the code stroke system (hazard ratio = 0.60, 95% confidence interval 0.47–0.79, $p < 0.001$).

Conclusion
The code stroke system was associated with reduced odds and instantaneous risk of poststroke epilepsy. Further studies are required to identify the contribution of the individual components and mechanisms against epileptogenesis after stroke.

Classification of evidence
This study provides Class III evidence that for people with acute ischemic stroke, implementation of a code stroke system reduces the risk of poststroke epilepsy.

MORE ONLINE

→ **Class of evidence**
Criteria for rating therapeutic and diagnostic studies
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